Molecular Devices

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MetaXpress® Software:
Neurite Outgrowth Module
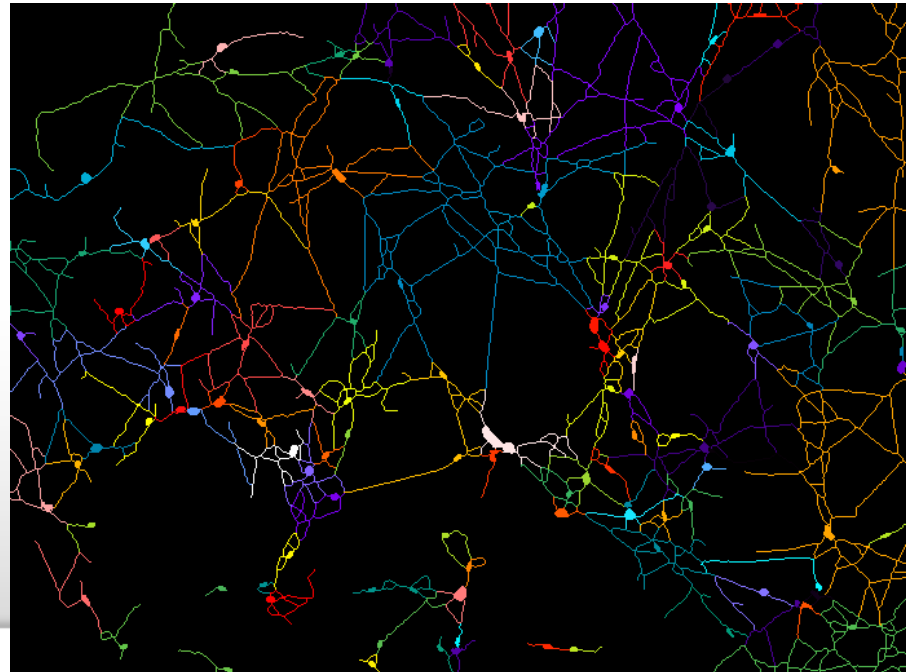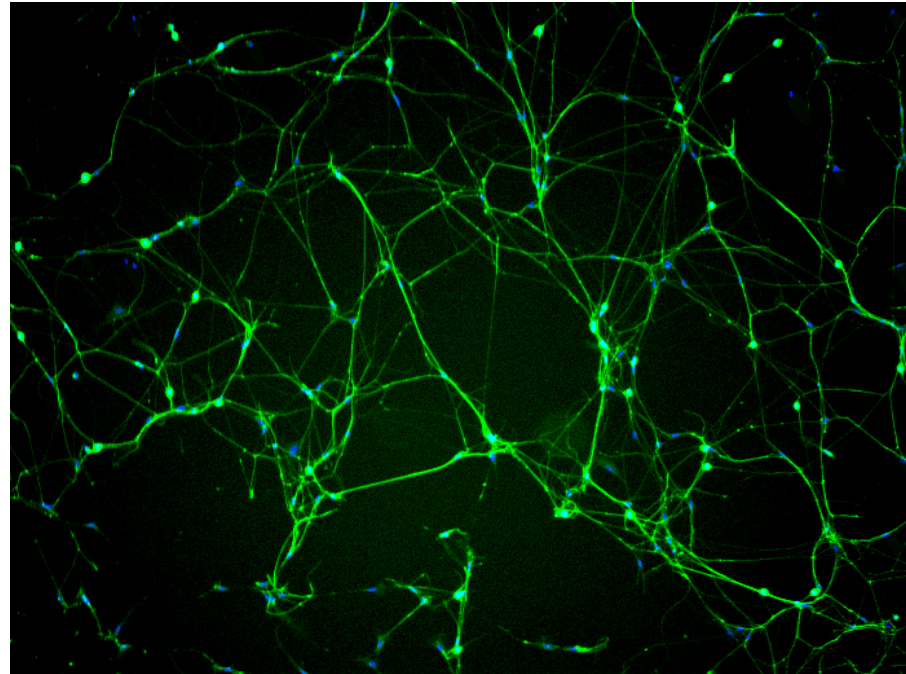
Neurite Outgrowth

- Identifies and measures processes connected to cell bodies

- Optional use of a nuclear stain for assisting cell body identification

- Cell bodies / outgrowths can be either fluorescent or transmitted light images

Module Settings

- **Neurite image**

- Select the wavelength for the **neurites** (outgrowths and cell bodies)

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Module Settings

- **Display result image**

- Leave "**Display result image**" deselected (this is generally only used when journaling)

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Module Settings

- **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

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Module Settings
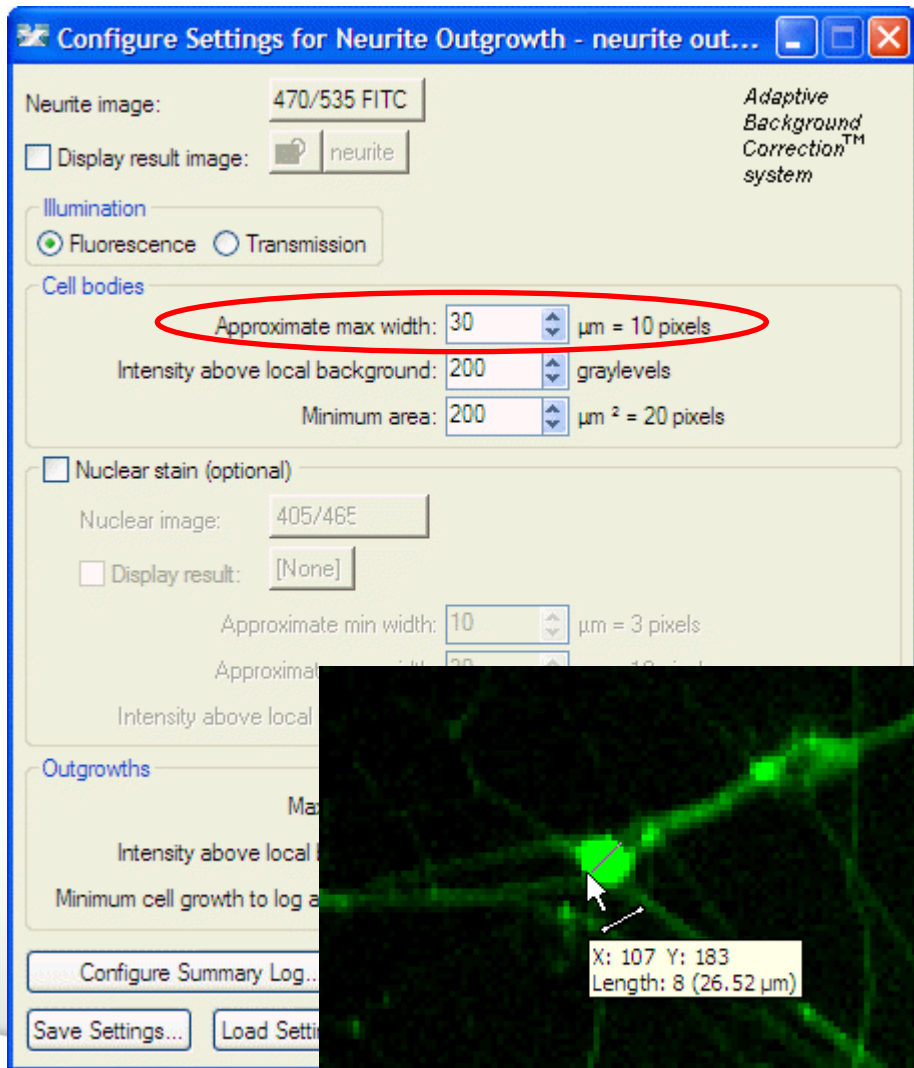
- **Cell bodies**
- **Set the Approximate max width** for the cell bodies that you want to detect
- The width is the short axis of a cell body (in um)
- The region tools can be used to measure widths
- If the setting is too large, clusters of cell bodies may be joined together
- If the setting is too small, thicker outgrowths or branch points may be identified as cell bodies

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Devices

Module Settings

- **Cell bodies**

- The **intensity above local background** is used for finding the cell bodies

- This value is a minimum and should be set slightly lower than the difference in intensity between a dim cell and its local background

- Draw a line across a cell into the background and use Measure > Linescan to determine intensity values; or simply mouse over the cell and the background and view the intensity values

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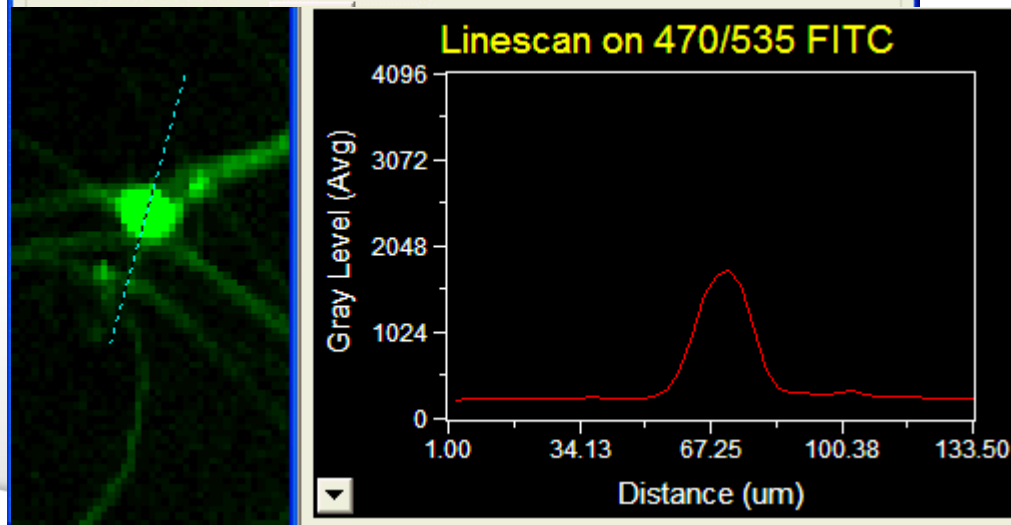
Module Settings
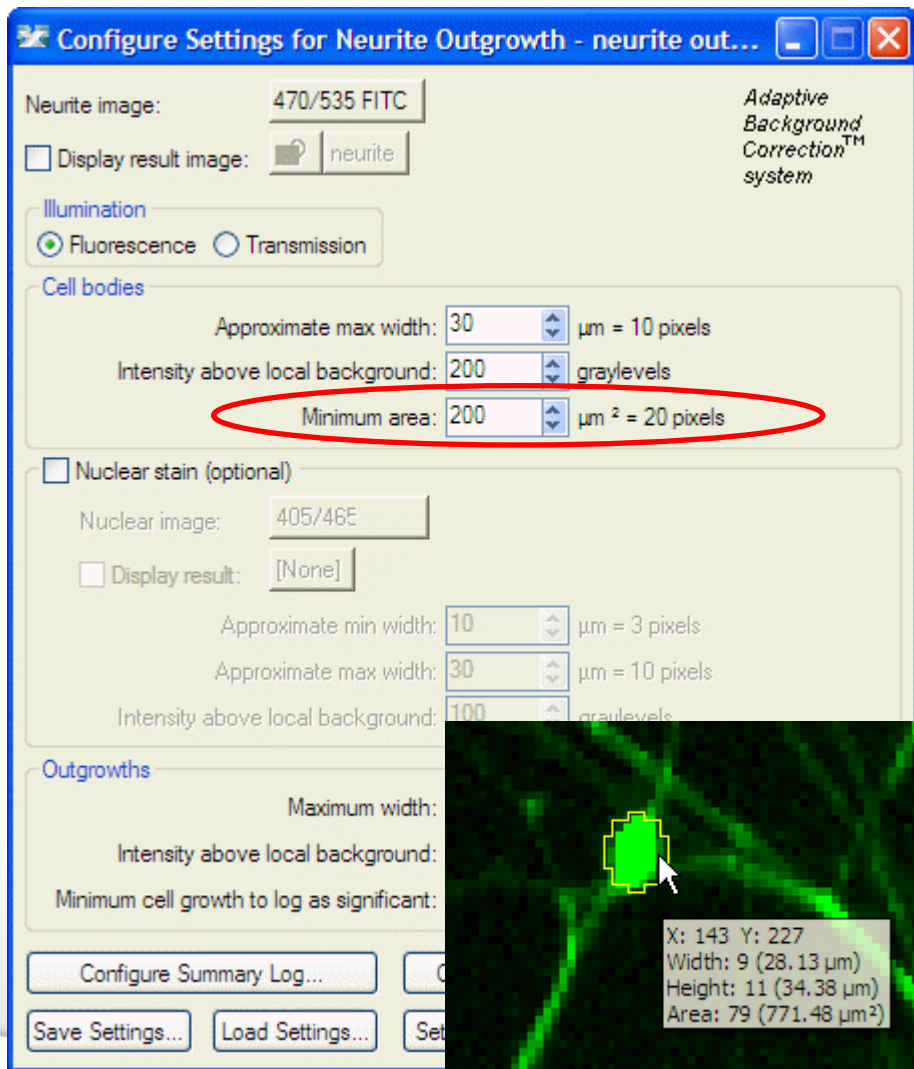
- **Cell bodies**

- The **minimum area** is an additional criteria to separate cell bodies from branch points / thick outgrowths

- Use the elliptical region tool to measure the area of a small cell in the image

- If the area is set too large, smaller cell bodies will be missed

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Module Settings

- **Outgrowths**

- The **maximum width** is used to help distinguish the beginning of outgrowths from cell bodies or branch points

- Use the line region tool to measure the width of a thick outgrowth

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Module Settings

- **Outgrowths**

- The **intensity above local background** is used for finding the outgrowths

- This value is a minimum and should be set slightly lower than the difference in intensity between the dimmest part of the outgrowth (typically the end) and its local background

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

Module Settings
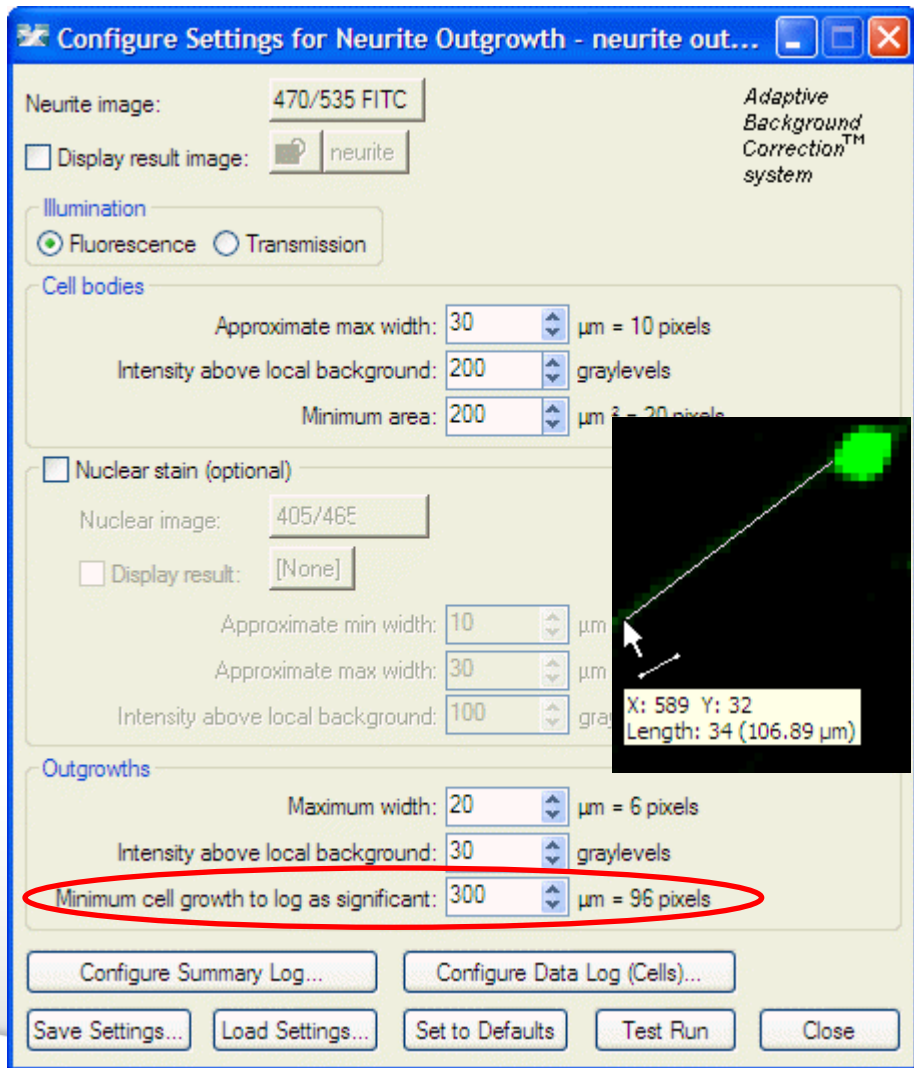
- **Outgrowths**

- The **minimum cell growth to log as significant** is 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

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Module Settings

- **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

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Module Settings

- **Nuclear stain (optional)**

- Leave "**Display result**" deselected (this is generally only used when journaling)

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Module Settings
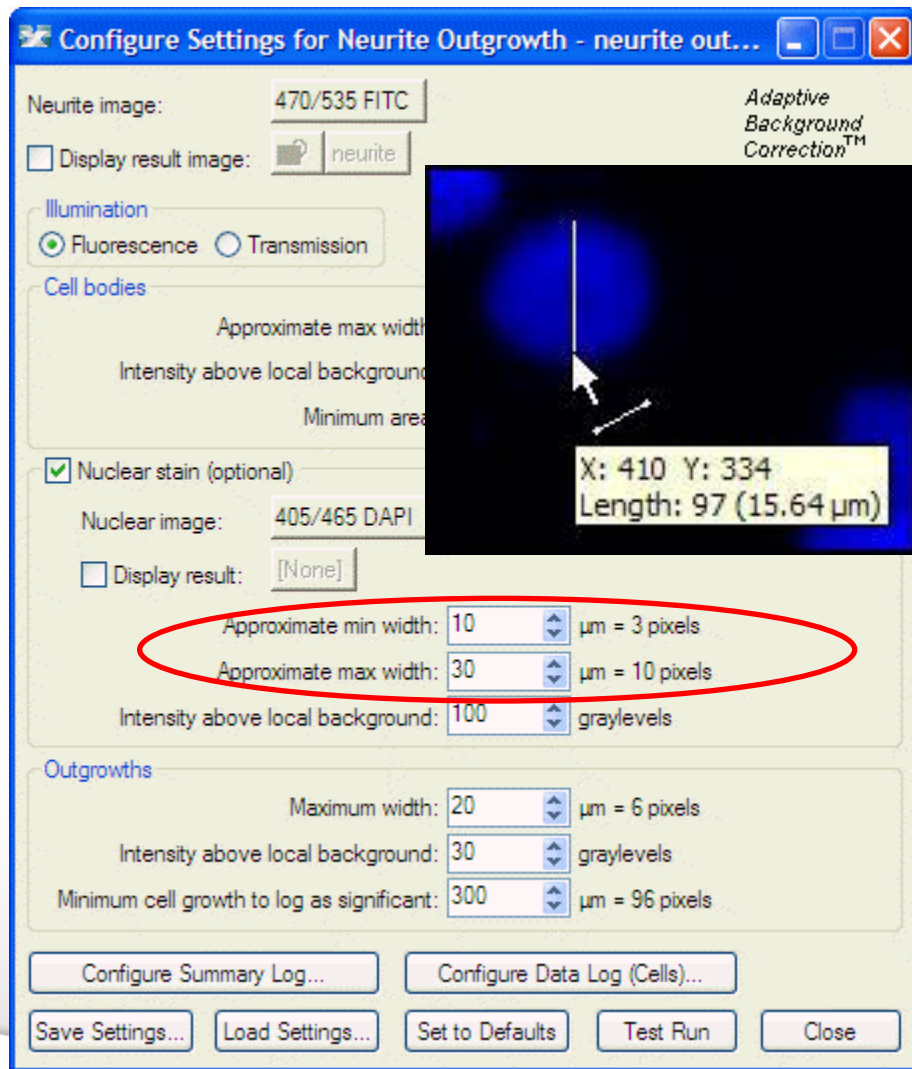
- **Nuclear stain (optional)**
- **Set the Approximate min width** and **Approximate max width** for the range of nuclei that you want to detect
- The width is the short axis of a nucleus (in um)
- The region tools can be used to measure widths
- Much smaller cells will be ignored
- Much larger cells will be split

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Module Settings

Effects of varying nuclear width settings
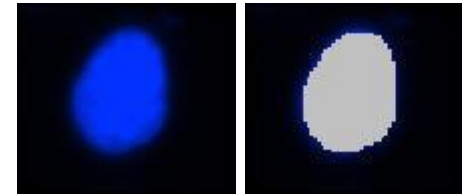
Min width too small: splits nuclei

Min width too large: omits smaller nuclei
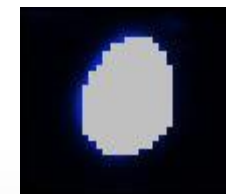
Max width too small: may shrink nuclear boundaries

Max width too large: may slightly enlarge nuclear boundaries

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Module Settings

- **Nuclear stain (optional)**

- The **intensity above local background** is used for finding the nuclei

- This value is a minimum and should be set slightly lower than the difference in intensity between a dim nucleus and its local background

- Measure the nuclear intensity just as for the cell bodies

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Regions for Measurement

Cell Body

Processes (4) connected to cell body
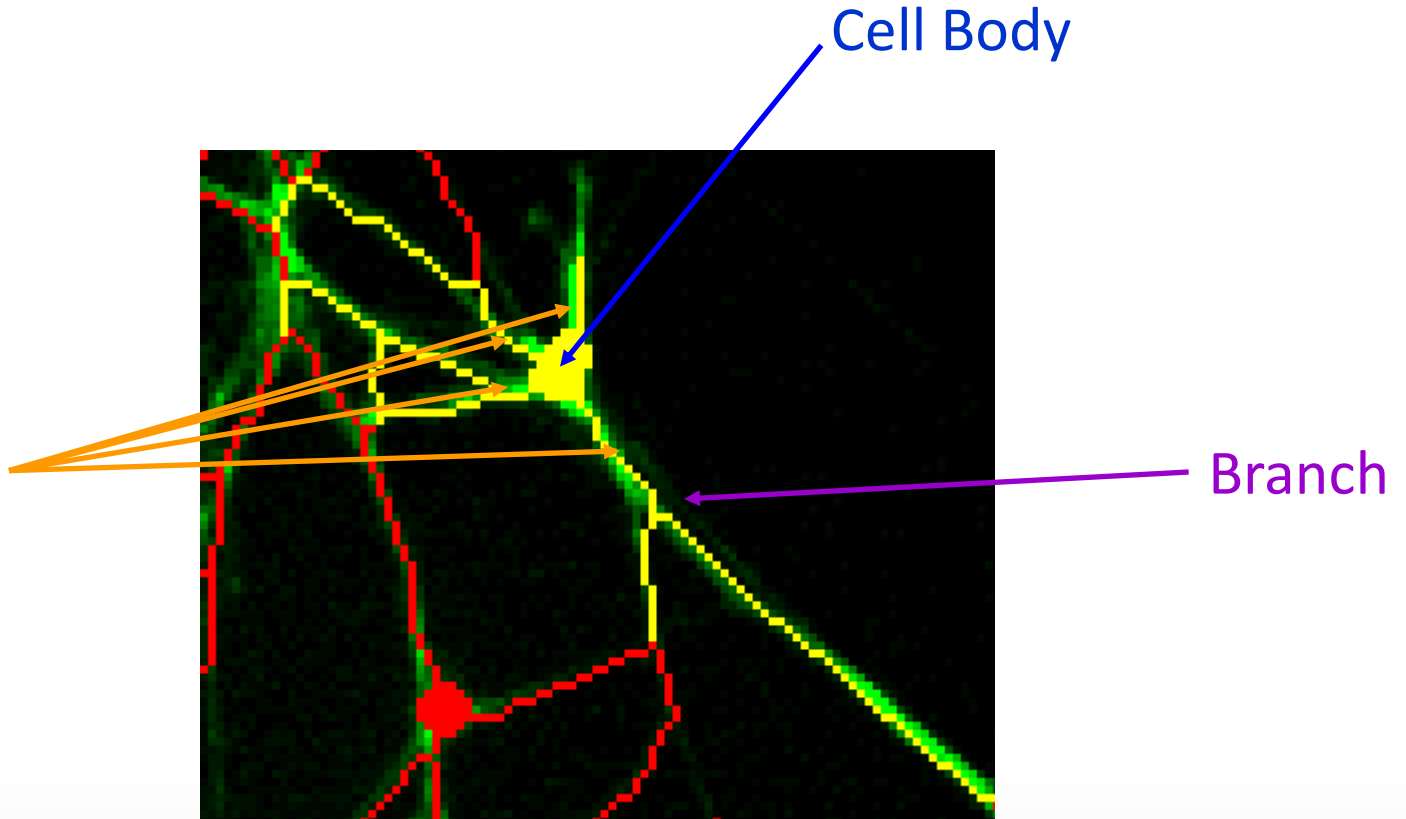
Branch

Summary Data (site-by-site measurements)

- Number of Cells
- Total Outgrowth
- Mean Outgrowth Per Cell
- Total Processes
- Mean Processes Per Cell
- Total Branches
- Mean Branches Per Cell
- Total Cell Body Area
- Mean Cell Body Area
- Straightness
- Cells Significant Growth
- %Cells Significant Growth
- Mean Outgrowth Average Intensity

- **Number of Cells**: Number of cell bodies in the image

- **Total Outgrowth**: Total length of skeletonized outgrowth in mm (corrected for diagonal lengths)

- **Mean Outgrowth Per Cell**: Average skeletonized outgrowth in mm corrected for diagonal lengths divided by the number of cells.

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Summary Data (site-by-site measurements)

- ✔ Number of Cells
- ✔ Total Outgrowth
- ✔ Mean Outgrowth Per Cell
- ✔ Total Processes
- ✔ Mean Processes Per Cell
- ✔ Total Branches
- ✔ Mean Branches Per Cell
- ✔ Total Cell Body Area
- ✔ Mean Cell Body Area
- ✔ Straightness
- ✔ Cells Significant Growth
- ✔ %Cells Significant Growth
- ✔ Mean Outgrowth Average Intensity

- **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

- **Mean Branches Per Cell**: Total Branches divided by Number of Cells

Summary Data (site-by-site measurements)

- Number of Cells
- Total Outgrowth
- Mean Outgrowth Per Cell
- Total Processes
- Mean Processes Per Cell
- Total Branches
- Mean Branches Per Cell
- Total Cell Body Area
- Mean Cell Body Area
- Straightness
- Cells Significant Growth
- %Cells Significant Growth
- Mean Outgrowth Average Intensity

- **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

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Summary Data (site-by-site measurements)

- ✔ Number of Cells
- ✔ Total Outgrowth
- ✔ Mean Outgrowth Per Cell
- ✔ Total Processes
- ✔ Mean Processes Per Cell
- ✔ Total Branches
- ✔ Mean Branches Per Cell
- ✔ Total Cell Body Area
- ✔ Mean Cell Body Area
- ✔ Straightness
- ✔ Cells Significant Growth
- ✔ %Cells Significant Growth
- ✔ Mean Outgrowth Average Intensity

- **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

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Cell Data (cell-by-cell measurements)

✔ Cell: Assigned Label #
✔ Cell: Total Outgrowth
✔ Cell: Processes
✔ Cell: Mean Process Length
✔ Cell: Median Process Length
✔ Cell: Max Process Length
✔ Cell: Branches
✔ Cell: Straightness
✔ Cell: Cell Body Area
✔ Cell: Mean Outgrowth Intensity

- **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

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Cell Data (cell-by-cell measurements)

- Cell: Assigned Label #
- Cell: Total Outgrowth
- Cell: Processes
- Cell: Mean Process Length
- Cell: Median Process Length
- Cell: Max Process Length
- Cell: Branches
- Cell: Straightness
- Cell: Cell Body Area
- Cell: Mean Outgrowth Intensity

- **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: Max Process Length** – Maximum value of the outgrowth lengths (in µm) associated with the cell's various processes

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Cell Data (cell-by-cell measurements)

✔ Cell: Assigned Label #
✔ Cell: Total Outgrowth
✔ Cell: Processes
✔ Cell: Mean Process Length
✔ Cell: Median Process Length
✔ Cell: Max Process Length
✔ Cell: Branches
✔ Cell: Straightness
✔ Cell: Cell Body Area
✔ Cell: Mean Outgrowth Intensity

- **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)

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Cell Data (cell-by-cell measurements)

- Cell: Assigned Label #
- Cell: Total Outgrowth
- Cell: Processes
- Cell: Mean Process Length
- Cell: Median Process Length
- Cell: Max Process Length
- Cell: Branches
- Cell: Straightness
- Cell: Cell Body Area
- Cell: Mean Outgrowth Intensity

- **Cell: Cell Body Area** – Total area in µm² 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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