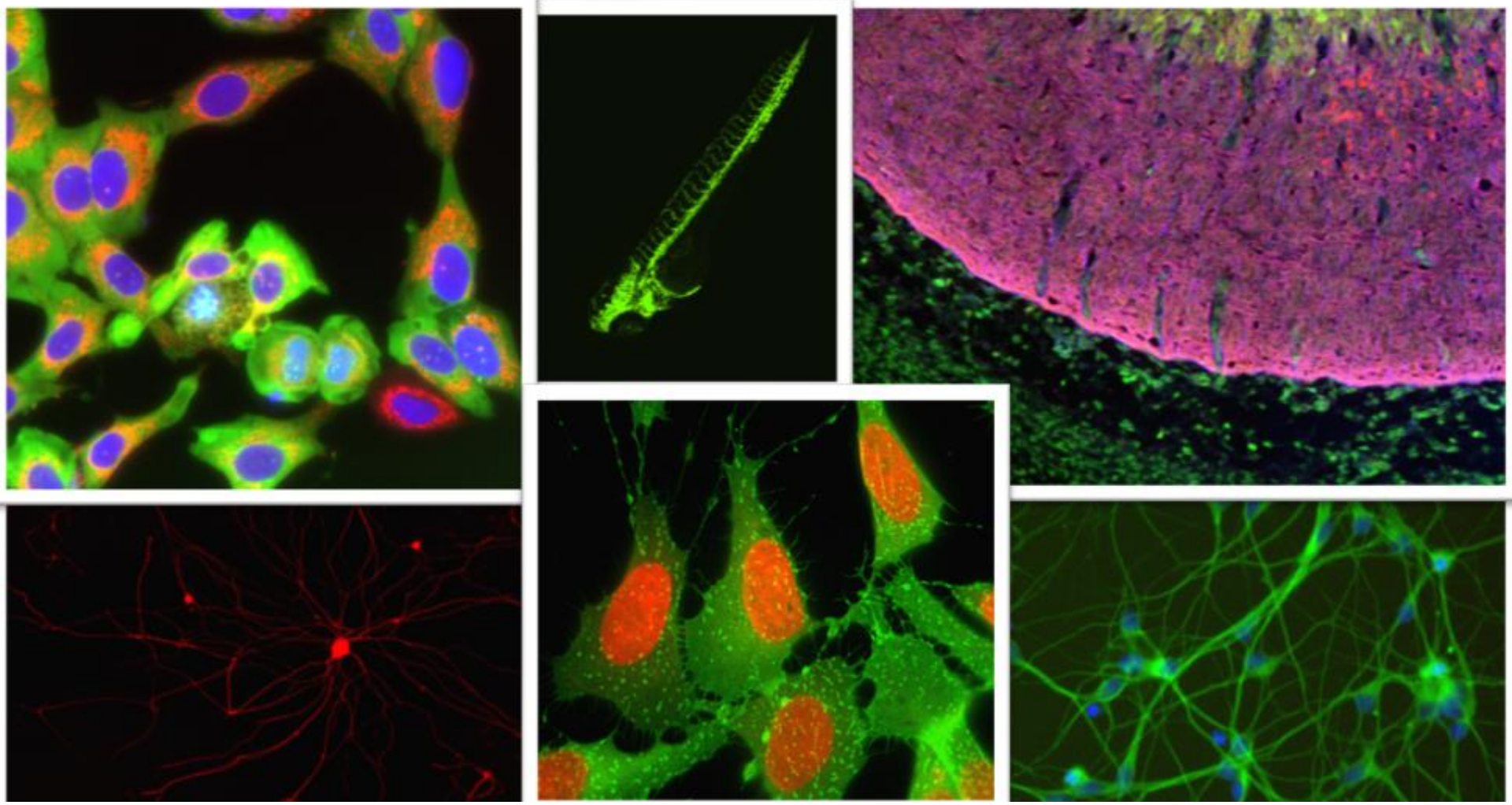

Molecular Devices

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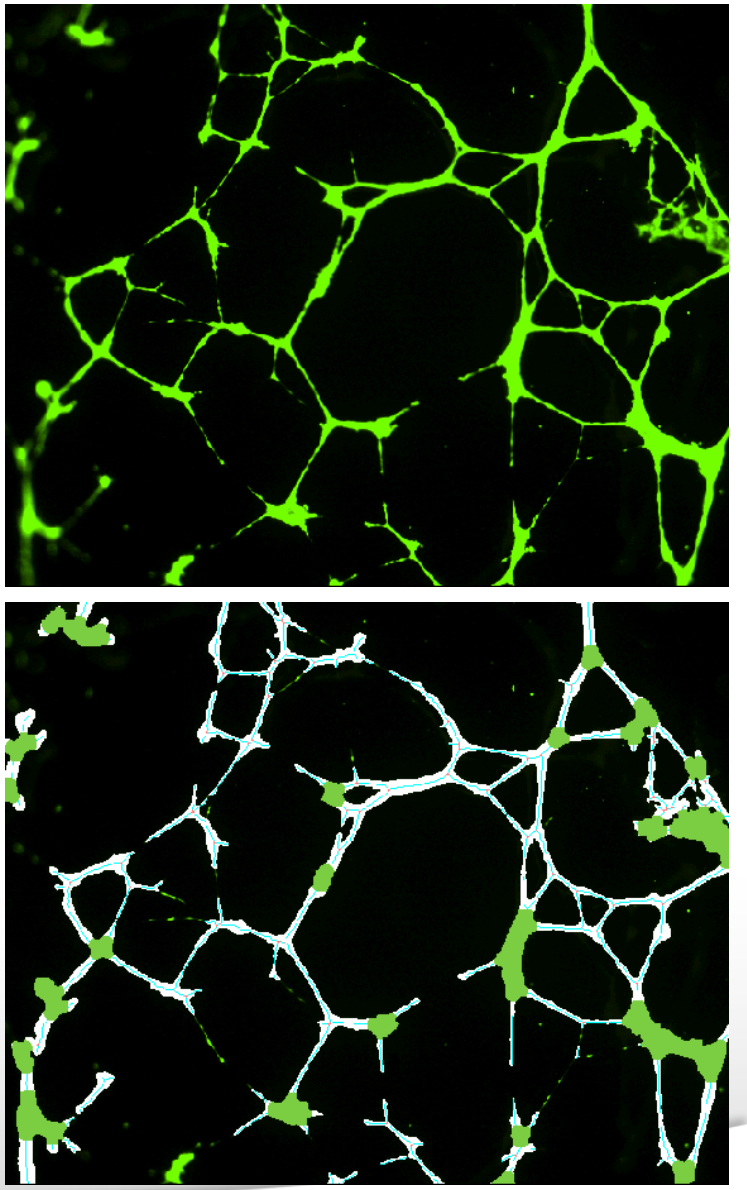
MetaXpress® Software:
Angiogenesis Module

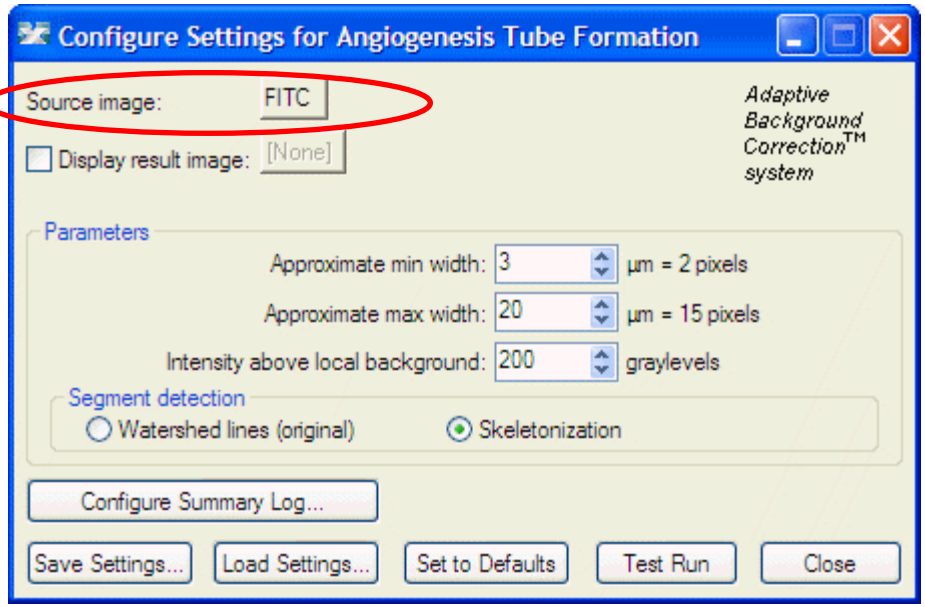
Angiogenesis

- Identifies and measures tubes (long thin objects) and nodes (connecting points between tubes) in a single wavelength

- In addition to tube formation assays, can be used for to measure neurite outgrowth in assays where the cell bodies are indistinct or outside the field of view

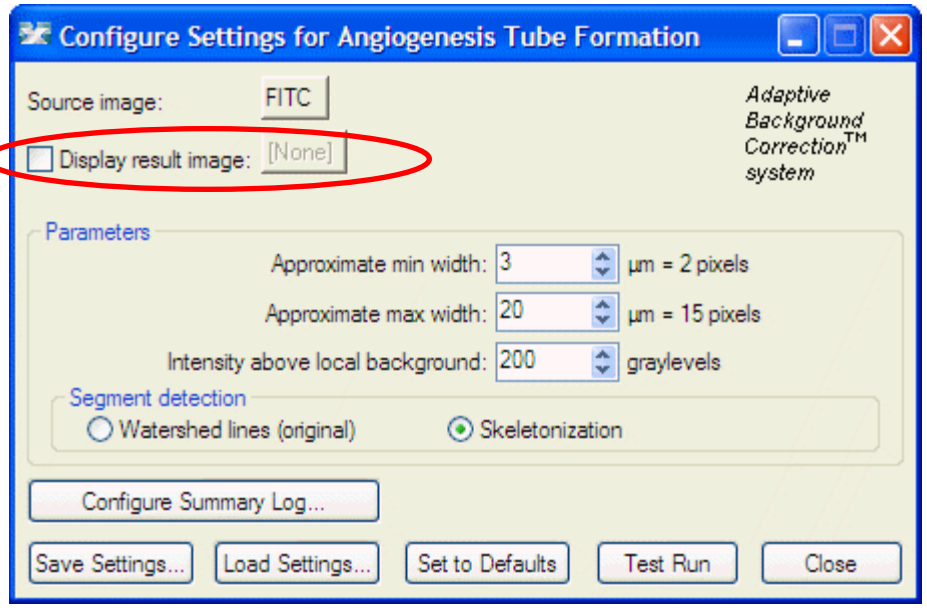


Module settings



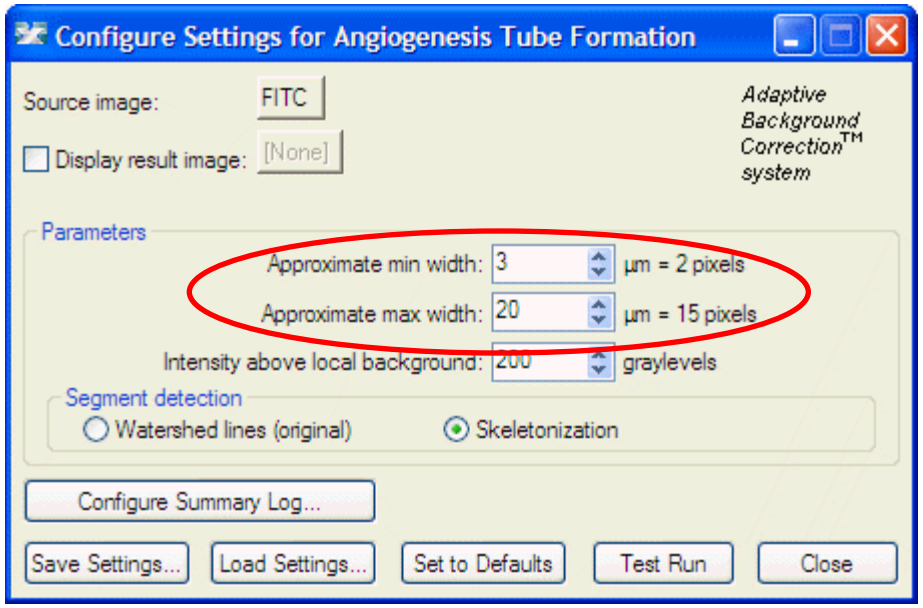
- **Source image**
- Select the wavelength for the **tubes** and **nodes**

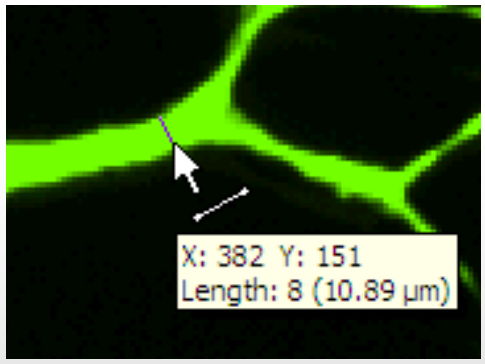
Module settings



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

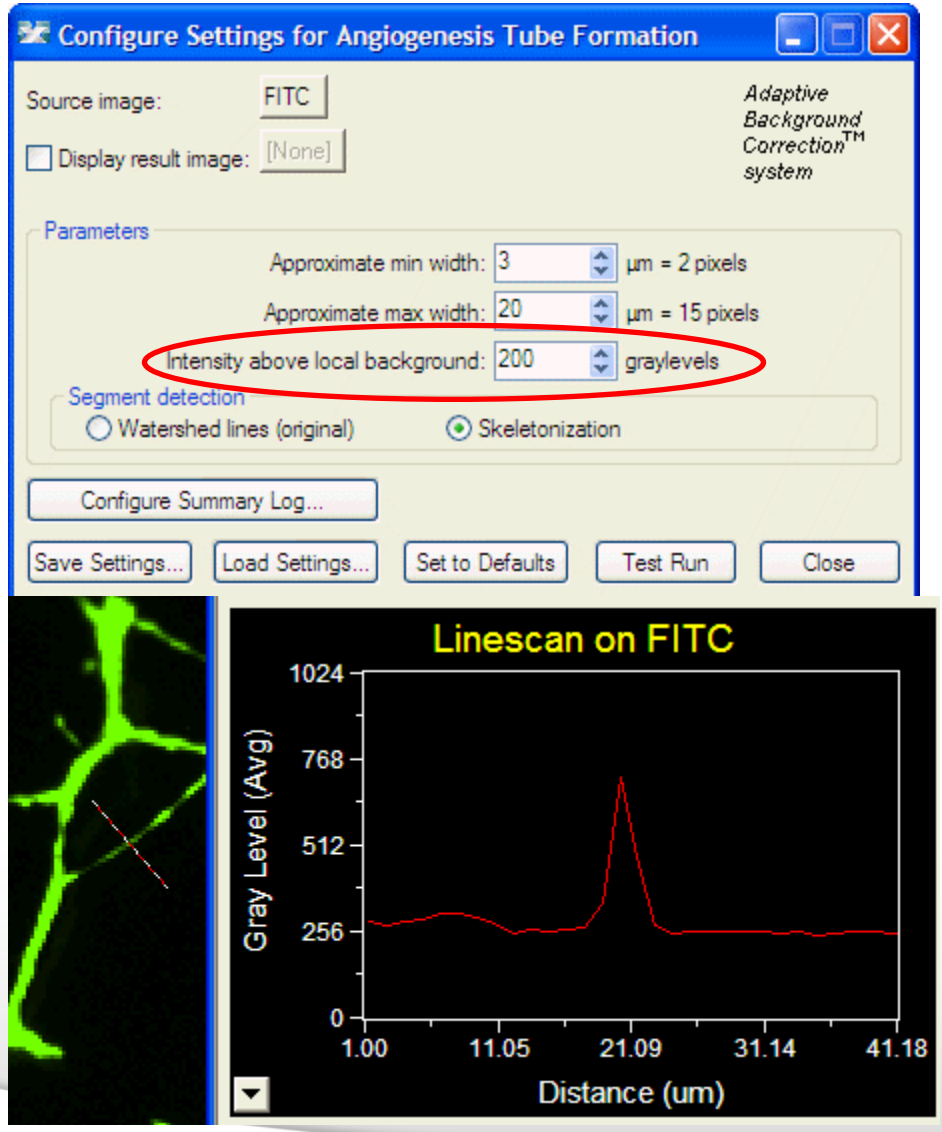
Module settings





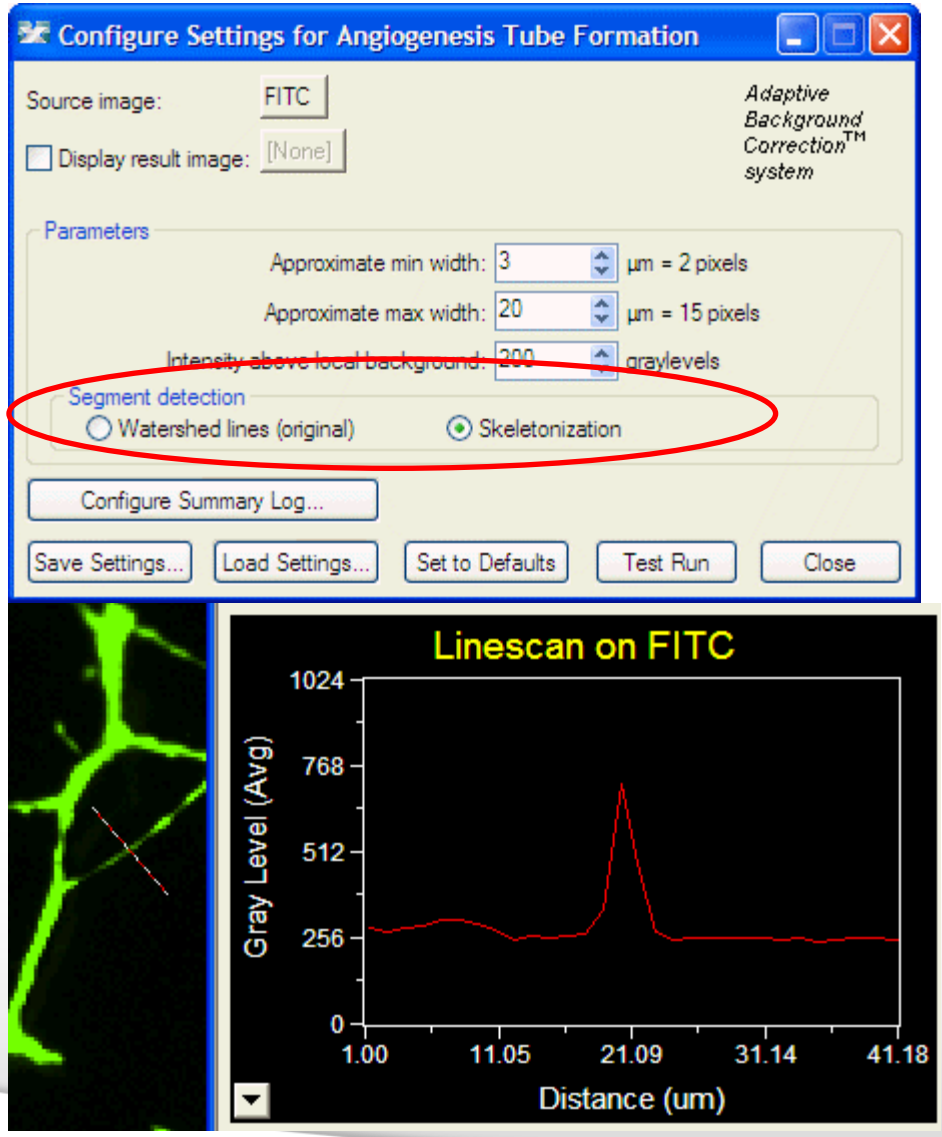
- **Parameters**
- **Set the Approximate min width** and **Approximate max width** for the range of tubes that you want to detect
- The width is the distance across a tube (in um)
- The region tools can be used to measure widths
- Much smaller tubes will be ignored
- Much larger tubes will be identified as nodes

Module settings



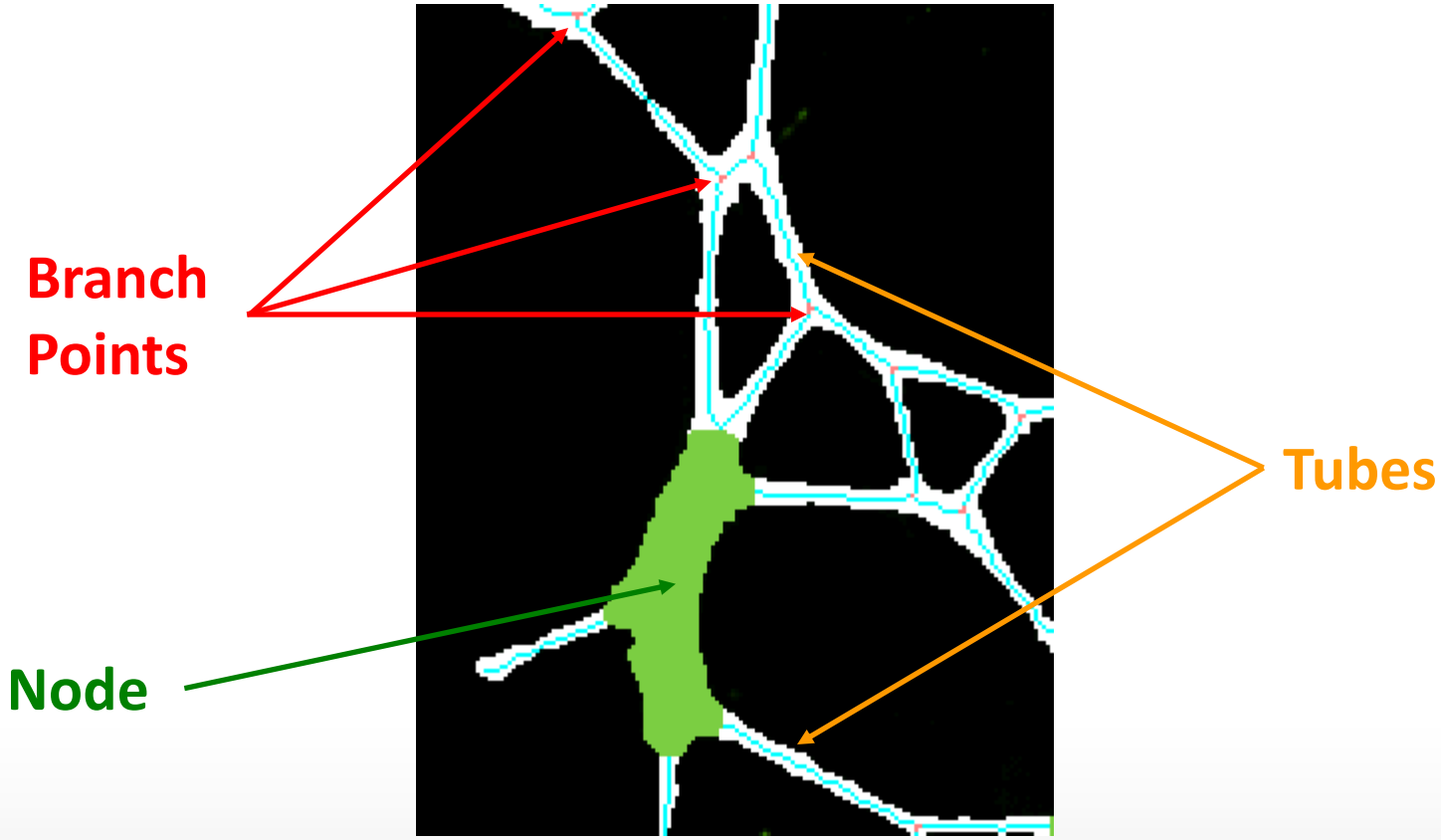
- **Parameters**
- The **intensity above local background** is used for finding the tubes and nodes
- This value is a minimum and should be set slightly lower than the difference in intensity between the dimmest part of the tube (typically the thinnest part) and its local background
- Draw a line across 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

Module settings

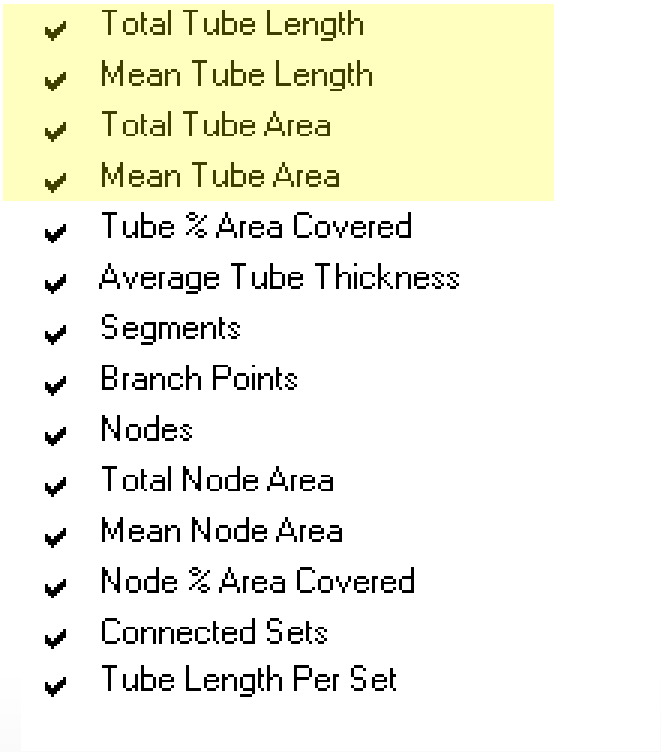


- **Segment detection**
- **Watershed lines (original)** uses the legacy segmentation method that was used in MetaMorph prior to version 7.6.4.0 and MetaXpress prior to version 3.1.0.69
- **Skeletonization** uses a new segmentation method, which may provide more accurate segmentation for some data sets that have thicker and sparser tubes

Regions for Measurements

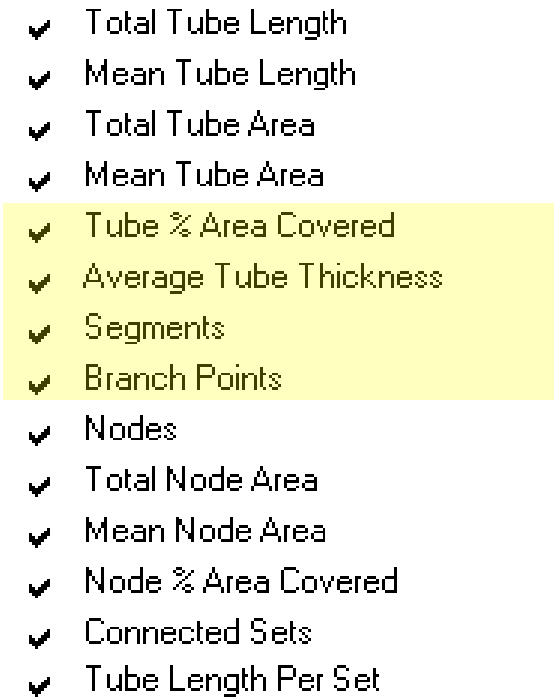


Summary Data (site-by-site measurements)



- **Total Tube Length**: Total microns of tube length (excluding nodes)
- **Mean Tube Length**: Total tube length divided by the number of segments
- **Total Tube Area**: Total square microns of tube area (excluding nodes)
- **Mean Tube Area**: Total square microns of tube area divided by the number of segments

Summary Data (site-by-site measurements)



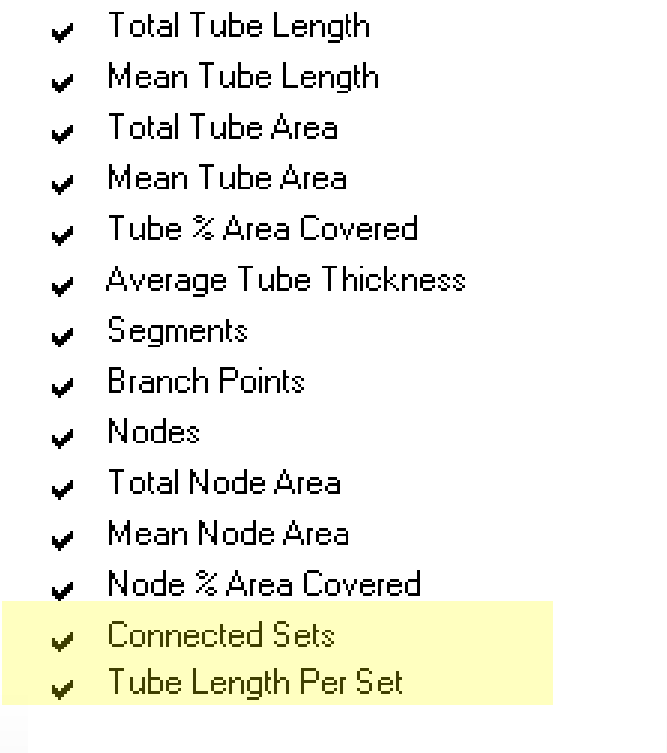
- **Tube % Area Covered**: Total tube area (excluding nodes) divided by total image area (width times height), times 100
- **Average Tube Thickness**: Average thickness of tubes computed as total area (excluding nodes) divided by total length (excluding nodes), and indicated in microns
- **Segments**: Total number of tube segments connecting branch points and/or ends
- **Branch points**: Total number of junctions connecting segments (nodes are not considered branches)

Summary Data (site-by-site measurements)

- Total Tube Length
- Mean Tube Length
- Total Tube Area
- Mean Tube Area
- Tube % Area Covered
- Average Tube Thickness
- Segments
- Branch Points
- Nodes
- Total Node Area
- Mean Node Area
- Node % Area Covered
- Connected Sets
- Tube Length Per Set

- **Nodes**: number of connected blobs with thickness exceeding maximum width; excluded from length and area measures
- **Total Node Area**: Total square microns of node area
- **Mean Node Area**: Total square microns of node area divided by the number of nodes
- **Node % Area Covered**: Total node area divided by total image area (width times height), times 100

Summary Data (site-by-site measurements)



- **Connected Sets**: Number of distinct objects detected in the image not connected to one another (no path of connected pixels of tubes or nodes connects the objects); measures the overall connectivity of the growth network (a completely connected network would have just one connected set of pixels)

- **Tube Length Per Set**: Total tube length in microns divided by the number of connected sets

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