

## Using the Angiogenesis Tube Formation application

### ABSTRACT

This document describes how to use the Angiogenesis Tube Formation application module from the *Apps* menu in MetaMorph® software (version 7.6r4 or higher) or MetaXpress® software (version 3.1 or higher).

This document describes the following procedures:

- Configuring the assay and saving settings
- Running the module and logging measurement data

### NOTES

- For more information about the procedures and dialog boxes described in this document, refer to the MetaMorph Help by pressing the [F1] key within the software to view help information about the active dialog box. You can also click the *See Also* button from within the Help window for additional information such as dialog box settings.
- This document assumes that the Angiogenesis Tube Formation drop-in has been loaded using the Meta Imaging Series Administrator.

ARTICLE #  
T20043

PRODUCTS  
MetaMorph  
MetaXpress

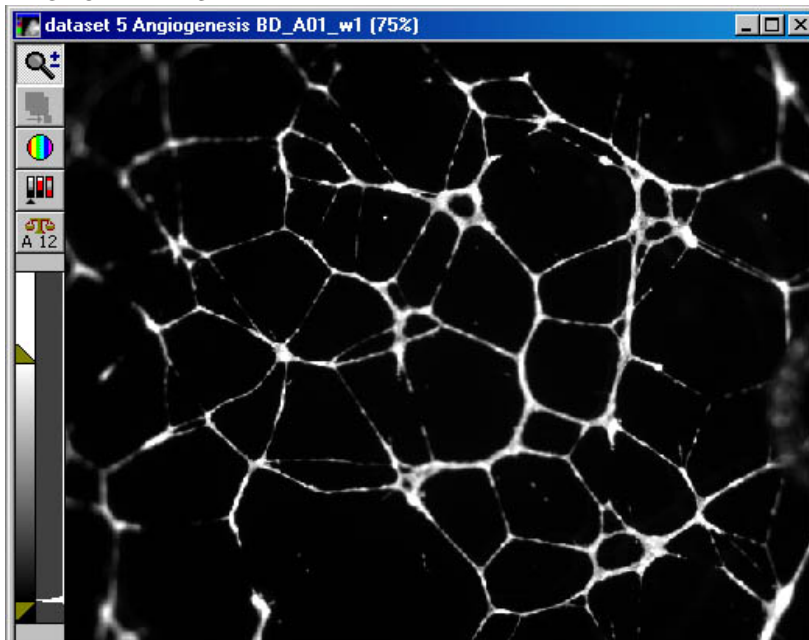
CREATED  
28-Jul-2005

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### GETTING STARTED

1. Start MetaMorph software.
2. Open an image of interest.

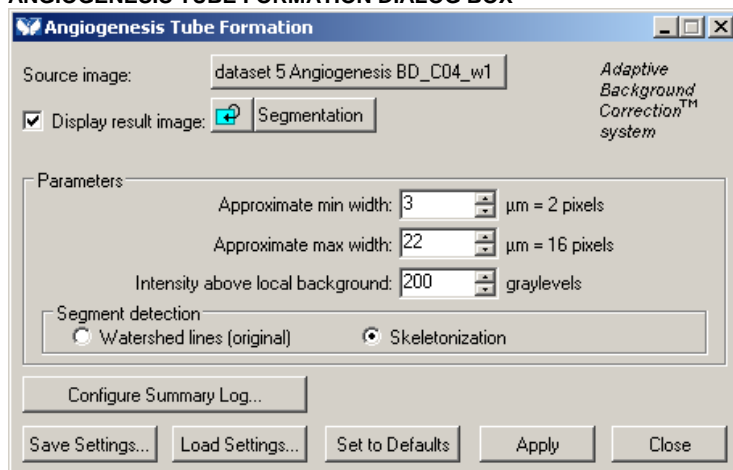
**FIGURE 1**  
**IMAGE OF INTEREST**



**CONFIGURING THE ANGIOGENESIS ASSAY**

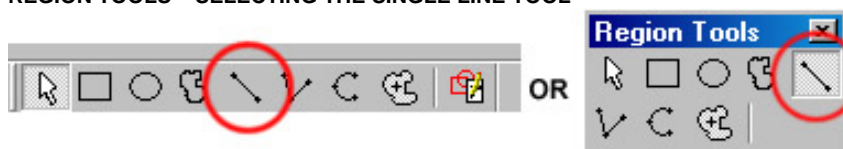
1. From the Apps Menu, select Angiogenesis Tube Formation. The Angiogenesis Tube Formation dialog box opens, as shown in Figure 2:

**FIGURE 2  
ANGIOGENESIS TUBE FORMATION DIALOG BOX**



2. Click *Source Image:* and select the image of interest. Valid source images are 16-bit images.
3. Optionally, check the box next to *Display result image*. This option will create a new result image called *Segmentation* once the analysis is run. You can change the result image name by clicking the *Segmentation* image selector and clicking *Specified*. The segmentation result image will be a 16-bit autoscaled image where a value of 2 (white) represents the vessels and a value of 1 (green) represents the nodes.
4. From the Region Menu, select Region Tools. The Region toolbar should appear if it is not already present.
5. Select the Single Line tool, as shown in Figure 3:

**FIGURE 3  
REGION TOOLS – SELECTING THE SINGLE LINE TOOL**



6. On the image, locate one of the smaller width tubes.
7. Zoom in, move the mouse pointer to one edge of the tube and left-click to start the line.
8. Move the mouse pointer to the other edge of the tube and read the value shown right after *Length*. In Figure 4, the value is 3 pixels or 3.82 microns (this sample image is calibrated). Left-click again to set the line:

**FIGURE 4**  
CLOSE UP OF A TUBE WITH A REGION TO DETERMINE THE WIDTH OF A TUBE

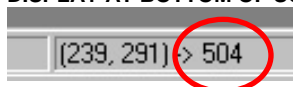


9. In the Angiogenesis Tube Formation dialog box, in the *Approximate Min Width* field, type the tube width in microns. The value for the number of pixels is displayed outside the box to the right.
10. Repeat steps 7-9 on the thickest tubule you can locate to determine the maximum width. Enter this measurement in the Angiogenesis Tube Formation dialog box, in the *Approximate Max Width* field. You can remove the regions of interest in the image by selecting Clear Regions from the Region Menu. **NOTE:** Another way to measure the width of an object is with calipers. For more information on calipers, see the *Additional Information* section at the end of this document.
11. If you do not want the nodes (junctions) detected and excluded from the tubule measurements, enter a value in the *Approximate Max Width* field larger than the tubes. This will include them for the tube length measurements.

**NOTE:** You can remove the region lines by going to the Regions menu and selecting Clear regions.

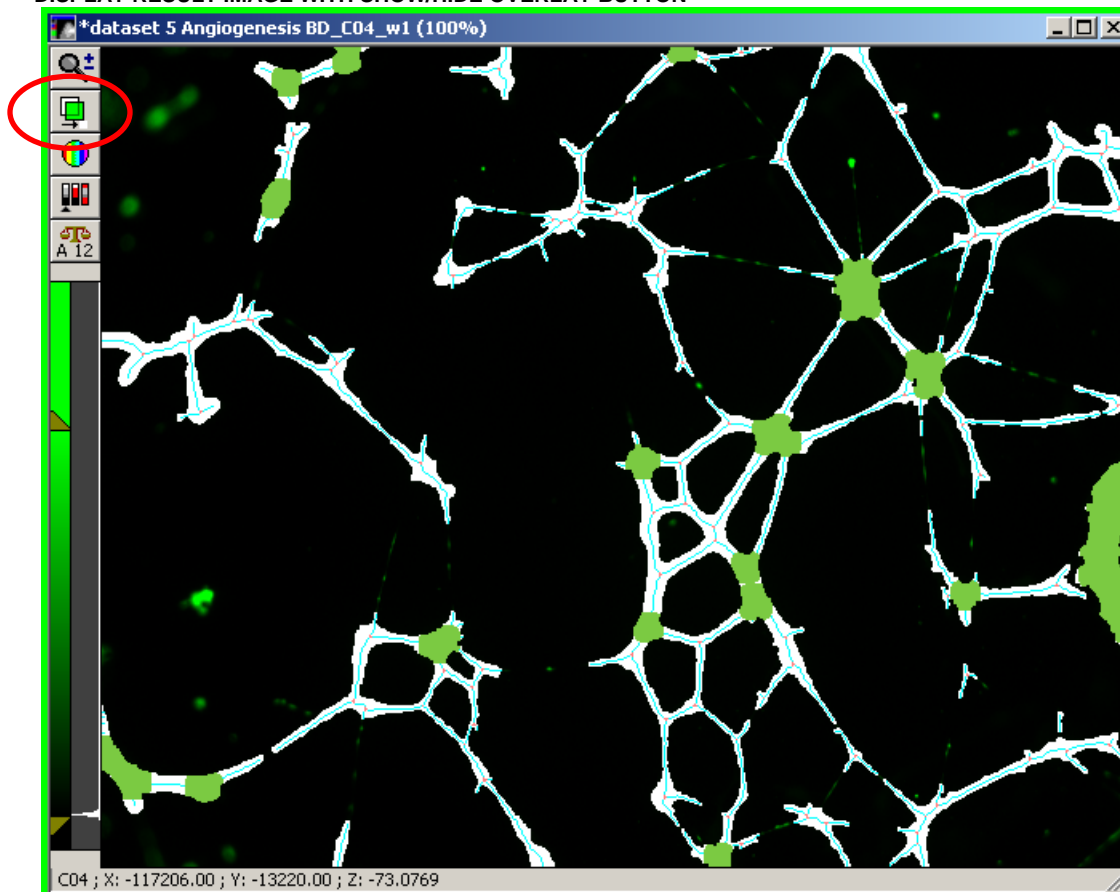
12. Now select the *Arrow* tool on the region toolbar and find the dimmest tube.
13. Move your mouse pointer over the dim tube and read the gray value for this tube displayed at the bottom of your screen. The gray value in Figure 5 is 504:

**FIGURE 5**  
DISPLAY AT BOTTOM OF SCREEN SHOWING INTENSITY



14. Move the mouse pointer just outside the tube to read the gray value of the background, displayed at the bottom of your screen.
15. Now calculate the difference between the signal and the background. For the sample image the calculation is as follows:  $504 - 283 = 221$ .
16. Select a slightly lower value and type it in the *Intensity above local background* field. For example, type 200.
17. Select the Segment detection algorithm. The Watershed algorithm is the original algorithm validated. The Skeletonization algorithm tends to be more accurate with data sets in which the tubule growths are thicker and sparser.
18. Click *Apply* to evaluate your settings. If the option *Display result image* was checked, a segmentation image window will appear. The tubes will be displayed in white. The tube segments will be displayed in blue. The branch points are displayed in orange and the nodes displayed in green. The original image also will have an overlay that can be toggled on and off using the *Show/Hide Overlay* button on the image window, as shown in Figure 6:

**FIGURE 6**  
**DISPLAY RESULT IMAGE WITH SHOW/HIDE OVERLAY BUTTON**



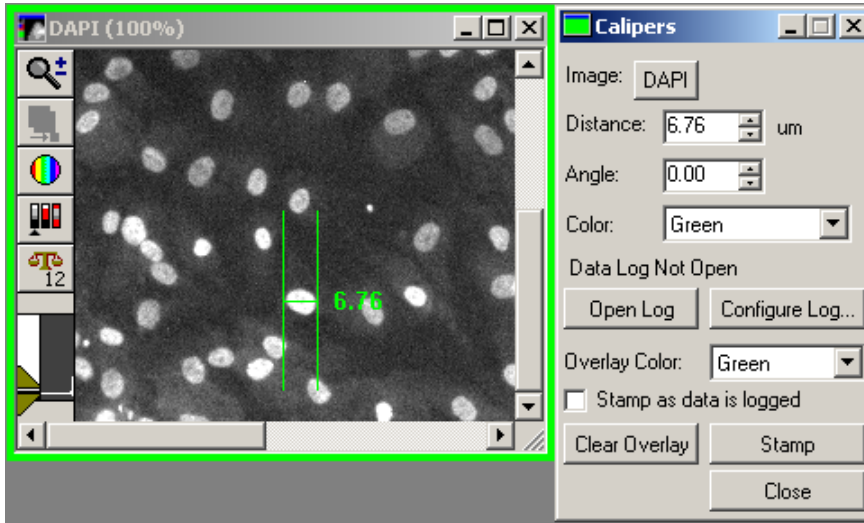
19. Click the *Show/Hide Overlay* button to see if all the tubes are detected or if too much background was detected.
20. Click *Configure Summary Log* in the Angiogenesis Tube Formation dialog box. The Configure Log dialog box opens. Check and/or uncheck individual measurement parameters and logging options. Refer to the description of these settings under *Dialog Box Options: Configure Summary Log* in the Help file, accessible by pressing the [F1] key. Click *OK* to close the Configure Log dialog box.
21. In the Angiogenesis Tube Formation dialog box, click *Save Settings* to name and save your settings for future use.
22. Optionally, you can log all your measurements and export them. Select the *Log* menu, *Open Summary Log*, check the box next to *Dynamic Data Exchange (DDE)* if using Microsoft® Excel®, and click *OK*. The Export Log Data dialog box will open. Click *OK* to open Excel.
23. In the Angiogenesis Tube Formation dialog box, click *Apply* to evaluate the measurements and export the data to Excel.
24. Change settings if needed and resave the settings.
25. You can now run the settings on other images by clicking *Load Settings* in the Angiogenesis Tube Formation dialog box.

**ADDITIONAL INFORMATION**

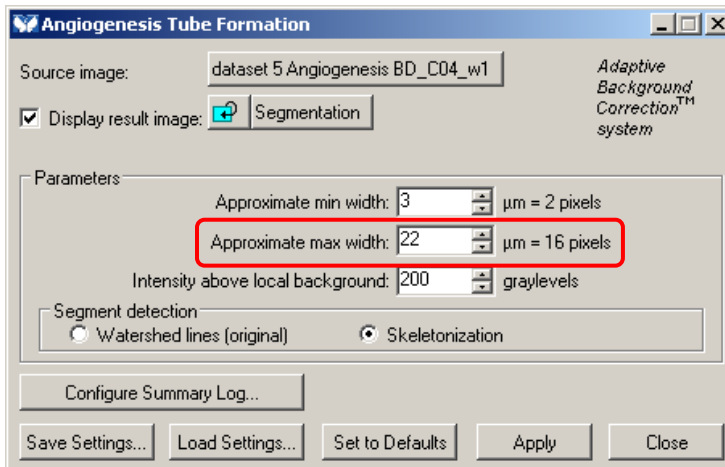
You can also use the Caliper tool to measure the width of an object. The caliper function is a drop-in that must be loaded into the software. If the command is not present in the *Measure* menu, please see your System Administrator to load it. Complete the following procedure to use the *Calipers* tool:

1. From the *Measure* menu, select *Calipers*. The Calipers dialog box opens.
2. Select the image to measure in the *Image* selector. The caliper appears on the selected image, as shown in Figure 7:

**FIGURE 7  
CALIPERS DIALOG BOX**



3. The calipers can be moved by single-clicking the cross-bar so that it is displayed as a blinking line, indicating that it is active, and then dragging (or rotating) the cross-bar to the desired location with your pointer.
4. Enter the value in the *Approximate max width* field of the application module dialog box.



If you have any further questions, please contact Technical support at 800-635-5577 x1820 or [support.dtn@moldev.com](mailto:support.dtn@moldev.com).