

## Using the Multi Wavelength Cell Scoring application module in MetaMorph

### ABSTRACT

The new Multi Wavelength Cell Scoring (MWCS) module is a flexible segmentation tool for the analysis of up to seven wavelengths — a nuclear stain and up to six additional stains. The following example uses two wavelengths — a nuclear stain and cytoplasmic stain.

### NOTES:

- Before starting, ensure that the Multi Wavelength Cell Scoring drop-in is loaded using the Meta Imaging Series Administrator.
- The MWCS module works with 10-, 12-, 14-, or 16-bit images. MetaMorph treats all of these formats as 16-bit images.
- All images used must be of identical height and width, with spatial calibration in microns.

### PROCEDURE

Complete the following procedure to use the MWCS module:

1. Open the appropriate images.
2. From the *Apps* menu, select *Multi Wavelength Cell Scoring*. The module's dialog box opens.
3. In the *Number of wavelengths* field, set the number to 2. A *W2* tab opens in the dialog box.
4. To create a new result image called Segmentation once the analysis is run, check the box next to *Display result image*.
5. In the *All nuclei* tab, click *W1 Source image* and select the nuclei image.
6. From the *Regions* menu, select *Region Tools* and select the *Single Line* from the toolbar.
7. On the nuclei image, locate one of the narrowest nuclei.
8. Zoom in, move the mouse pointer to one edge of the cell and left-click to start the line.
9. Move the mouse pointer to the other edge and read the value shown right after *Length* in the tooltip. In Figure 1, the value is 9 pixels or 2.89 microns (this sample image is calibrated). Left-click again to set the line:

**FIGURE 1  
MEASURING THE NUCLEI**



10. In the MWCS dialog box, in the *Approximate min width* field, type the nucleus width in microns. The value for the number of pixels is displayed outside the box to the right.
11. Repeat steps 7-9 for one of the widest nuclei. Enter this measurement in the MWCS dialog box, in the *Approximate max width* box.
12. Click the Arrow tool on the Region toolbar.
13. Move the mouse pointer over one of the dimmest nuclei and read the gray value displayed at the bottom of the screen. The gray value in Figure 2 is 330:

ARTICLE #  
T20045

PRODUCTS  
MetaMorph®

CREATED  
11-Nov-2005

LAST UPDATED  
15-Nov-2005

**FIGURE 2**  
**GRAY VALUE DISPLAYED AT THE BOTTOM OF THE SCREEN**



14. Move the mouse pointer just outside the nucleus to measure the background and calculate the difference between the nucleus signal and the background. In this example the background was approximately 230. The difference between the gray value of the nucleus and the gray value of the background is  $330 - 220 = 110$ . Type this approximate value in the *Intensity above local background* field of the MWCS dialog box. In this example, a value of 100 was used.
15. Click *Preview* to process the image using the current settings. The results are overlaid on the W1 source image. Press the *Show/Hide* overlay button on the image window to toggle the results. Note that if you save the image at this time, you will save it with the overlay.
16. Click the W2 tab. You can optionally name this wavelength by specifying a name in the *Name* field. Click the *W2 Source image* to select the cytoplasm image.
17. Specify the *Stained area* as Nucleus, Cytoplasm or Nucleus and Cytoplasm. You can optionally change the legend color.
18. Find the smallest cell and repeat steps 7-14.
19. Under the *Scoring* section, set the *Minimum stained area* to the size of the cells you want to score as positive for this staining. *Region Tools* such as *Ellipse* or *Trace* can be used to interactively measure an appropriate area on the image and read its value from the tooltip. Steps 15-18 can be repeated for each additional wavelength.
20. Click *Apply* to evaluate your settings. The Cellular Results table opens, as well as a segmentation image window (if the option *Display result image* is checked), as shown in Figure 3:

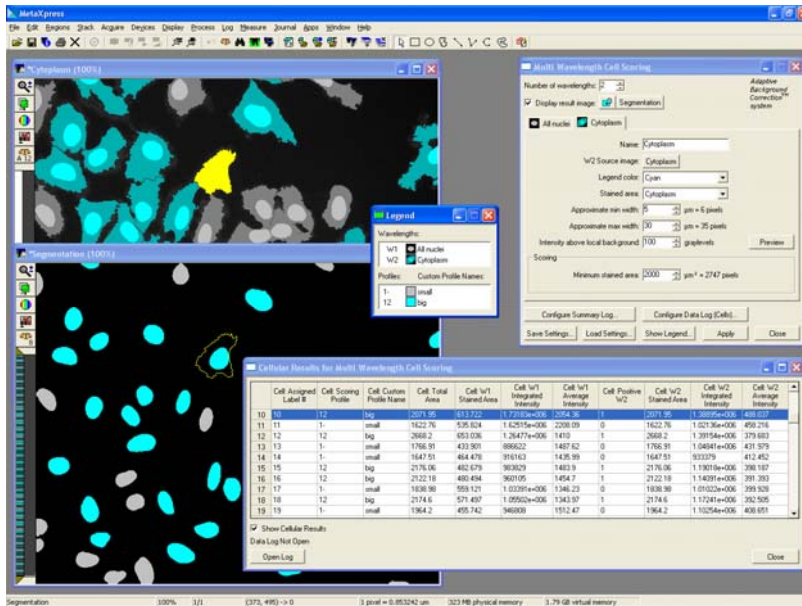
**FIGURE 3**  
**THE MODULE SEGMENTS THE IMAGE**

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21. You can now tweak your settings until you are satisfied with the results, then click *Save Settings* in the MWCS dialog box to name and save your settings for future use.
22. The legend can be customized with a biologically meaningful name for each stain and/or profile. To modify your legend, click the *Show Legend* button on the *Multi Wavelength Cell Scoring* dialog box.

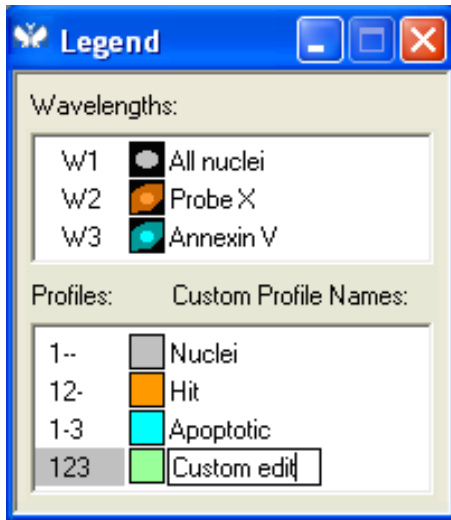
**FIGURE 3**  
**CUSTOMIZABLE LEGENDS**

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23. Double-click each wavelength name or each profile name to modify them. When you are finished, close the *Legend* dialog box, and click *Save Settings* in the *Multi Wavelength Cell Scoring* dialog box.

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