

## Using the Count Nuclei application module in MetaMorph version 6.3 and higher

### ABSTRACT

This document describes how to use the Count Nuclei Assay using the Count Nuclei application module from the Apps menu in MetaMorph version 6.3 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 the 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 Count Nuclei drop-in has been loaded using the Meta Imaging Series Administrator.

ARTICLE #  
T20042

PRODUCTS  
MetaMorph® 6.3 and higher

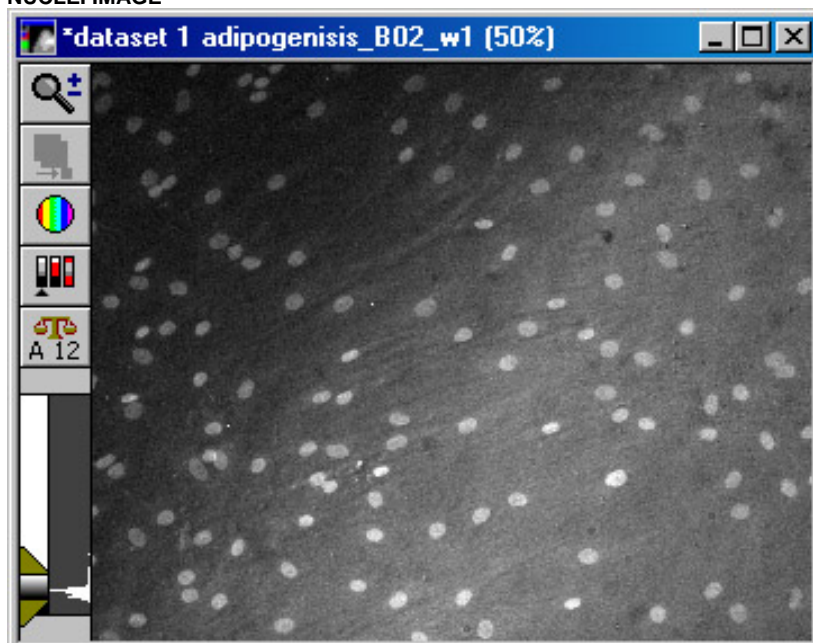
CREATED  
28-Jul-2005

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23-Sep-2005

### GETTING STARTED

1. Start MetaMorph.
2. Open a nuclei image of interest.

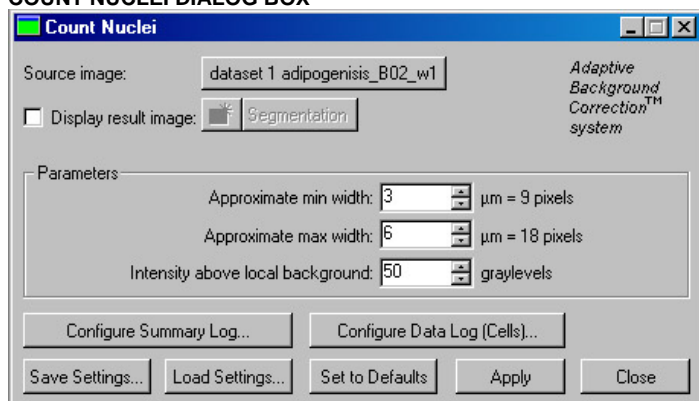
FIGURE 1  
NUCLEI IMAGE



**CONFIGURING THE COUNT NUCLEI ASSAY**

1. From the Apps Menu, select Count Nuclei. The Count Nuclei dialog box opens, as shown in Figure 2:

**FIGURE 2  
COUNT NUCLEI 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* after the analysis is run. You can change the result image name by clicking the *Segmentation* image selector and clicking *Specified*. The result image shows segmentation of the nuclei drawn as different indexed colors according to nucleus size on a black background.
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 DIALOG BOX – SELECTING THE SINGLE LINE TOOL**



6. On the image, locate one of the narrowest nuclei.
7. Zoom in, move the mouse pointer to one edge of the cell and left-click to start the line.
8. Move the mouse pointer to the other edge and read the value shown right after *Length*. In Figure 4, the value is 9 pixels or 2.89 microns (this sample image is calibrated). Left-click again to set the line:

**FIGURE 4**  
**MEASURING THE NUCLEI**

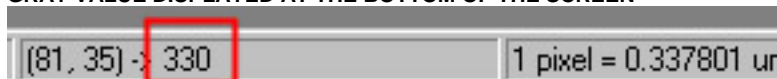


9. In the Count Nuclei dialog box, in the *Approximate Min Width* field, type the nucleus width in microns. The value for the number of pixels is displayed outside the box to the right.
10. Repeat steps 7-9 for one of the widest nuclei. Enter this measurement in the *Count Nuclei* dialog box, in the *Approximate Max Width* box. 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.

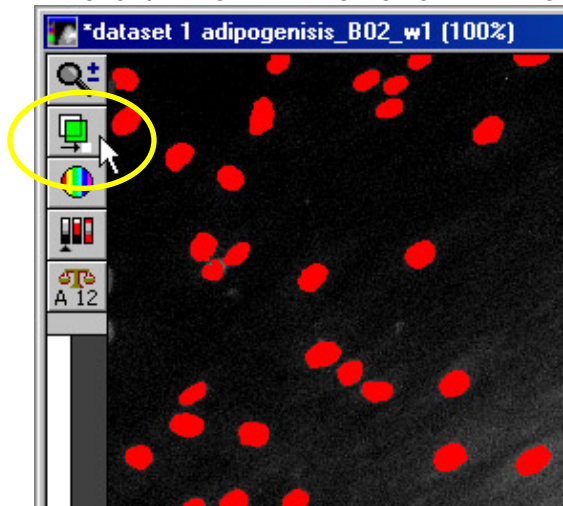
11. Click the Arrow tool on the Region toolbar.
12. Find one of the dimmest nuclei.
13. Move the mouse pointer over the nucleus and read the gray value displayed at the bottom of the screen. The gray value in Figure 5 is 330:

**FIGURE 5**  
**GRAY VALUE DISPLAYED AT THE BOTTOM OF THE SCREEN**



14. Move the mouse pointer just outside the nucleus to measure the background.
15. Calculate the difference between the nucleus signal and the background. In this example the background was approximately 290. The difference between the gray value of the nucleus and the gray value of the background is  $330 - 290 = 40$ .
16. Select a slightly lower value than 40 and type it in the *Intensity above local background* field of the Count Nuclei dialog box. In this example, a value of 35 was used.
17. 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). The gray value of the nuclei on the segmentation image refers to the cell's assigned label number as it appears in the Cellular Results table. The assigned label is based on cell area and numbered according to increasing cell area measurements. 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**  
**THE SHOW/HIDE OVERLAY BUTTON ON THE IMAGE WINDOW**



18. Click the *Show/Hide Overlay* button to see if all the nuclei are detected or if too much background was detected. Possible problems:
  - a. Not all nuclei were found: try lowering the *Intensity above local background* or if only large nuclei were found, try decreasing the *Approximate min width*.
  - b. Too much background was included with your nuclei: try raising the *Intensity above local background* or try decreasing the *Approximate max width*.
  - c. If nuclei are split into pieces: try increasing the *Approximate min width*.
19. Repeat steps 12-18 until the appropriate results are obtained.
20. To log the summary data, select the *Log* menu, select *Open Summary Log*, check the box next to *Dynamic Data Exchange (DDE)* if using Microsoft<sup>®</sup> Excel<sup>®</sup>, and click *OK*. The *Export Log Data* dialog box opens. Click *OK* to open Excel.
21. In the *Count Nuclei* dialog box, click *Configure Summary Log*. 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.
22. To log individual cell data, select the *Log* menu, *Open Data Log*, check the box next to *Dynamic Data Exchange (DDE)* if using Microsoft<sup>®</sup> Excel<sup>®</sup>, and click *OK*. The *Export Log Data* dialog box will open. Click *OK* to open Excel.
23. In the *Count Nuclei* dialog box, click *Configure Data Log (Cells)* 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 Data Log (Cells)* in the Help file, accessible by pressing the [F1] key. Click *OK* to close the *Configure Log* dialog box.
24. In the *Count Nuclei* dialog box, click *Save Settings* to name and save your settings for future use.
25. In the *Count Nuclei* dialog box, click *Apply* to evaluate the measurements and export the data to Excel.
26. Change settings if needed and resave the settings.
27. You can now run the settings on other images by clicking *Load Settings* in the *Count Nuclei* dialog box.

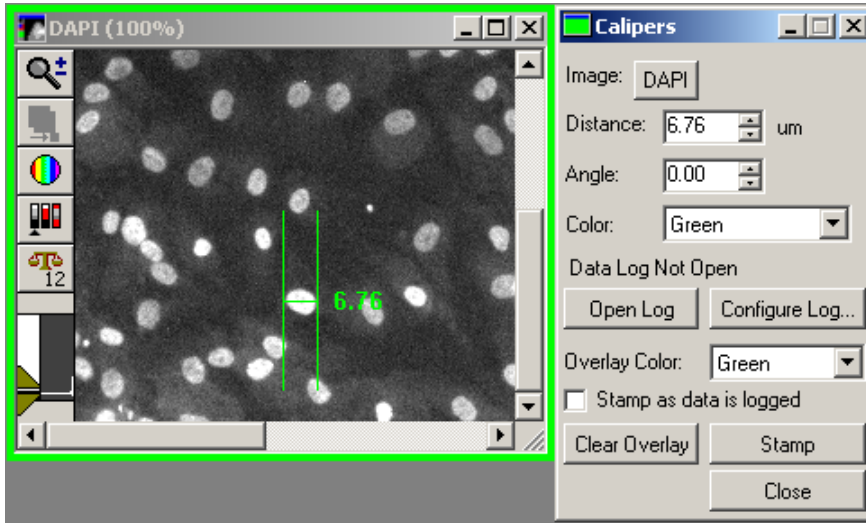
**ADDITIONAL INFORMATION**

FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

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.

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