

MetaMorph application modules overview

OVERVIEW

This document describes some common procedures that can be applied to all MetaMorph application modules. Most application modules share similar configuration steps, such as selecting images to process or determining an object's width. An understanding of these similar steps helps when learning to use different application modules. This document describes the following procedures:

- Configuring an application module
- Testing and saving settings
- Running an application module

NOTES

- The application modules discussed in this document can be purchased as drop-ins to your existing MetaMorph installation. Contact your MDC representative for more information.
- For more information about the procedures and dialog boxes described in this document, refer to the MetaMorph online help system. Press [F1] to access help for the active dialog box.
- This document does not cover using database versions of application modules with Discovery-1 or MetaXpress. Refer to the application note [General Tools for use with Screening Modules](#) for information on using database versions of application modules.
- All source images used in the application modules must be 16-bit. Note that images taken with 10-, 12-, or 14-bit cameras are treated as 16-bit images in MetaMorph and can be used with the application modules.
- The sample image in this document was calibrated using the Calibrate Distances command. For best results, source images in all application modules should be calibrated in microns. If more than one image is used as a source image, both images must have identical distance calibrations.

INSTRUCTIONS

CONFIGURING AN APPLICATION MODULE

The following procedures explain how to configure an application module prior to running it:

- Open the appropriate images on the MetaMorph desktop
- Open the application module and select the source image(s) and result image
- Determine measurements

Complete the following procedure to open the image(s) to process with the application module:

1. From the File menu, select Open. The Open dialog box appears.
2. Select the appropriate folder, then select the image name and click *Open*. Your selected image opens in a MetaMorph image window. You can also select the image files with your mouse and drag them into the MetaMorph window.
3. Repeat Steps 1 and 2 until all needed images are open.

ARTICLE #
T20036

PRODUCTS
MetaMorph® 6.2 or later

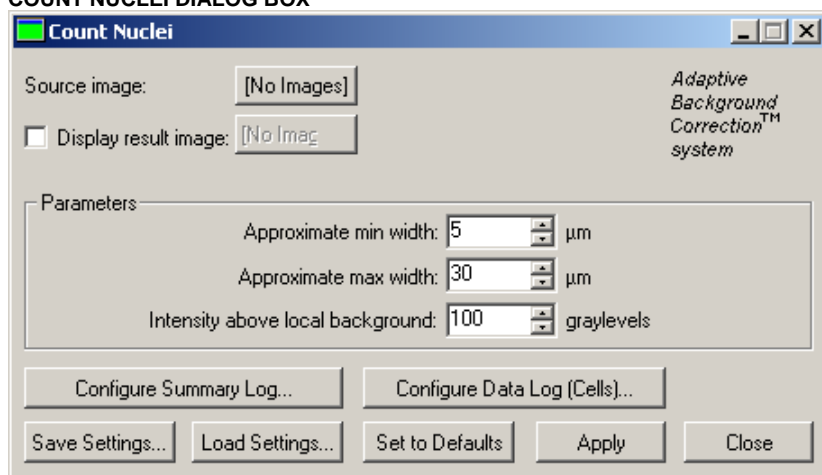
CREATED
04-Feb-2005

LAST UPDATED
28-Feb-2005

Complete the following procedure to open the application module and select source and result images:

1. From the Apps menu, select the application module to configure. This document uses the Count Nuclei application module as an example. Figure 1 illustrates the Count Nuclei dialog box:

FIGURE 1
COUNT NUCLEI DIALOG BOX



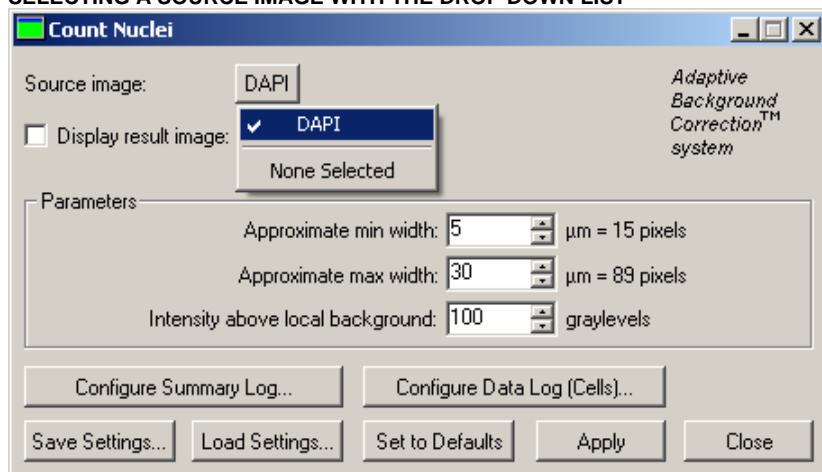
Each application module needs at least one Source image to process. Some modules need more than one source image. These images must be open on the MetaMorph desktop in order to be processed (unless you are using journals and variables to run your application module, which is beyond the scope of this document). Examples of source image names in various application modules include:

- Count Nuclei: Source image
- Angiogenesis: Source image
- Neurite Outgrowth: Neurite image and nuclear image


NOTE: The appropriate type of source image(s) vary depending on the application module used. Refer to the online help for the module you are using for more information about source images.

2. Click *Source image* and select the appropriate image name from the drop down list. Figure 2 shows an example of selecting an image:

FIGURE 2
SELECTING A SOURCE IMAGE WITH THE DROP DOWN LIST



There are two ways to view the results of each application module: Displaying a result image or using an image overlay. Enabling *Display result image* will open or overwrite (see Step 4) a new image depicting what was measured. Using image overlay creates an overlay on the main image.

You can toggle the image overlay on or off with the Show/Hide overlay button  on the side of your image window.

NOTE: To display a result image the *Display result image* check box must be selected and an image must be selected.

3. To create a separate result image, select the *Display result image* check box
4. Open the image selector for *Display result image* and if needed change it from *None* to *New*, *Overwrite*, or *Add to*. *Overwrite* is the recommended selection.
 - **Overwrite:** Overwrites the selected image or creates a new image if one does not exist. This is the recommended selection.
 - **Add to:** Adds a plane to a stack.
 - **New:** Creates a new image every time the assay is run.
5. Click on the image name (typically indicated with [Source]).
6. Select *Specified* and give the image an appropriate name, such as Result.
7. Repeat steps 1-4 for all needed result images.
8. Select *Apply/ Test run* to check your result image.

Most application modules require you to specify a number of size and contrast measurements before processing the image(s). These measurements often include the following:

- Minimum, average, and/or maximum width of an object
- Area of an object
- Intensity above background of an image

Complete the following procedure to determining the width of objects using the Region tools Line tool:

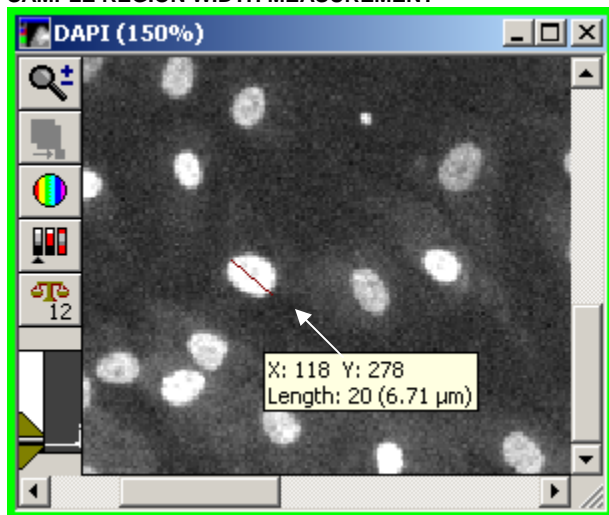
1. If the Region toolbar is not open, select Region Tools from the Region menu. The region toolbar opens, as shown in Figure 3:

**FIGURE 3
REGION TOOLBAR WITH LINE TOOL SELECTED**



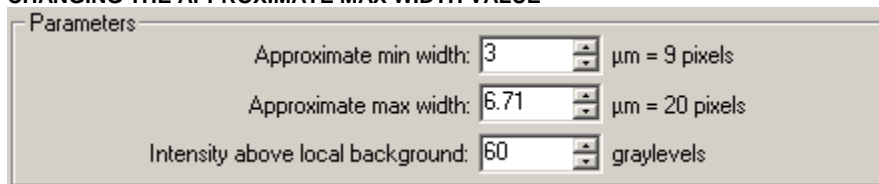
2. Select the Line tool as show above.
3. Place your mouse pointer on the image and locate the object that you want to measure.
4. Move the mouse pointer to one of the edges and click. A tool-tip appears showing the current X and Y values of the pointer, as well as the length.
5. Move the mouse pointer to the opposite edge of the object and note the Length value. In the following example the value is 20 pixels (or 6.71 μm). This number represents the cell width in pixels. If the image is calibrated, the length in pixels and calibrated units.

**FIGURE 4
SAMPLE REGION WIDTH MEASUREMENT**



6. Type or select the value in microns in the *Approximate max width* field. Figure 5 show the new *Approximate max width* value.

**FIGURE 5
CHANGING THE APPROXIMATE MAX WIDTH VALUE**

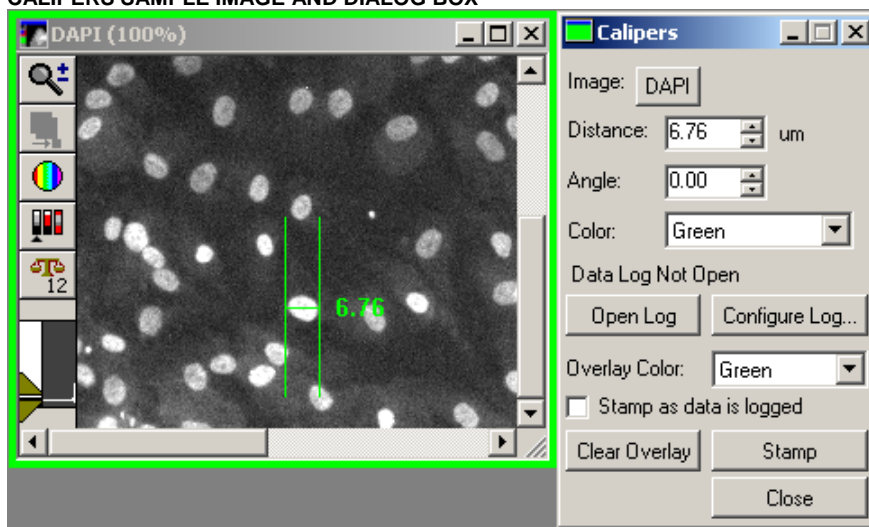


7. You can remove the region lines by selecting Clear Regions from the Regions menu, or right-click on the region and select Delete Region.

You can also use the Caliper tool to measure the width of an object. The caliper function is a drop-in and may need to be loaded into the software. If the command is not present in the Measure menu, please see your System Administrator to have this drop-in loaded. Complete the following procedure to use the Caliper tool to measure the width of an object:

1. From the Measure menu, select Calipers. The Calipers dialog box opens.
2. Select the image to measure from the Image selector. The calipers appear on the selected image, as shown in Figure 6:

FIGURE 6
CALIPERS SAMPLE IMAGE AND 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 the cross-bar to the desired location with your pointer.
4. Single-click one of the caliper edge lines so that it is displayed as a blinking line. Then drag the line to the desired distance. The other caliper line will remain anchored.
5. Double-click the caliper cross-bar so that "nodes" appear at each end. With your pointer, drag one of nodes away from the other to the desired distance. The size of the line is displayed in the image as well as in the Caliper dialog box.
6. Enter the value in the *Approximate max width* field of the application module dialog box.

A third way to determine the width of an object is by using the Show Region Statistics dialog box. This also displays the area of the selected object. Complete the following procedure to use the Show Region Statistics dialog box to view the width and area of an object:

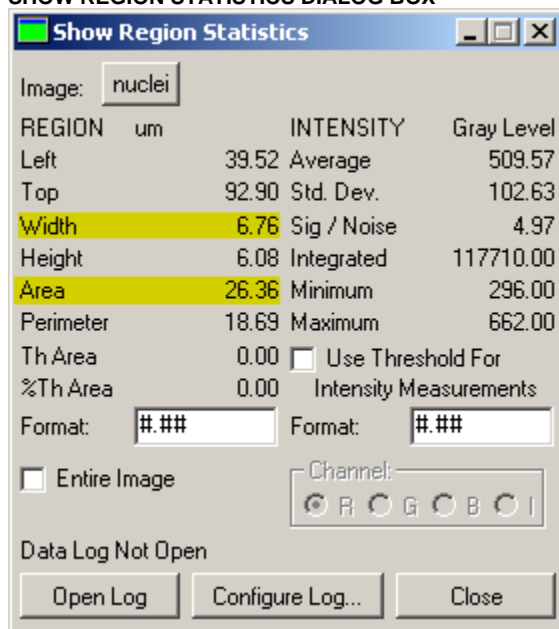
1. If the Region toolbar is not open, select Region Tools from the Region menu. The region toolbar opens, as shown in Figure 7:

FIGURE 7
REGION TOOLBAR WITH TRACE REGION TOOL SELECTED



2. Select the Trace Region tool as show above.
3. Place your mouse pointer on the image, click and hold the left mouse button to draw a region of interest around a typical compartment. Double-click the left mouse button to close the region.
4. From the Measure menu, select Show Region Statistics. The Show Region Statistics dialog box opens, as shown in Figure 8:

FIGURE 8
SHOW REGION STATISTICS DIALOG BOX

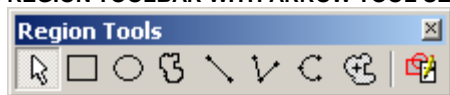


5. Ensure that *Entire Image* is not selected in order to view the area and/or width measurements for the active region created in Step 3.

The *Intensity above local background* field is common for all application modules. It specifies a value for the intensity threshold of the object(s) of interest compared to the neighboring background gray values. This setting is for controlling the sensitivity of the detection and/or segmentation. Complete the following procedure to determine accurate intensity above background values:

1. If the Region toolbar is not open, select Region Tools from the Region menu. The region toolbar opens, as shown in Figure 9:

FIGURE 9
REGION TOOLBAR WITH ARROW TOOL SELECTED



2. Select the Arrow tool as show above (this should be selected by default).
3. Place the arrow cursor in the image window and move it over the visibly dimmest part of the dimmest object in the image.
4. Note the gray-level value of the object. The current gray-level value, along with the current X and Y coordinate values, are displayed on the bottom of MetaMorph desktop, as shown in Figure 10:

FIGURE 10
BOTTOM OF METAMORPH DESKTOP WITH CURRENT GRAY-LEVEL HIGHLIGHTED

(101, 80) -> 366

5. Move the mouse just outside the object to the background of the image and note the gray-level value of the background.
6. Calculate the difference between the cell signal and the background. For the example, using the cell signal value of 366 noted in Figure 10 and a background value of 304, the calculation would be as follows: $366 - 304 = 62$.
7. Enter a slightly lower value in the *Intensity above local background* field of the application module dialog box. In this example, a value of approximately 60 is appropriate.
8. Move on to the next section, *Testing and saving settings*. Do not close the application module dialog box that you have configured.

TESTING AND SAVING SETTINGS

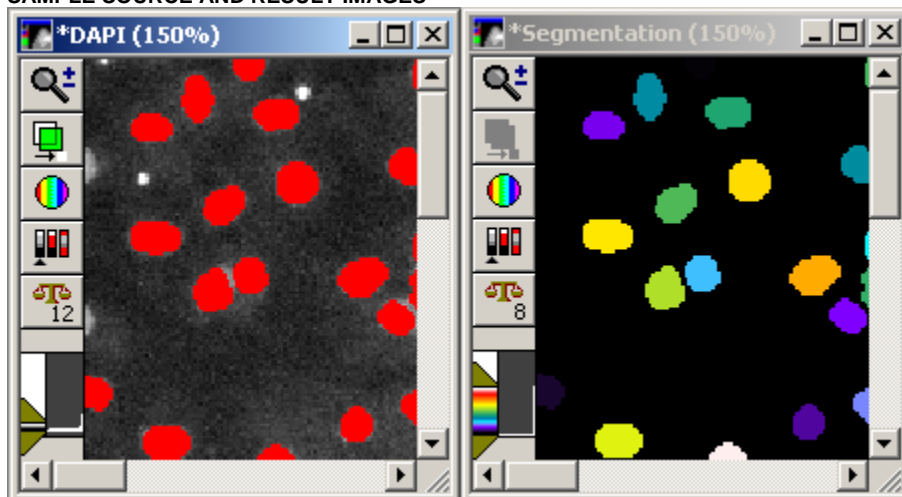
After the module is configured, you should test the settings, make adjustments if needed, and then save the settings.

NOTE: Configuring and saving settings only needs to be done before you run an application module for the first time. After the settings are saved, they can be loaded as needed without further configuration.

Complete the following procedure to test and save your settings:

1. Click *Apply* in the application module to measure using the settings that you just configured. The application module will run and an overlay image will appear on the original image. A result image will also open if the *Display result image* checkbox was selected. Figure 11 illustrates a sample source image with an overlay and a result image with the cells individually colored:

FIGURE 11
SAMPLE SOURCE AND RESULT IMAGES



2. Compare the result image with the source to determine if all the objects of interest have been detected. If not, lower the value in the *Intensity above background* field and run the module again before modifying the other parameters.
3. To configure logging the application module data, click *Configure Summary Log*. The Configure Log dialog box opens.

4. Check and/or uncheck individual parameter configuration settings. For more information on the settings that can be logged, refer to the online help Dialog Box Options page for the application module you are using.
5. Click *Configure Data Log* and configure the Data log if needed.
6. To save you settings, click *Save Settings*. The Save As dialog box opens.
7. Navigate to the folder where you want to save the settings.
8. Enter a name for the settings in the *File name* field and click *Save*. The setting is saved and can be restored using the *Load Settings* command.

RUNNING AN APPLICATION MODULE

The following procedure provides an overview on running an application module interactively or recording it in a journal. It is based on the instructions in this document. It assumes that you are using Microsoft Excel to log the data:

1. From the Log menu, select *Open Summary Log*. The Open Summary Log dialog box opens.
2. Select *Dynamic Data Exchange (DDE)* and click *OK*. The Export Log Data dialog box opens.
3. Enter name for the sheet and click *OK*. Excel opens.
4. If you need to open a Data log as well, repeat Steps 1-3, replacing Open Summary Log with Open Data Log in step 1.

NOTE: If you open a data log, ensure that you change the sheet name from the default if your summary log already has the default name. Otherwise, the summary log data will be overwritten.

NOTE: Individual object data is exported to a data log. Summary data for the entire image is exported to the summary log.

5. From the Apps menu, select your assay (for example, Count Nuclei).
6. Set the appropriate parameters and select the images as described earlier in this document.
7. Click *Apply*. The module runs, the source and result image(s) are displayed, and the data is exported to Excel. A Cellular Results table containing individual object data also opens. If the table is not opening when you run an application module, ensure that Show Cellular Results is select in the Window menu.
8. Repeat steps 6-7 as needed for remaining images.