Using the MetaMorph Morphology Filters

ABSTRACT

The Morphology Filters in MetaMorph provide a set of tools that can be used to transform binary and grayscale images through morphology filtering and analysis. Both of these processes can make the data that you need to extract from your images more accessible. This document explains how to perform two typical segmentation applications using MetaMorph's Morphology Filters. The following procedures are explained in this document:

- Creating a single-pixel wide set of partition lines around segments
- Creating a binary mask image that separates cells from background

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PRODUCTS
MetaMorph® 6.1 or later

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This document assumes the following:

- You have the **`frogeggs.tif`** and **`muscle.tif`** sample image files located in the MetaMorph images directory. These images are installed by default.
- The Morphology drop-in has been loaded using the Meta Imaging Series Administrator.
- You have some familiarity with image thresholding and the Binary and Arithmetic dialog boxes located in the Process menu.

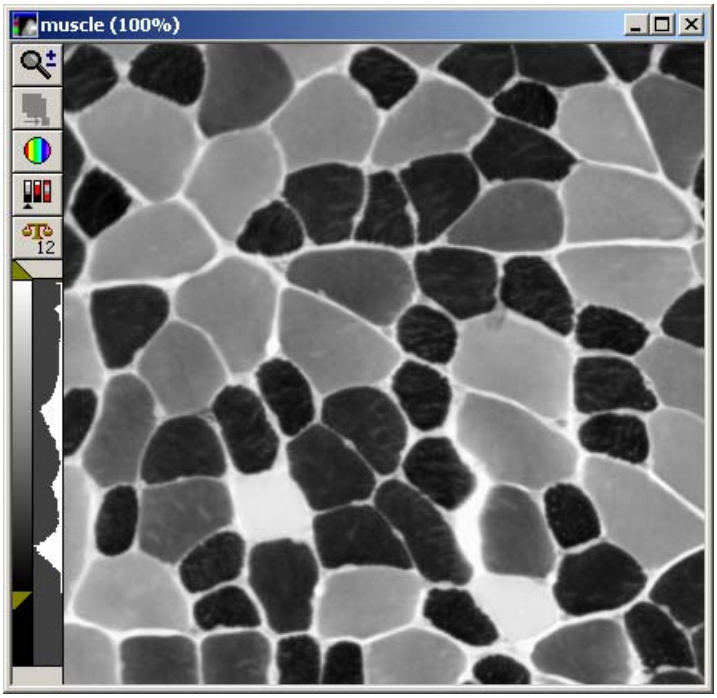
NOTE: The examples in this document were done using MetaMorph 6.3r1.

CREATING A SINGLE-PIXEL WIDE SET OF PARTITION LINES AROUND SEGMENTS

Complete the following procedure to create a single-pixel wide set of partition lines around segments using the sample image file **`muscle.tif`**:

1. From the File menu, select Open. The open dialog box opens. Navigate to the images directory (C:\MM\images by default) and open the image file **`muscle.tif`**, as shown in Figure 1:

FIGURE 1
MUSCLE IMAGE

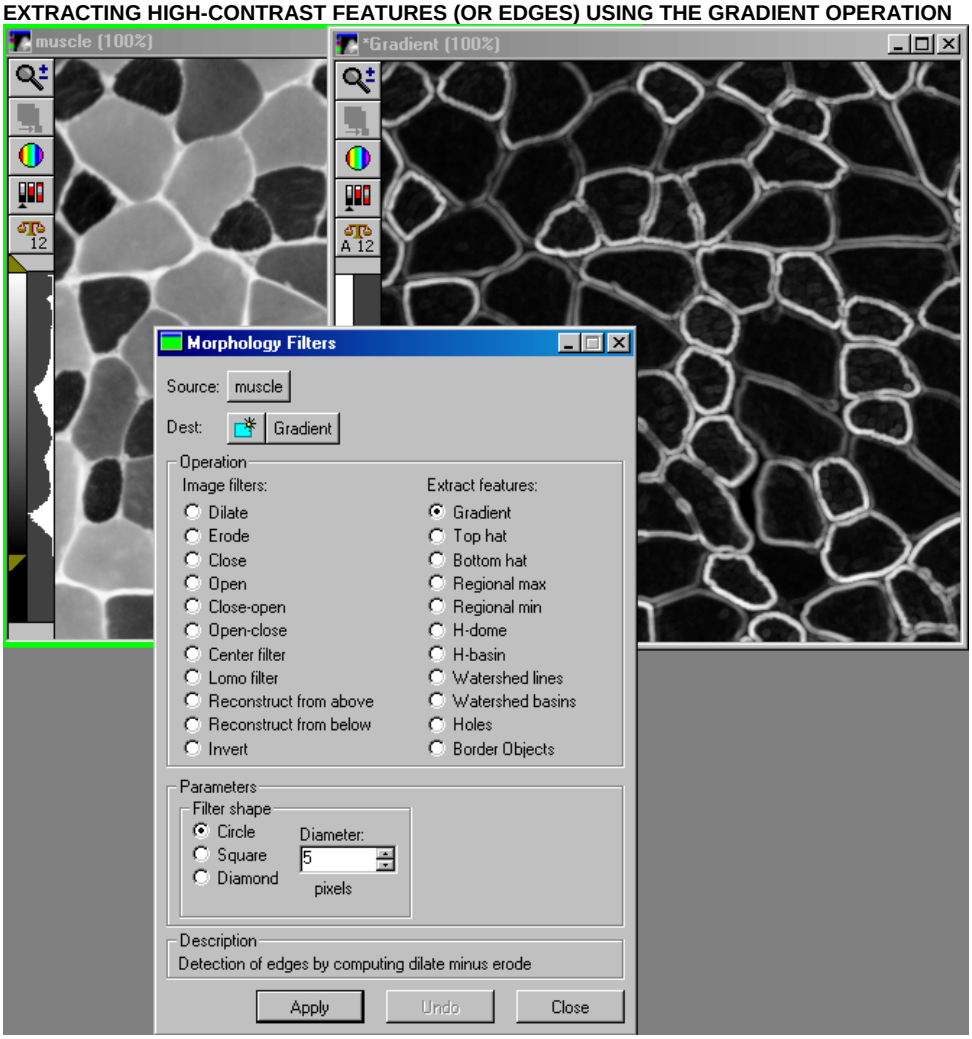


The tissue seems divided into compartments separated by thin white boundaries, but thresholding for light objects doesn't extract these boundaries easily -- some of the compartments are actually lighter than some of the boundary tissue.

Note that visually, the boundary tissue is distinct because of its high contrast with its neighboring compartments. We can extract high contrast features (or *edges*) using the Morphology Filters dialog box's *Gradient* operation.

2. From the *Process* menu, select Morphology Filters.
3. Select *Gradient* from the *Operation* field.
4. In the *Parameters* field, select *Circle* and enter a value of 5 pixels in the *Diameter* field.
5. Click the *Apply* button. This will create a new image called Gradient, as shown in Figure 2:

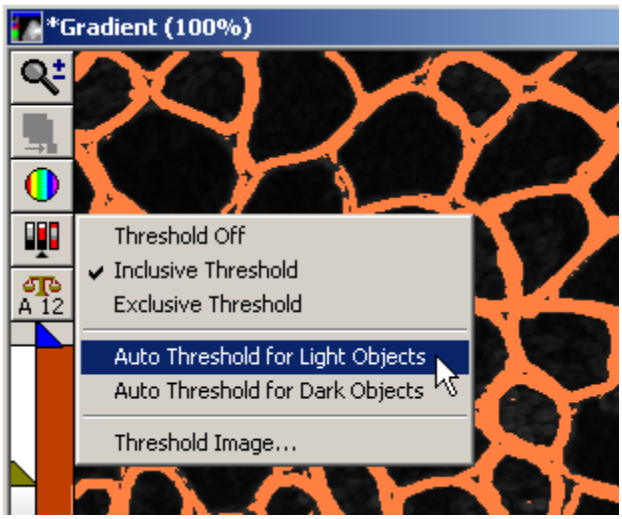
FIGURE 2
EXTRACTING HIGH-CONTRAST FEATURES (OR EDGES) USING THE GRADIENT OPERATION



To threshold the Gradient image:

1. Click the *Threshold* button on the Gradient image toolbar and select *Auto Threshold for Light Objects*, as shown in Figure 3:

FIGURE 3
THRESHOLDING GRADIENT

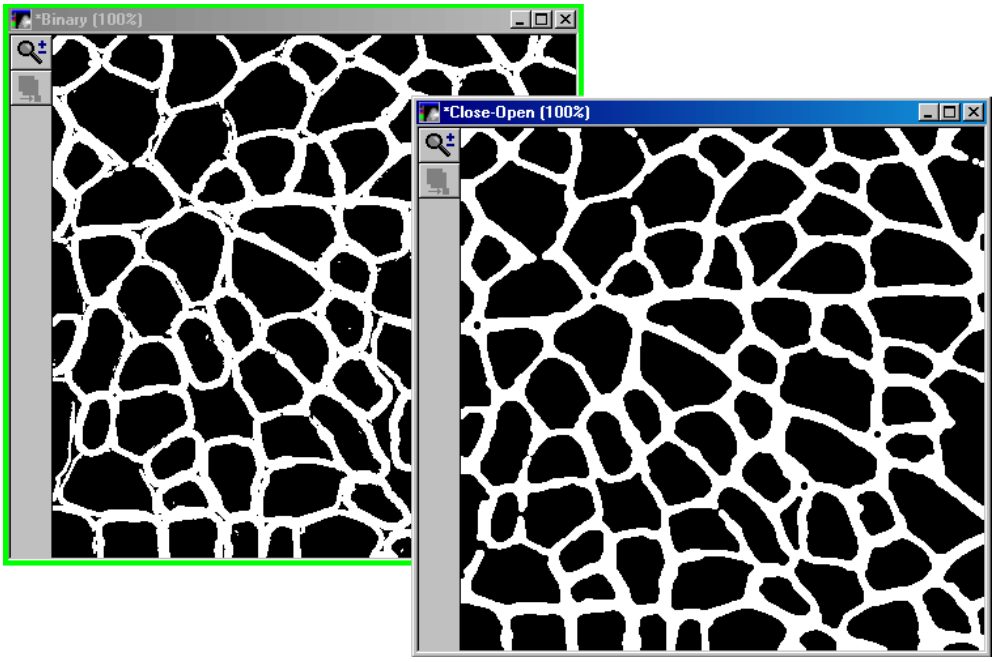


2. Go to the *Process* menu and select *Binary*.
3. Select the Gradient image as the *Source* image.
4. Select *Binarize* from the *Operation* field.
5. Set the *Low* threshold value to 300 and click *Apply*. This creates an image named Binary.

The Binary process effectively separates the compartments, but there are still some spots to clean up. Complete the following procedure to use a smoothing filter that cleans up both dark and light "junk."

1. In the Morphology Filters dialog box, select the Binary image from the *Source* image selector.
2. From the Operation field, select the *Close-open* operation. This filter, along with the Open-close, Center, and Lomo filters are used for general smoothing of both light and dark detail within images.
3. In the *Parameters* field, select *Circle* and enter a value of 5 pixels in the *Diameter* field.
4. Check the *Use sequential filtering* checkbox. This option processes the image by gradually increasing the filter size until the desired scale is reached rather than directly applying only the final filter size. It's good practice to use this option when performing a general smoothing operation because it is more robust.
5. Click the *Apply* button. A new image called Close-open is created, as shown in Figure 4:

FIGURE 4
THRESHOLDING THE GRADIENT IMAGE AND CLEANING UP WITH CLOSE-OPEN FILTERING



To turn the compartments white:

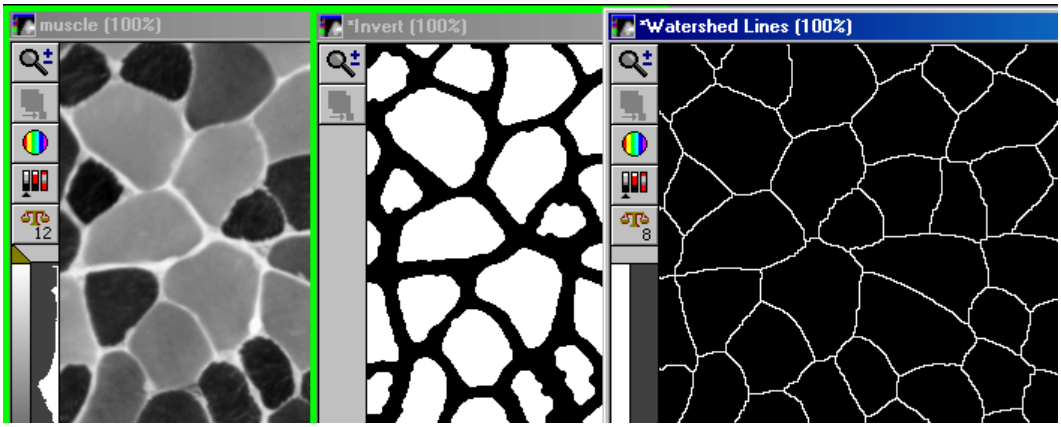
6. In the Binary dialog box, select the Close-Open image as the *Source* image.
7. Select *Invert* from the Operation field and click *Apply*.

Now you can use this result as a mask or create regions with MetaMorph. But perhaps you want to segment the original image into compartments, separated by only single-pixel wide boundaries You can select the Binary dialog box's skeletonize operation, or get some practice with the Morphology Filters dialog box's watershed segmentation operation.

Watershed operations require one image where intensity represents altitude on a topographical map (we have that in the muscle image already -- those bright boundary tissues separating darker compartments, also in the gradient image, etc.), and a recommended second image marking or "seeding" the final segmented regions (in the inverted image you just made -- each white blob serves to roughly mark one region). Imagine water flowing downhill (towards darker pixels) and draining into a marked basin. Where water parts on peaks and ridges, watershed lines are created, naturally segmenting the image into watersheds.

1. In the Morphology Filters dialog box, select the original Muscle image as the Source image.
2. Select *Watershed lines* from the Operation field.
3. In the *Parameters* field, ensure that *Marker* is checked and select the Invert image as the Marker.
4. Click the *Apply* button. A Watershed image opens, as shown in Figure 5:

FIGURE 5
INVERT TO MAKE WHITE "MARKERS" FOR USE WITH WATERSHED ON THE MUSCLE IMAGE.



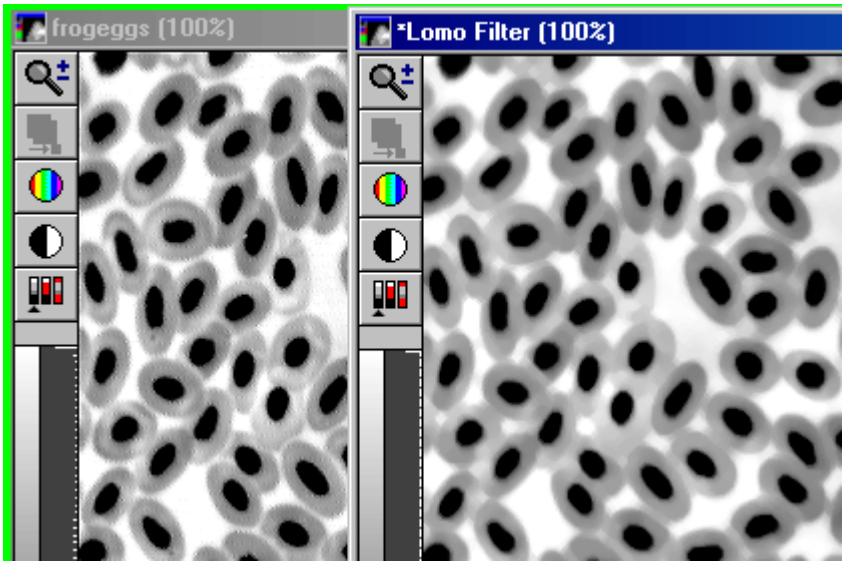
CREATING A BINARY MASK IMAGE THAT SEPARATES CELLS FROM BACKGROUND

The goal in this procedure is to create a binary mask image, separating the cells from the background as well as from each other. Such a mask image allows for accurate counting and for further measurements of individual cell sizes and shapes.

Before you begin, close all the images from the previous example by clicking the Close All button on the standard toolbar, select *Close without saving open images*, then click *Close All.*

1. From the File menu, select Open. The open dialog box opens. Navigate to the images directory (C:\MM\images by default) and open the image file **`frogeggs.tif`**.
2. Under the Process menu, select Morphology Filters to open the Morphology Filters dialog box.
3. Select the *Lomo filter* from the Operation field.
4. Check the *Use sequential filtering*. This option processes the image by gradually increasing the filter size until the desired scale is reached rather than directly applying only the final filter size. It's good practice to use this option when performing a general smoothing operation because it is more robust.
5. Click the *Apply* button. A new image called Lomo Filter is created, as shown in Figure 6:

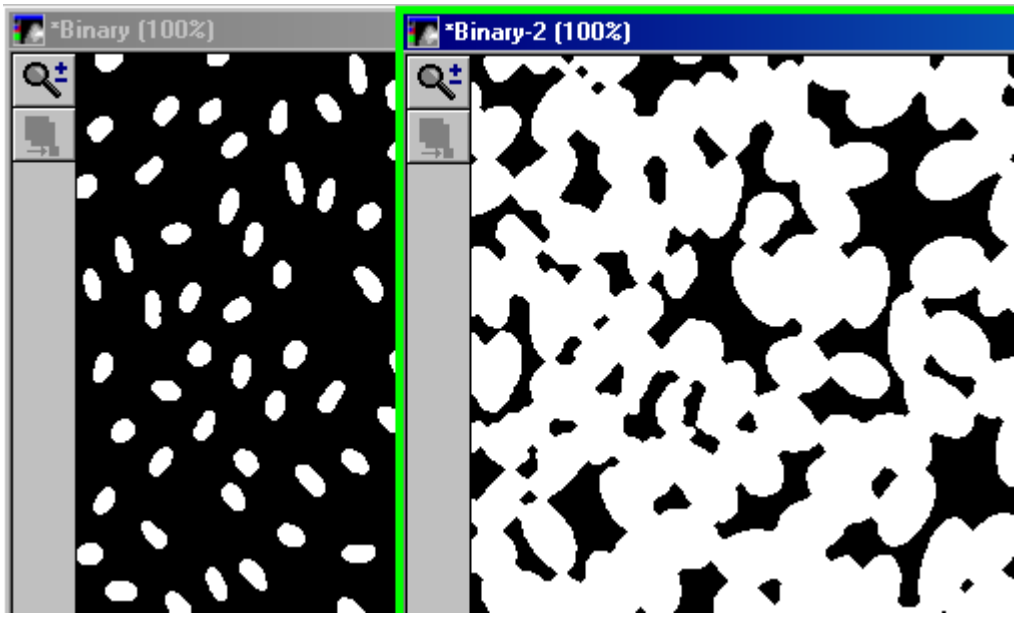
FIGURE 6
SMOOTH OUT BOTH DARK AND LIGHT EXTRANEOUS DETAILS USING THE LOMO FILTER



Now you can make two thresholded images from the Lomo filtered result image:

6. From the Process menu, select Binary.
7. Select the Lomo Filter as the Source image and select *Binarize* from the *Operation* field.
8. Set the *Low* threshold value to 0 and the High threshold value to 30 and click *Apply*. This creates an image named Binary.
9. In the Binary Image Operations dialog box, again select the Lomo Filter image.
10. Set the *Low* threshold value to 0 and the High threshold value to 200 and click *Apply*. This creates an second image named Binary-2, as shown in Figure 7:

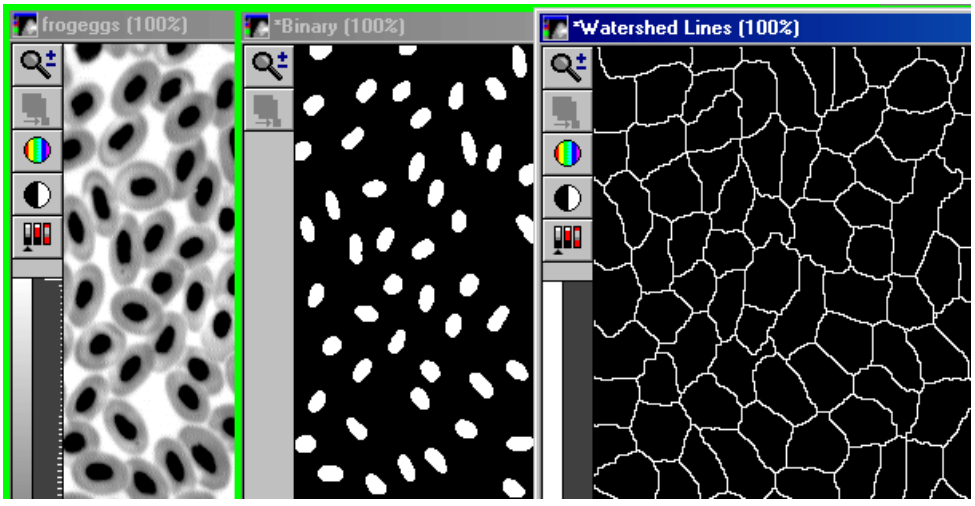
FIGURE 7
APPLY THRESHOLDING TO OBTAIN BOTH NUCLEI ONLY AND ENTIRE CELLS IMAGES



The original frog eggs image has bright background and dark objects -- imagine rainwater parting on this topographical surface into watersheds. The nuclei only image (Binary-2) does a good job of marking the watershed basins we want.

11. In the Morphology Filters dialog box, select the original frogeggs image as the source.
12. Select the *Watershed Lines* from the *Operation* field.
13. Under the Parameters field, select the Binary image as the Marker.
14. Click the *Apply* button. This creates a new image called Watershed Lines, as shown in Figure 8:

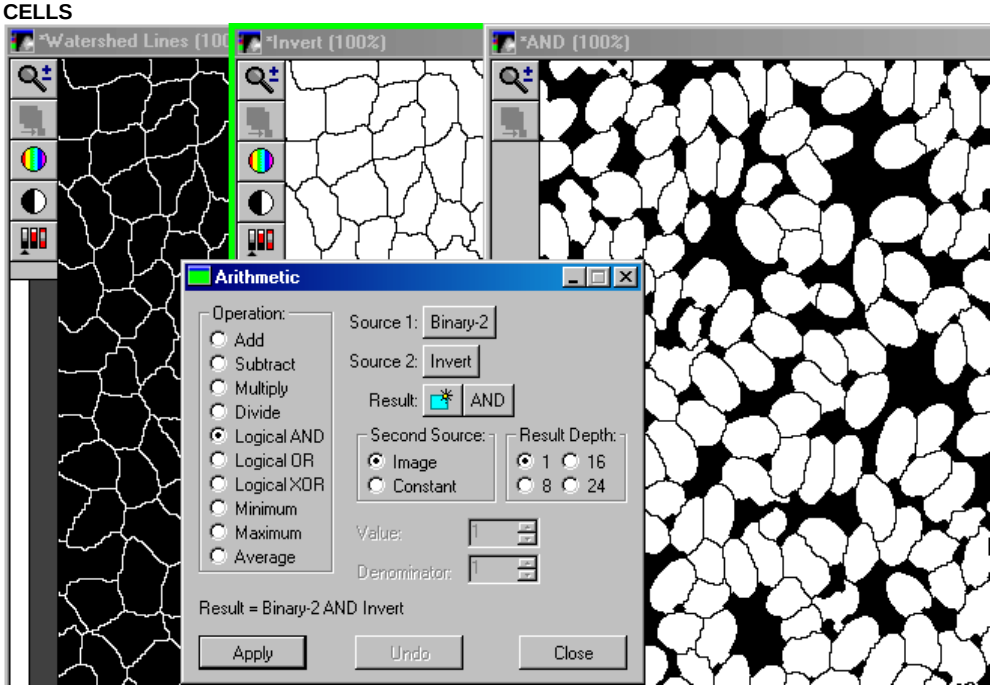
FIGURE 8
WATERSHED LINES SEPARATE THE ORIGINAL IMAGE AS WATER PARTS AT BRIGHT RIDGES AND FLOWS INTO MARKED BASINS



Use the Watershed Lines image to separate touching cells:

1. In the Morphology Filters dialog box, select Watershed Lines as the *Source* image and select Invert from the *Operation* field.
2. Click *Apply*. This creates a new image called Invert.
3. From the Process menu, select *Arithmetic*. The Arithmetic dialog box opens.
4. Select the Binary-2 image as *Source 1* and the *Invert* image as *Source 2*.
5. Select *Image* from the *Second Source* field.
6. Select *Logical AND* from the Operation field. This will separate the touching cells.
7. Click *Apply*. This will create a new image called AND, as shown in Figure 9:

FIGURE 9
WATERSHED LINES (INVERTED) SERVE AS BLACK LINES SEPARATING THE THRESHOLDED TOUCHING CELLS



The thresholded (and now separated) cells do not look very rounded, but biologically we know they are – no sharp corners or jagged edges. The Morphology Filters Open operation will make this correction – it repaints the white objects only where the filter shape "fits" inside.

Imagine cleaning a white kitchen floor with a certain shape mop – if the floor space between the black stove and the black fridge is too narrow, it doesn't get cleaned and eventually goes black. The circular mop can never reach into the corners of the kitchen floor either, so they too turn black.

To correct the image using the Open operation:

1. In the Morphology Filters dialog box, select the AND as the *Source* image.
2. Select *Open* from the Operation field.
3. In the *Parameters* field, select *Circle* and enter a value of 9 pixels in the *Diameter* field to round off corners and narrow parts of the white objects in your segmentation result.

4. Click *Apply*. This creates a new image called Open. Note that the cells are rounded off and "cleaned," as shown in Figure 10:

FIGURE 10
OPEN FILTERING WITH A CIRCLE SHAPE ROUNDS OFF CORNERS AND THIN PROTRUSIONS TO CLEAN UP THE SEGMENTATION RESULT