

Correcting for Uneven Background in an Image Using Morphology Filters

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Abstract

This document provides a sample exercise in using MetaMorph's Morphology Filters and Arithmetic commands to correct an uneven background in an image. Correcting the image background increases your ability to isolate areas of interest, such as individual cells, in your image.

Instructions

The basic techniques included in this exercise include the following:

- ♦ Removing image noise—this is a good starting point for most image analysis
- ♦ Estimating the image background
- ♦ Subtracting the background from the image

Notes

- This document assumes that the Morphology drop-in has been loaded using the Meta Imaging Series Administrator.
- Color images (24- and 48-bit) cannot be used with Morphology Filters. If you have a color image that you want to apply background correction to, use the Color Separate command to create an 8- or 16-bit copy of the image.
- The example given produces similar results to the Flatten Background command; however, the given method allows for more manual control over the estimated background image.

Instructions

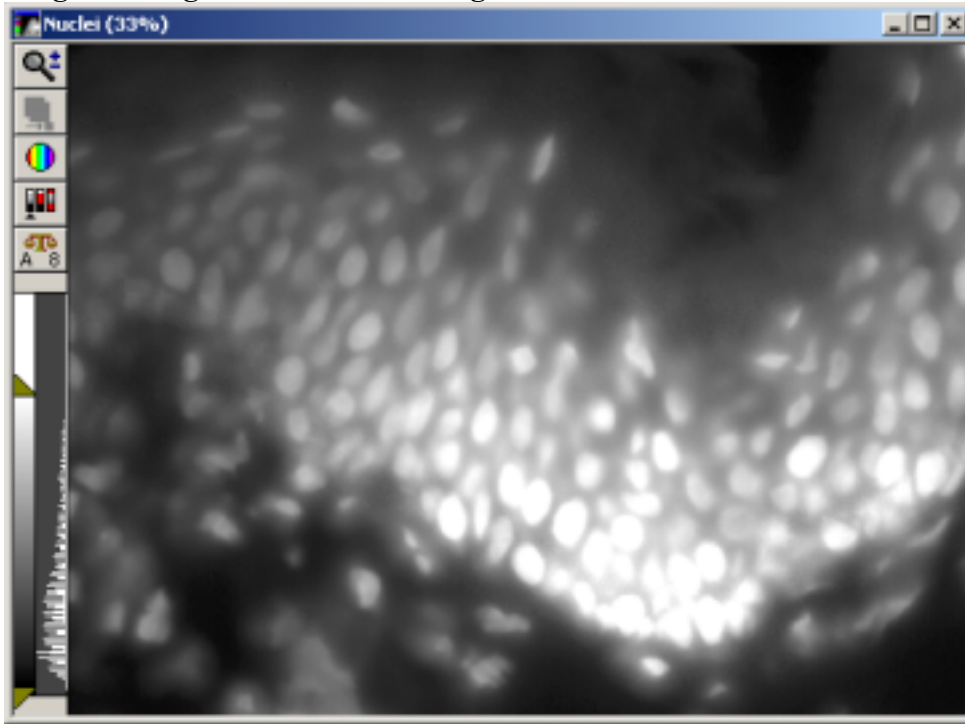
Removing Noise and Estimating the Image Background

Complete the following steps to remove the noise and estimate the background for an image:

1. From the Windows Start menu, choose Programs> Meta Imaging Series 6.x> MetaMorph to open MetaMorph.
2. Open the image that needs correction. The image used in this exercise is shown in Figure 1:

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Figure 1
Original Image with Uneven Background

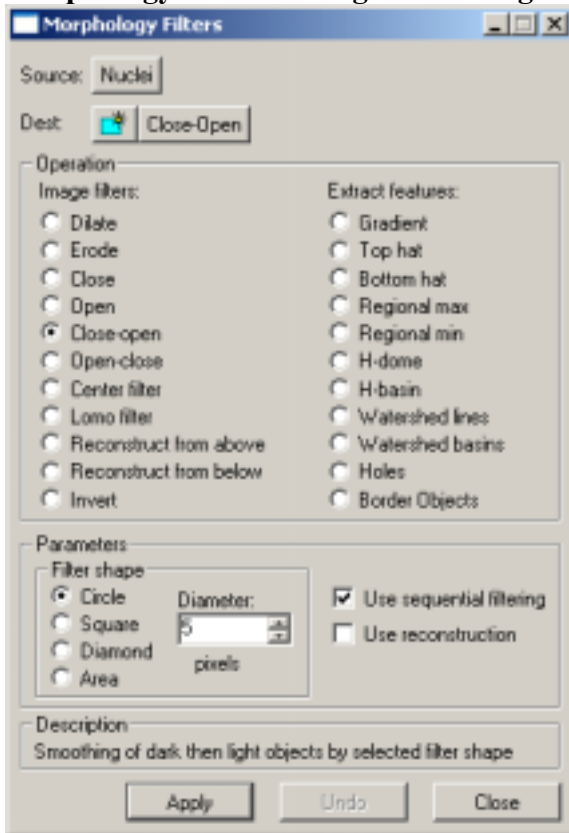


3. From the Process menu, select Morphology Filters; the Morphology Filters dialog box opens.
4. Select *Close-Open* from the *Image Filters* list in the *Operation* group.

Note: As noted in the *Description* field, the Close-Open option applies smoothing to both dark and light objects in the image. You can use other filters that also work on both light and dark objects, including the Open-Close, Center, and Lomo filters.

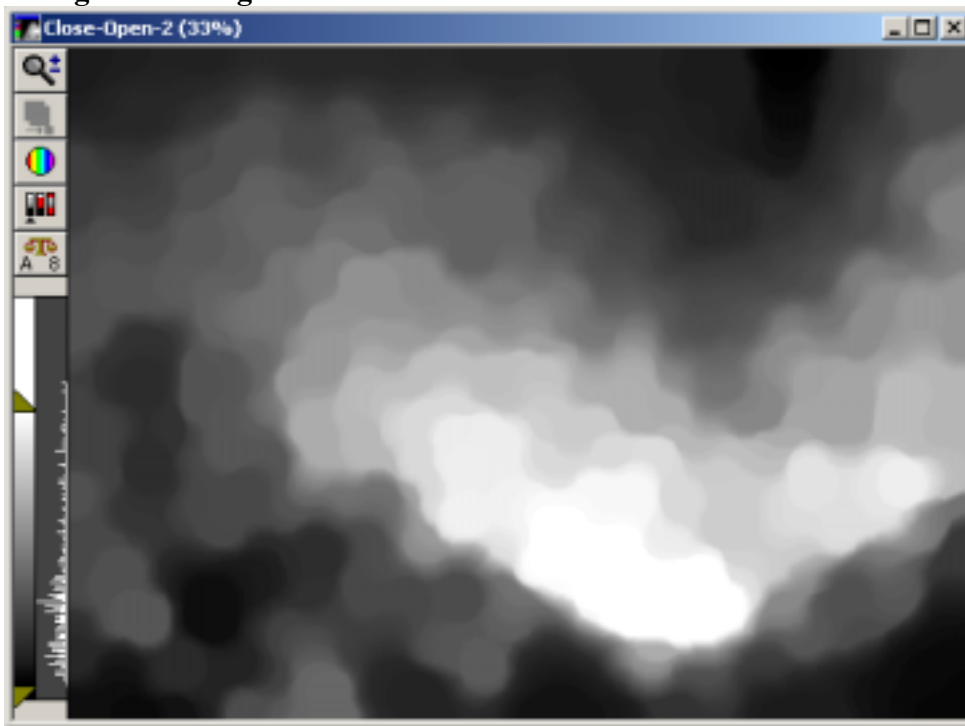
5. Select *Circle* from the *Filter shape* list in the *Parameters* group and enter *5* in the *Diameter pixels* field. These settings result in smoothing the image and removing features below the scale of interest (in this case below 5 pixels).
6. Check *Use Sequential filtering*. This option provides more robust filtering by gradually filtering more detail, up to the size selected in the *Diameter pixels* field. The dialog box should now be configured as shown in Figure 2:

Figure 2
Morphology Filters Dialog Box Settings



7. Click *Apply*. The filter is applied and a new image titled **Close-Open** opens on the desktop.
8. Now change the value in the *Diameter pixels* field from 5 to 80. Leave all other options the same and click *Apply*. The filter is applied and a new image titled **Close-Open-2** opens on the desktop, as shown in Figure 3:

Figure 3
Background Image



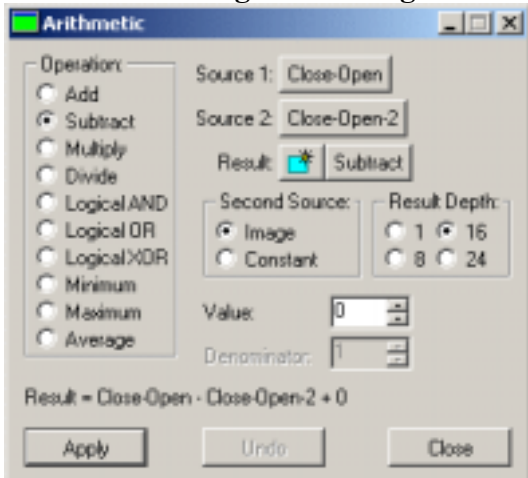
Changing the pixel diameter to 80 results in a heavily filtered image that will serve as your background image. The next step is to use the Arithmetic command to subtract the background from the image.

Subtracting Background from an Image

Complete the following steps to Subtract the background from the image:

1. From the Process menu, select Arithmetic; the Arithmetic dialog box opens.
2. Select *Subtract* from the *Operation* list and *Image* from the *Second Source* group.
3. Ensure that **Close-Open** is selected in the *Source 1* field and **Close-Open-2** is selected in the *Source 2* field. Figure 4 shows the dialog box with the correct settings:

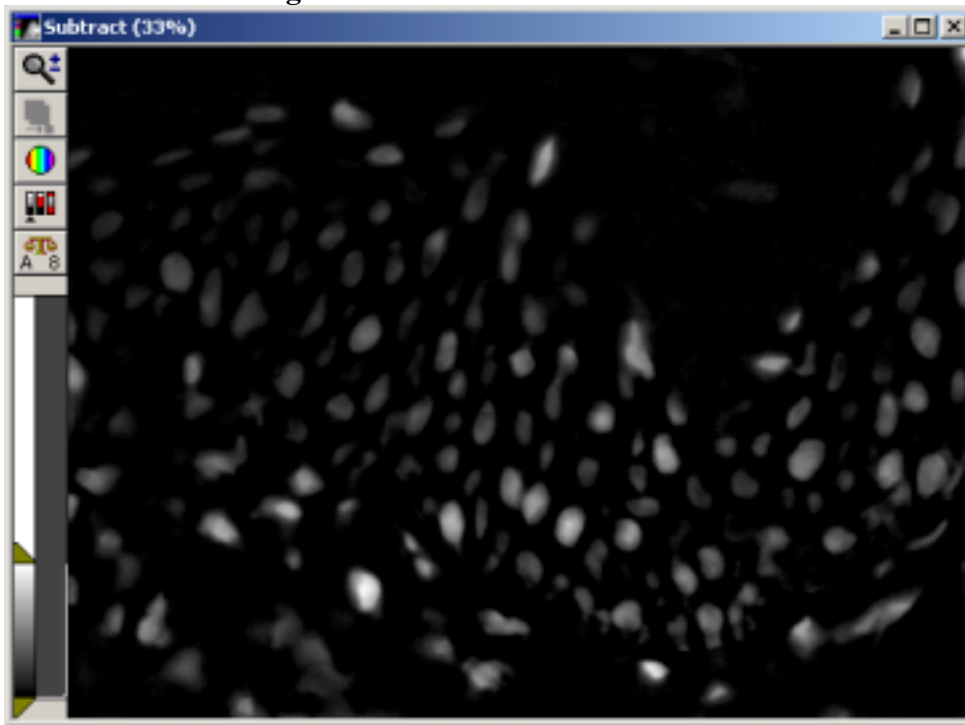
Figure 4
Arithmetic Dialog Box Settings



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4. Click *Apply*. The Arithmetic function is applied and the final, corrected image titled **Subtract** opens on the desktop, as shown in Figure 5:

Figure 5
Final Corrected Image



The final image has a much more even background than the original image and the cells can now be clearly thresholded for further analysis. Note that the settings used in the Morphology Filters dialog box are specific to this example; you will need to experiment to determine the optimal settings for your images.