

## Using References in NIS-Elements

### Overview

This technical note describes general usage of **References** in NIS-Elements for image processing and analysis.

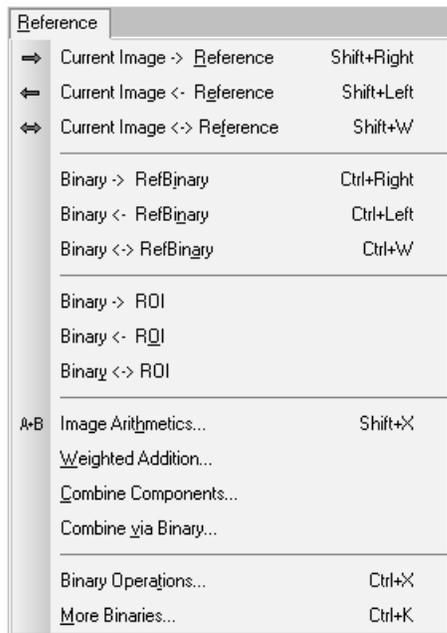
The *Reference* feature in NIS-Elements refers to a “clipboard” where the program temporarily stores an image or binary mask. Most arithmetic operations require the use of storing an image or binary mask to the clipboard (reference) beforehand.

In conjunction with the clipboard, found in the Reference pull-down menu, are several arithmetic function dialog boxes.

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 D with AutoMeasure Module

### The Reference pull-down menu

The Reference pull-down menu in NIS-Elements is subdivided into several categories:



*Copies the current image to the clipboard*  
*Makes the clipboard image the current image*  
*Swaps the current image and clipboard image*

*Copies the current binary to the clipboard*  
*Makes the clipboard binary the current binary*  
*Swaps the current binary and clipboard binary*

*Draws ROI(s) around the current binary*  
*Converts ROI(s) to binary*  
*Swaps binary layer and ROIs*

*Provides arithmetic and Boolean functions*  
*Performs weighted arithmetic of color images*  
*Allows swap of reference with R,G,B channels*  
*A binary masking function*

*Provides boolean operations on binary v. Refbinary*  
*Enhanced clipboard with more places to copy/swap*

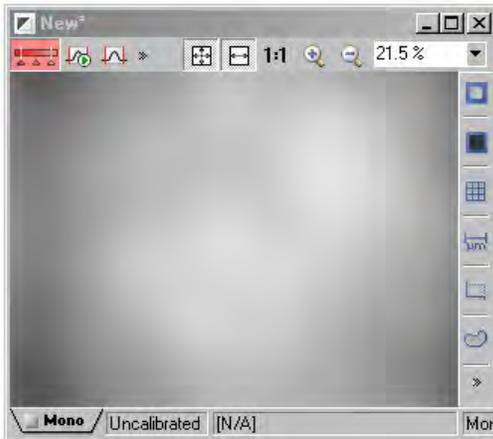


## Using References Images

A common reason for using reference images is to perform arithmetic operations on images, for example – a post-acquisition shading correction.

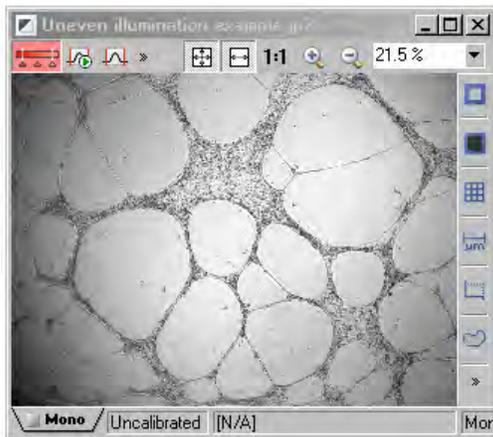
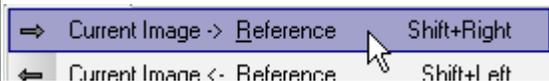
Consider an example where an image was acquired with uneven illumination, then a second image was acquired as a background image:

In order to perform shading correction using **References**, the following steps would be performed:



First, the shading image would be selected and active.

Then select **Reference > Current Image to Reference** to save this image to the clipboard

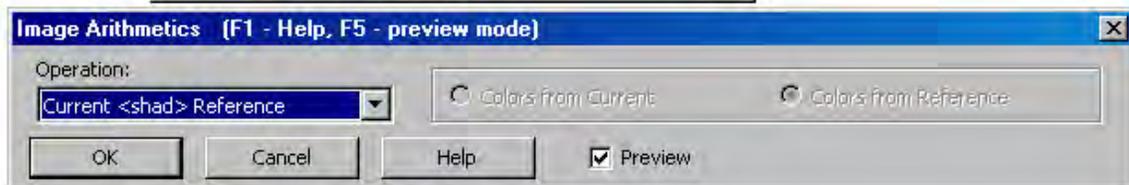
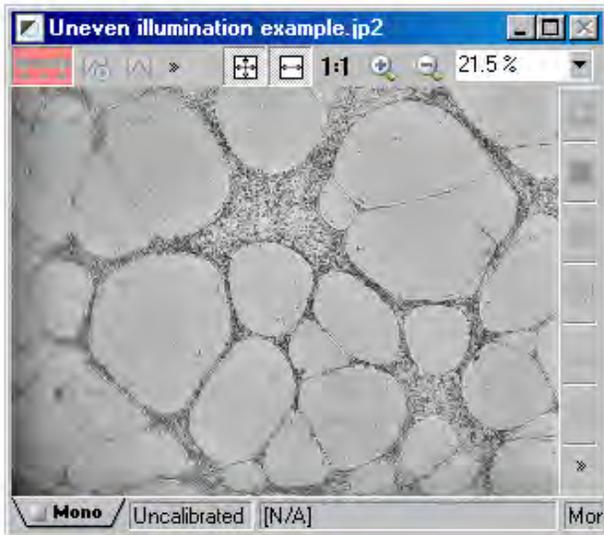


Next, select and make active the image that requires shading correction.



From the **References** pull-down menu, select **Image Arithmetics...**

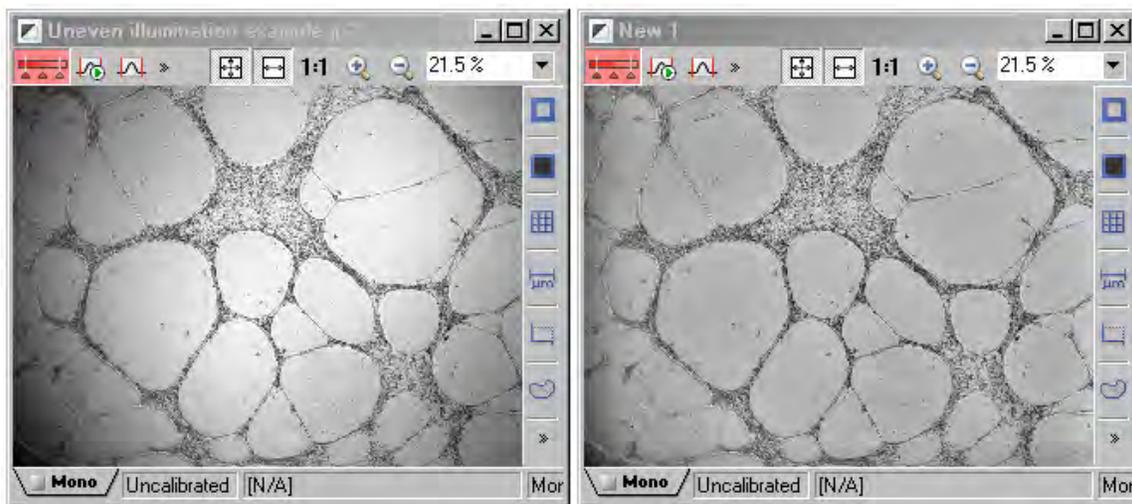




In the **Image Arithmetics** dialog box, there is an **Operation** pull-down menu that allows the user to select from several arithmetic functions. For shading correction, the function chosen is **Current <shad> Reference**.

Checking the **Preview** box allows the user to see real-time what the results of the arithmetic function will be.

Clicking '**OK**' will apply the arithmetic functions to the image.



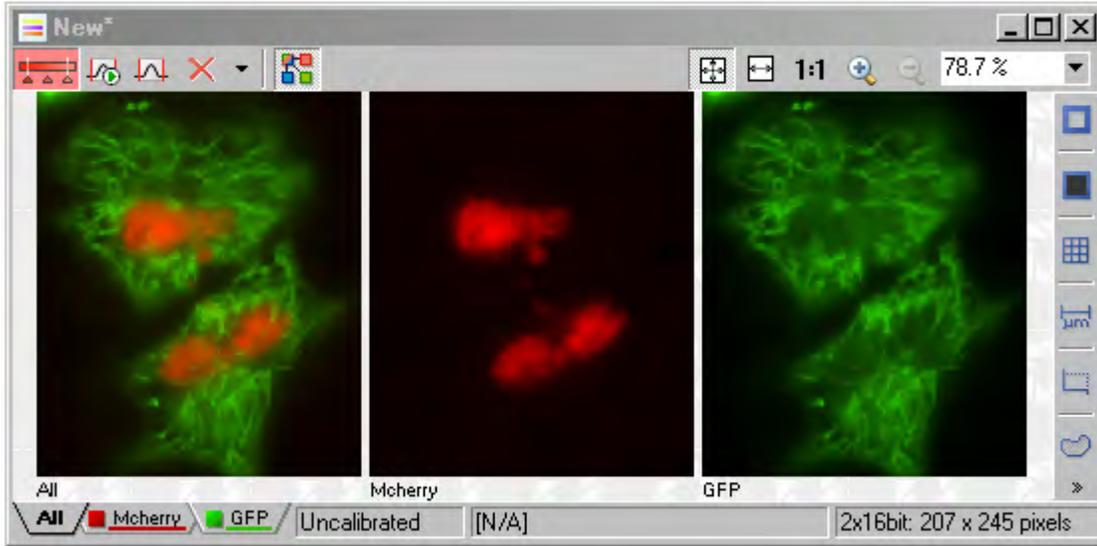
(Left) Before and, (Right) After Shading correction via References



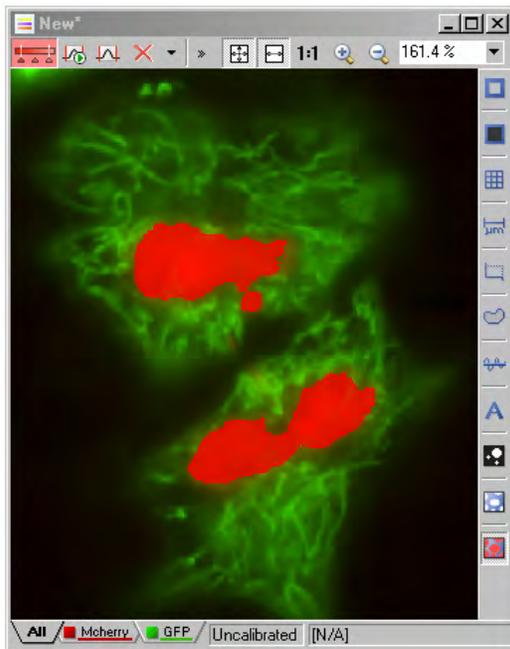
## Using Reference Binaries

Arithmetic operations may also be performed using reference binary layers. These may be used in conjunction with the Binary layers dialog box, and some functions described here are redundant to the Binary layers dialog box functions.

An example of using *Reference binaries*:



A user wishes to subtract the area occupied by nuclear stain (red) from the area occupied by cytoplasmic marking (green), so that the resulting binary layer only is reflective of the area occupied by the cytoplasmic stain.

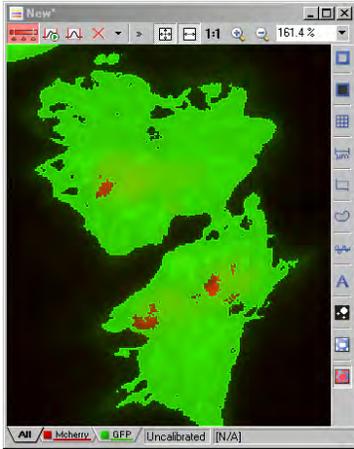


First, the area occupied in red is thresholded using the Define threshold tool: **Binary** pull-down menu > **Define Threshold**

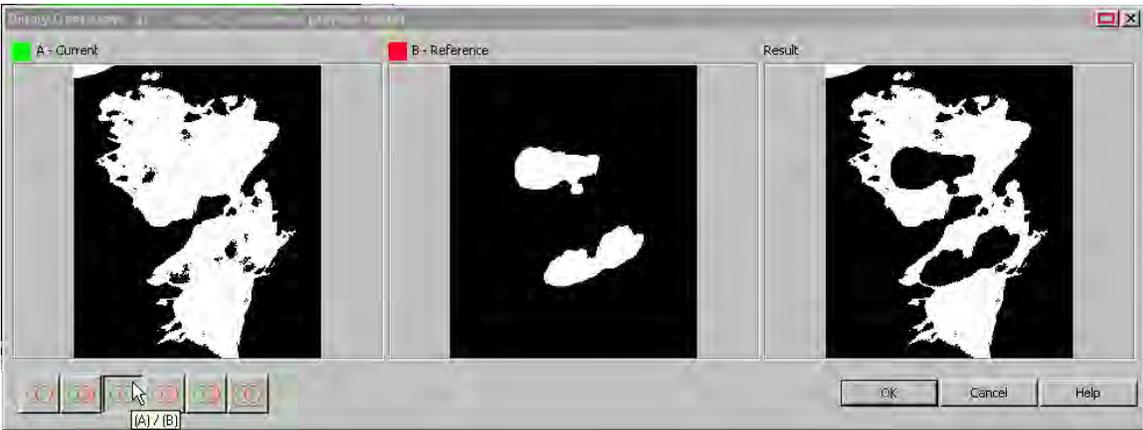


From the **Reference** pull-down menu, copy this binary to the clipboard using **Binary to RefBinary**



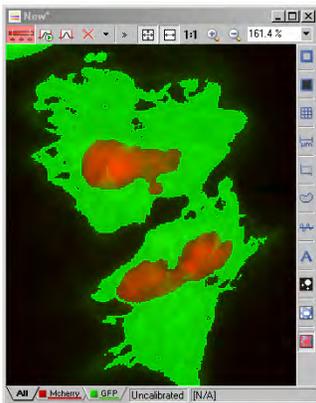


Next, threshold the image in the green channel to select all areas occupied by green.



From the **Reference** pull-down menu, selecting **Binary Operations** will bring up the **Binary Options** dialog box. In it, the current binary and the reference binary will be displayed. Selecting an icon below the current binary will select which arithmetic or Boolean operation should be performed between the two binaries.

In this example, **A / B** is selected to create the desired result. Hitting **OK** replaces the current binary layer on the image with the result binary layer.



The new binary layer is a result of the arithmetic operation performed.

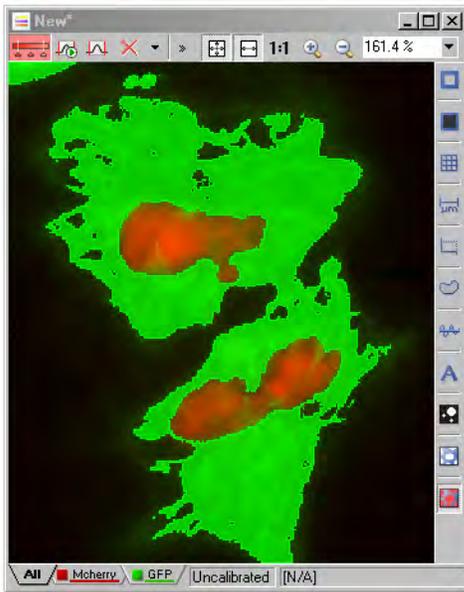


## Using Binary to ROI functions

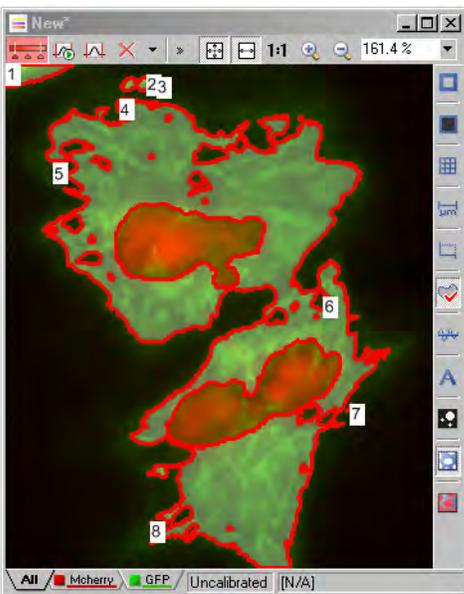
Binary masks can be converted to regions-of-interest (ROI), or ROIs can be converted to binary masks using the **References** pull-down menu.

Commonly, complex shapes can easily be thresholded and binary masks can be generated. It is much easier to convert a binary mask to ROIs than to manually draw ROIs on the image.

Consider the previous example where the cell cytoplasmic area was thresholded, and the nuclear area was removed using binary layers:



If the user wishes to create regions-of-interest (ROIs) around the cells, using the Reference menu option Binary to ROI is a very easy method to accomplish this.



Regions-of-interest are automatically drawn around the area selected in the binary layer.

*Note: Binary processing tools can assist in smoothing, filling holes, or separating touching objects before generating ROIs for best results.*





### **Other Reference Functions**

The other Reference menu functions are less-commonly used and are used for specific purposes, typically embedded in macro functions. Some of the functions are now 'legacy' due to the introduction of multiple binary layers in NIS-Elements 3.0.

