

Using Binary Layers with NIS-Elements

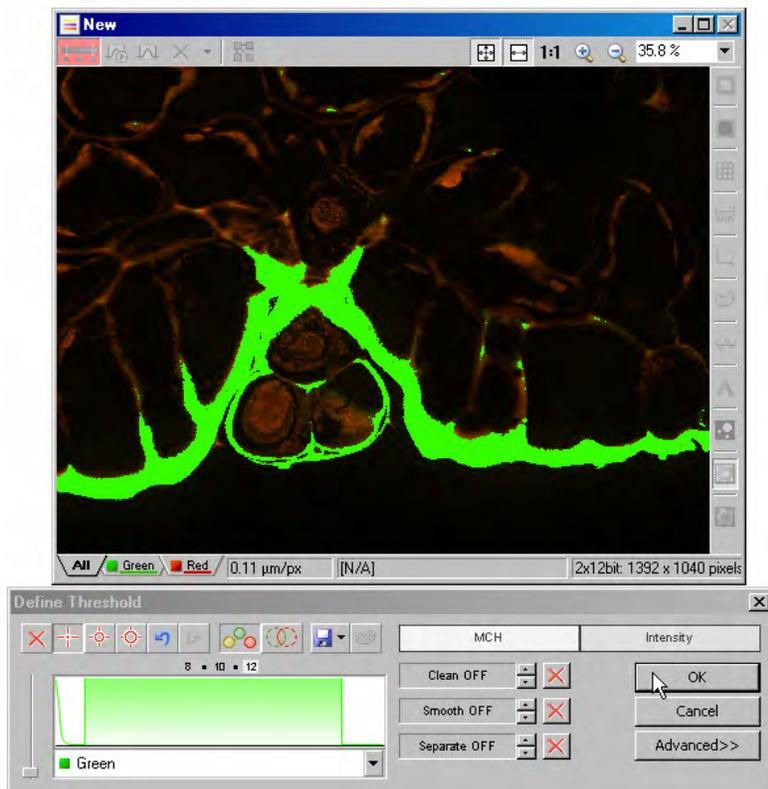
Overview

This technical note describes the usage of **Binary Layers** with NIS-Elements. Binary layers form an extension of simple intensity thresholding technique, allowing the user to save and recall multiple separate thresholds, or to use multiple thresholds to perform arithmetic or Boolean operations.

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D with Automeasure Module

Simple Binary Layers

Most commonly, a single binary layer can be used in NIS-Elements to define areas of interest. This layer is created by performing an intensity threshold on an image or image channel:



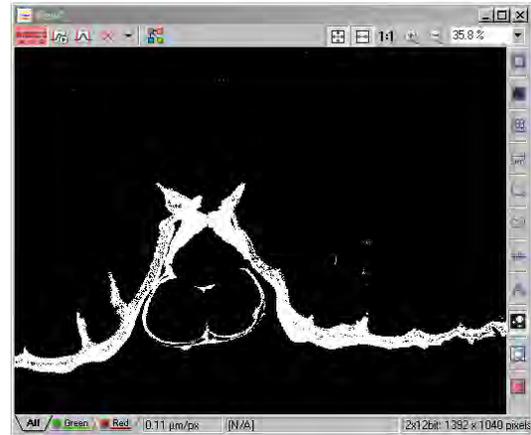
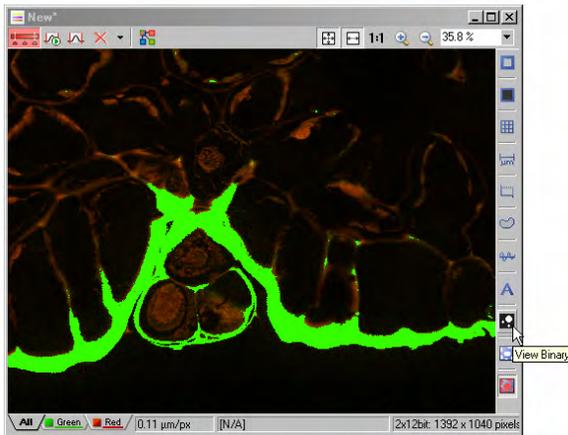
Here, a two channel image is opened. The intensity thresholding dialog box is used to define an area of interest in the green channel.

Choose **Binary > Define Threshold** to open the threshold dialog box, then select a range of intensities to use for the threshold.

At left, a range of intensities in the green channel is highlighted in green.

Click '**OK**' to confirm the threshold.





Once an image had an active threshold on it, the binary layer can be viewed by clicking on the **View Binary** icon on the image window.



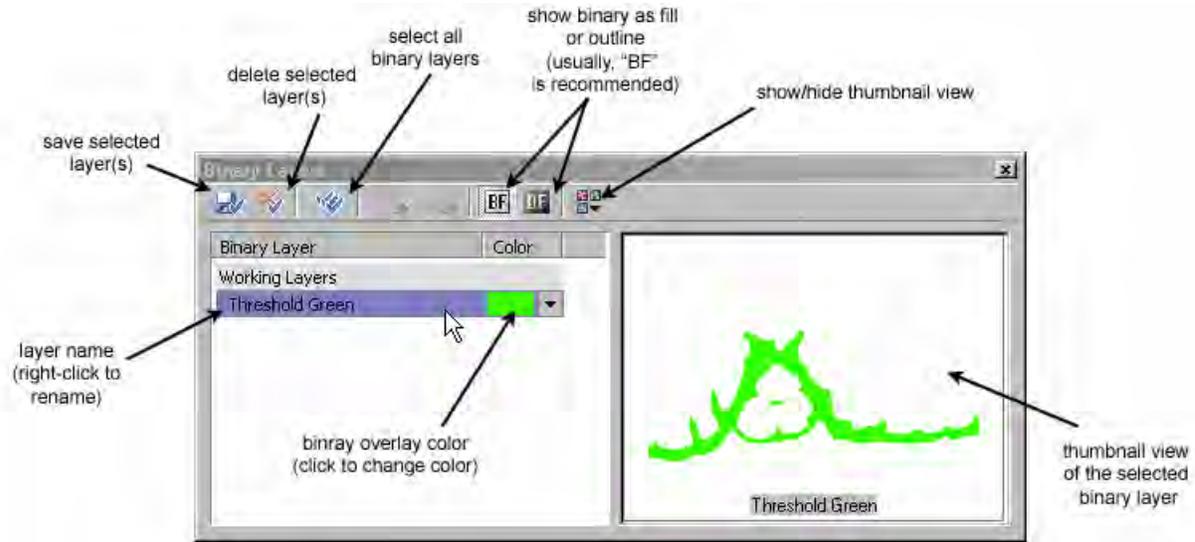
Several Binary processing tools can be used to modify and process the binary layer. These are located in the **Binary** pull-down menu of NIS-Elements. The image above was processed to remove all holes and small objects using the binary functions **Close** and **Clean**.



Alternate Binary Layer view

Alternatively, binary layers can be referenced using the **Binary Layers** dialog box, accessible via the **right-click context menu** in the Visualization Controls section, hitting **CTRL-ALT-B**, or via the pull-down menu **View > Visualization Controls > Binary Layers**.

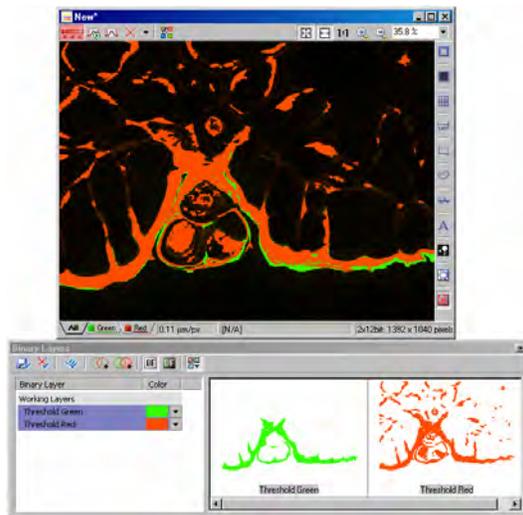
The **Binary layers** dialog box shows the binary layer(s) applied to an image, and offers some additional tools. Note: Some tools are active only if more than one binary layer is present on the data set.



More than One Binary Layer

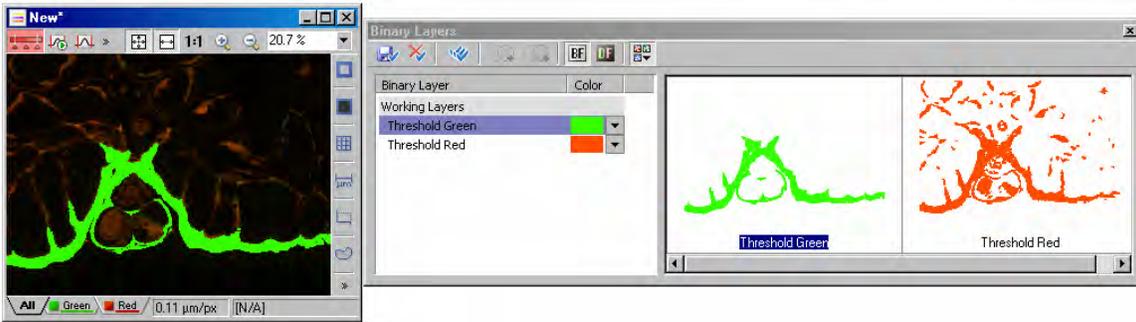
An image can contain several binary layers. Usually, a binary layer is associated with a channel in the image, but this is not mandatory.

If another channel is thresholded, another binary layer will be added to the image, and the Binary layers dialog box will be updated to reflect the new addition.



After the image was thresholded in the green and the red channels, both channels are visible in the **Binary layers** dialog box.

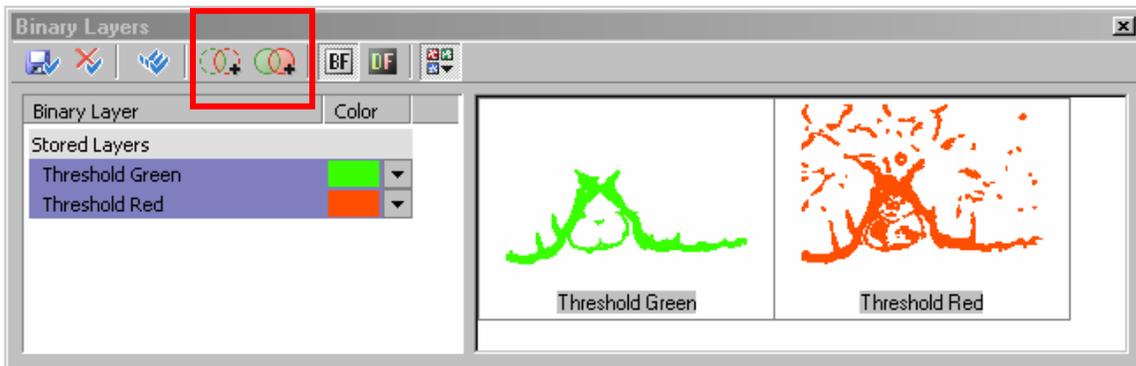




Users can select a specific binary layer by clicking on the layer name or the thumbnail in the **Binary layers** dialog box.



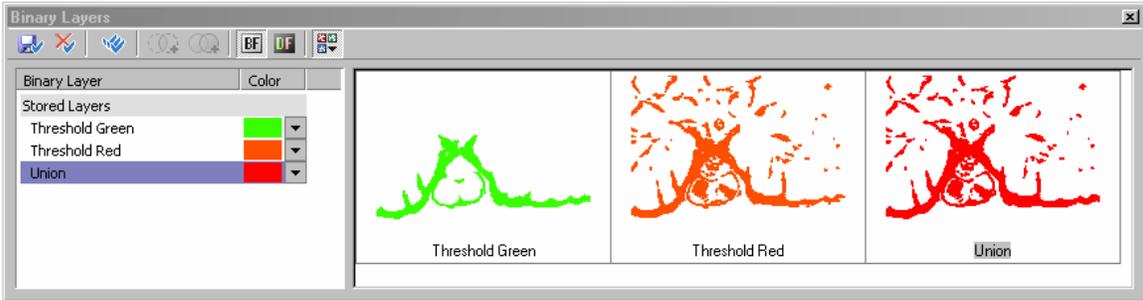
More than one binary layer can be viewed by CTRL+clicking on the layer name or the thumbnail in the **Binary layers** dialog box.



When more than one layer is selected, additional tools are available in the **Binary layers** dialog box: **Intersection** and **Union**.

- **Intersection** generates a Boolean AND as a new layer (only where the two layers both are present)
- **Union** generates a Boolean OR as a new layer (where either of the two layers is present)





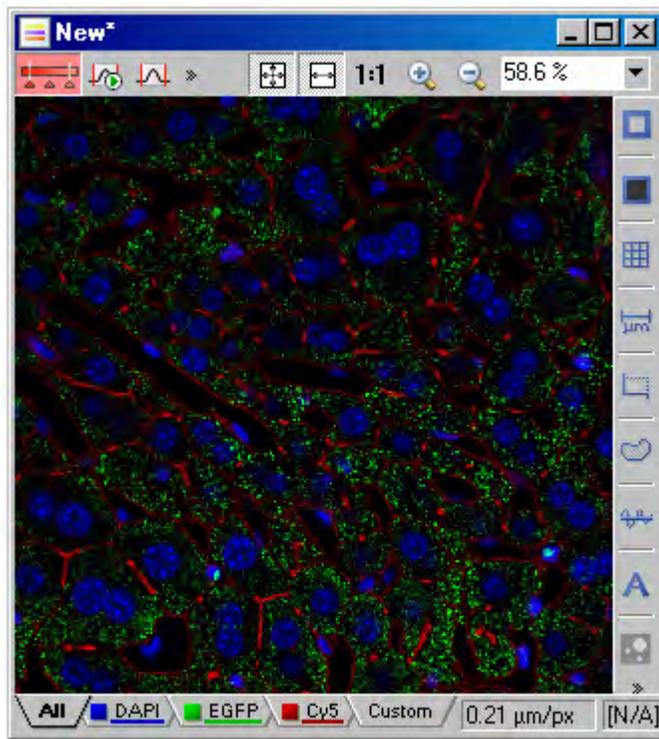
Selecting more than one layer, then clicking on the tool (above, Union was chosen) will generate a new binary layer.

Practical Applications of Binary Layers

The power of multiple binary layers is its use with multichannel images, and with images that have been processed using the *Pixel Classifier* or *Object Classifier* analysis tools.

Any binary layer (or group of binary layers, if more than one is selected) can easily be counted and quantified using *Object Count* or *Automated Measurement Results* analysis tools.

Here is an example walkthrough:

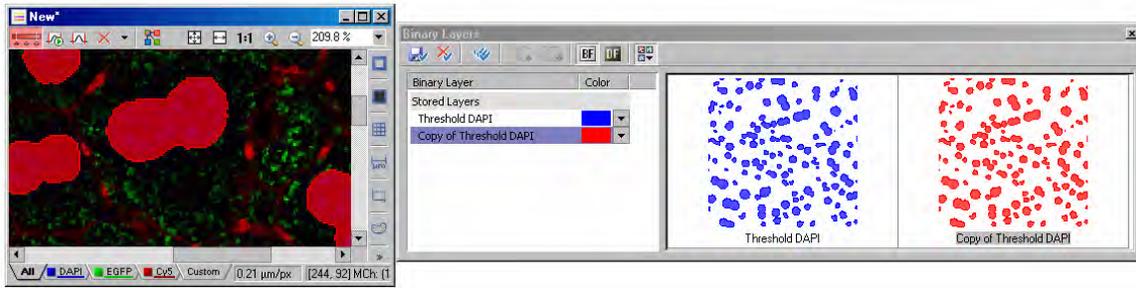


Binary layers can be used to help segment this data set.

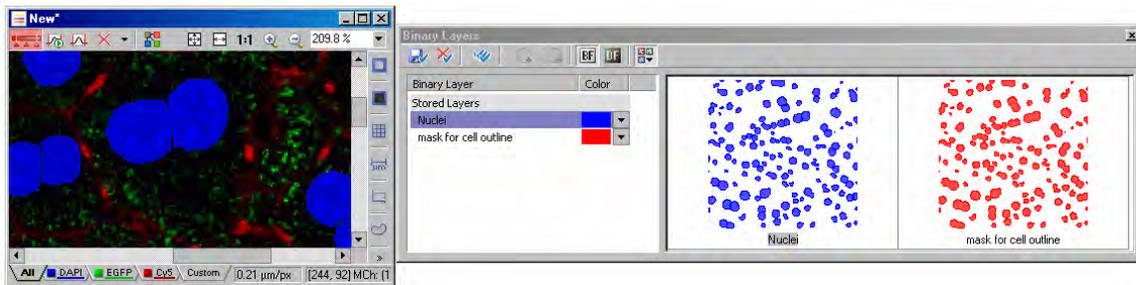
In this example, the number of green objects per cell is of interest.

Cell borders are not clearly defined, so binary tools can be used to help improve the contrast.



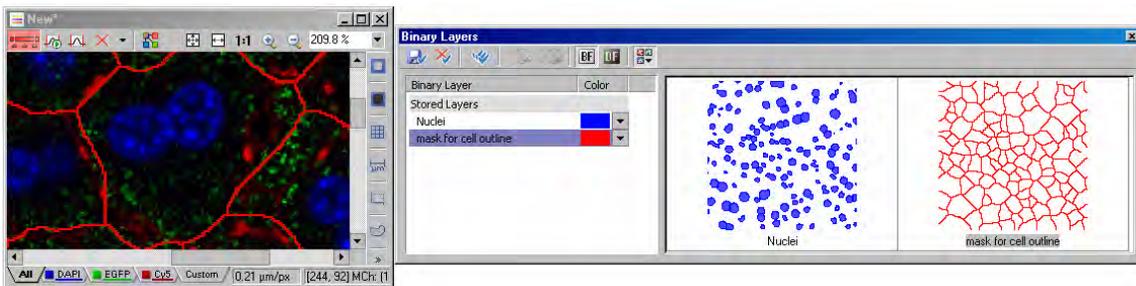


First the image is thresholded in the blue channel. Above, two copies of the same threshold are stored in the Binary Layers dialog box.



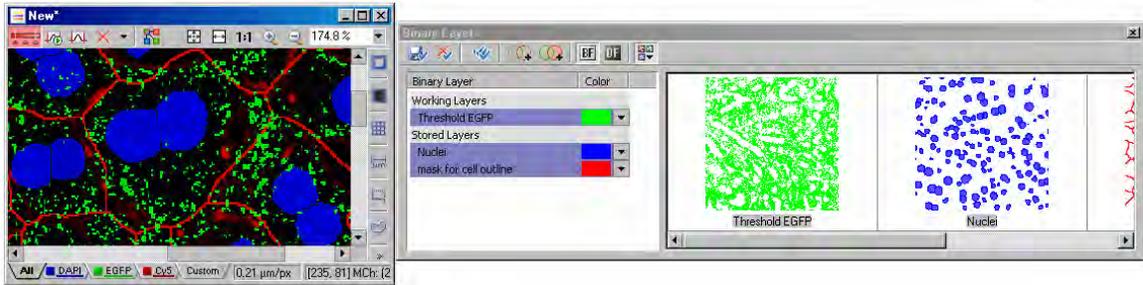
The stored layers are renamed: the blue layer will represent the nuclei. An additional tool, **Binary > Morpho Separate Objects**, was used to split touching nuclei in the blue layer.

The red layer will be used as a starting point to help create borders.

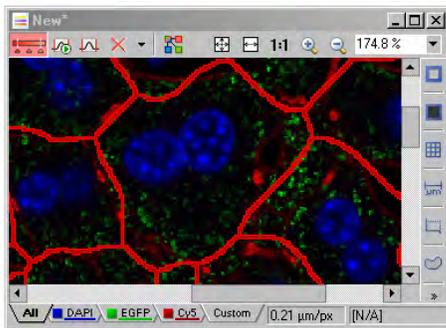


Using the red layer, predicted cell borders were made using the tool **Binary > Advanced Morphology > Zones of Influence**. This tool dilates the red layer until the objects are large enough that they almost touch, then inverts the selection. This method is oftentimes useful for estimating cell boundaries in tissues, when a nuclear marker is available (the Dapi/blue channel)



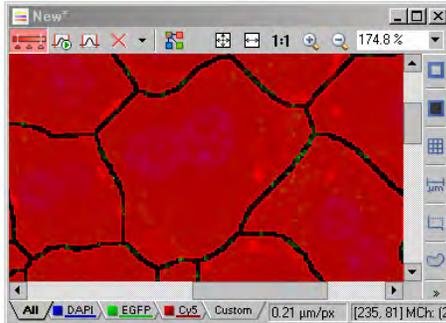


Now, the green layer is thresholded to select all of the green objects in the image. That layer is also now available in the **Binary Layers** menu. All binary layers can be selected, and their overlays seen in the image (selection was made by CTRL+clicking on the layer names, or the thumbnails)

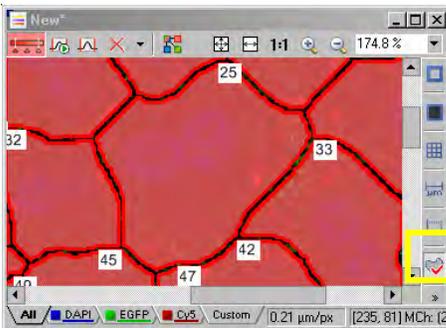


From the **Binary Layers** dialog box, the cell boundary layer is again selected (red). All other layers are deselected.

Because it is only 1 pixel wide, it is dilated to make the line slightly thicker using **Binary > Dilate**

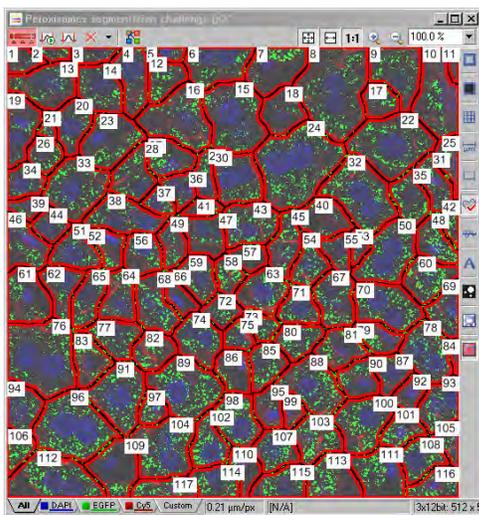


Because the area of interest is actually the cell, not the cell border, the binary layer must be inverted. Using **Binary > Invert**, this is easily done.



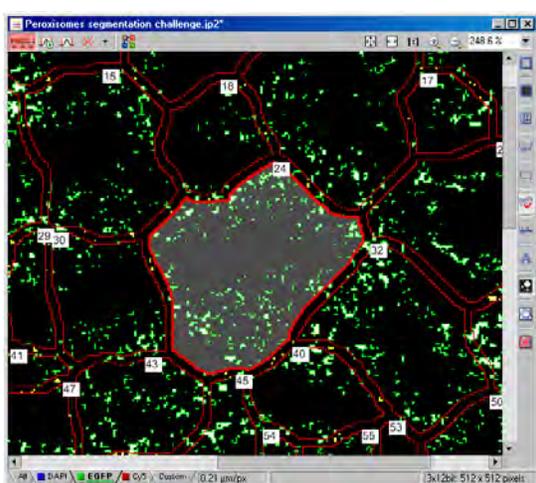
Finally, the binary area, now neatly divided into discrete areas, can be converted to regions-of-interest (ROIs). By selecting the **ROI tool** in the image window, and choosing **Copy Binary to ROI**, ROIs are drawn around each segmented binary part.





From the **Binary Layers** dialog box, now the red layer can be deselected, and the green layer now reselected.

Each cell is segmented by a region-of-interest.



Threshold Multichannel

Binary ID: 050

Channel: EGFP

Smooth OFF | Separate OFF

Restrictions: Area (µm²)

Equilateral In 0 ∞

Circularity In 0 1.00

Results

Total Area: 11102.75 µm²

Area Fraction: 7.88 %

5044

| ROI ID | Binary ID | # | Area | Perim |
|--------|----------------|-----|------|-------|
| ROI 1 | Threshold EGFP | 1 | 0.47 | |
| ROI 1 | Threshold EGFP | 41 | 0.13 | |
| ROI 1 | Threshold EGFP | 42 | 0.21 | |
| ROI 1 | Threshold EGFP | 90 | 0.04 | |
| ROI 1 | Threshold EGFP | 112 | 0.00 | |
| ROI 1 | Threshold EGFP | 117 | 0.04 | |
| ROI 1 | Threshold EGFP | 117 | 0.00 | |
| ROI 1 | Threshold EGFP | 146 | 0.17 | |
| ROI 1 | Threshold EGFP | 147 | 0.17 | |

NIS-Elements analysis functions can now be performed on the image, and counts of number of objects can be segmented by each region-of-interest (ROI) in the image.

In the example above, the Object Count dialog is shown, which incorporates and uses the binary and ROI information.

