NIS-Elements: Object Count

Automated Counting & Analysis with Object Count

In the Object Count dialog, NIS-Elements combines intensity thresholding, feature restriction and result/dataview in a simple concise interface. Object Count is designed for automated object detection and counting. Measurement results are easily exported.

This dialog is best used to address the following questions:
- How many objects are in this image or set of images?
  - i.e. Counting Nuclei, Cell Proliferation
  - Export data across an nd2 file as an option
- Using a multi-channel image, how many objects are expressing a TRITC probe, how many objects are expressing a FITC probe, how many objects are expressing both the TRITC and FITC probe?
  - This uses the Mutli-Channel thresholding option and the intersection or union function of thresholding options.
- How many objects are in this area (ROI) of the image vs. another area (ROI) of the image?
- How many round objects vs. elongated objects are in this image?
  - This uses the "restriction" portion of the dialog. There are 10 possible restrictions including shape features, intensity, area, length, etc.

Access to Object Count Dialog:

To open the Object Count dialog, right click on the desktop (not on the image). Select 'Analysis Controls' and then select 'Object Count.'
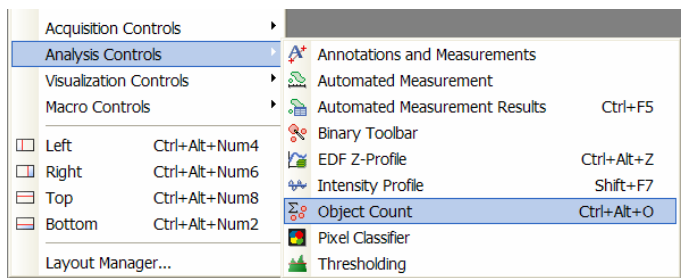
Note: The dialog can be docked (anchored) to the bottom or top docking panel. Double click on the top frame of the dialog to quickly dock the dialog to the bottom docking panel.
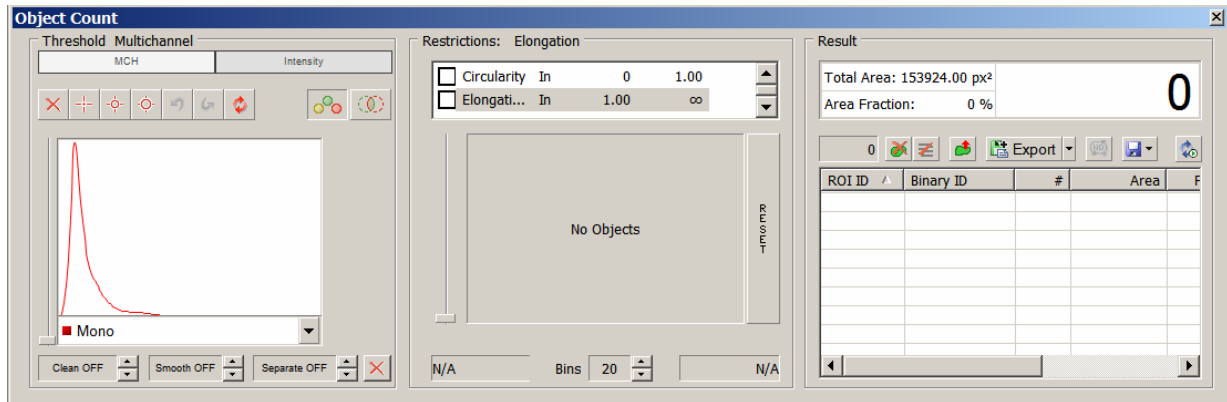
Object Count Components

The Object Count dialog has three components:

1) Thresholding   2) Restrictions   3) View of Results & Exporting

The recommended workflow through the dialog is to move from left to right, starting with thresholding, then using restrictions and finally reviewing the result and export out of NIS-Elements to a text file or an Excel spreadsheet.

Object Detection by Thresholding

Use the thresholding picking tools and click on the image to sample areas of interest. These pixels selected will determine what intensity/ part of histogram is considered accepted.

- The cross hair will sample 1 pixel for finer sampling.
- The small circle will sample 3 pixels for sampling more than 1 pixel.
- The larger circle will sample 6 pixels for sampling more pixels per click.
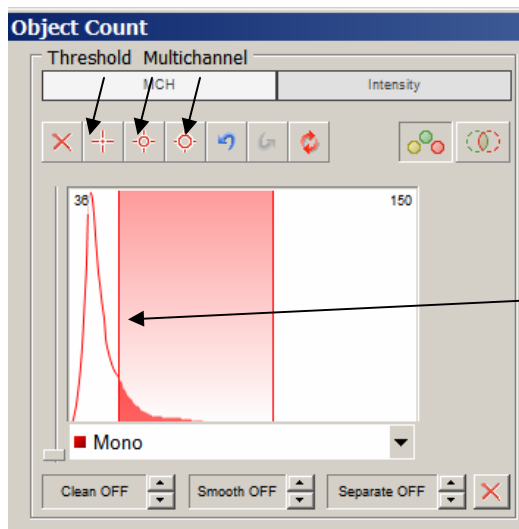
Thresholding is also possible by dragging the upper and lower limit on the histrogram to adjust what area is "accepted" for the threshold.

The threshold will "pick up" several specs or smaller objects such as noise or background. If desired, the 'Clean' and 'Smooth' filters will exclude smaller objects.

<u>Here is an example of how to use the picker tools on the image to create a threshold/ binary layer:</u>

1) Select the cross hair ⊞ tool.

2) Left click on the image over a pixel of the image that should be considered part of the threshold/ binary layer. Notice that once the mouse click is released, all pixels with similar intensity values of the image will also be highlighted. An outline will also display around the object(s) as well as the building solid colored threshold/ binary layer.
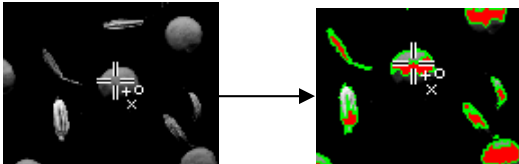
3) Continue to click on areas of the image that should be part of the threshold/ binary layer.

4) Alternatively, the small circle ⊙ and large circle ⊙ can be used to sample larger areas of pixels to build the threshold/ binary layer.

Another option for creating the threshold/ binary layer is to use the histogram.
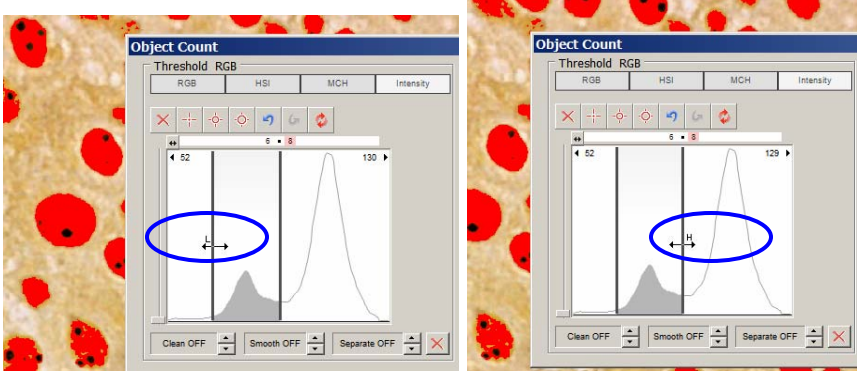
1) Mouse over the histogram and hover over the vertical line labeled 'L.' Use the vertical (low limit) to move the vertical line left or right to set the lower limit of the threshold. Use the image and its building threshold to provide feedback on whether the limit is set in the correct place. 'Accepted' areas of the histogram for the threshold will be colored gray.

2) Mouse over the histogram and hover over the vertical line labeled 'H.' Use the vertical (high limit) to move the vertical line left or right to set the upper limit of the threshold. Use the image and its building threshold to provide feedback on whether the limit is set in the correct place. 'Accepted' areas of the histogram for the threshold will be colored gray.

Another possibility is to also use the picker tools in combination of using the histogram to create the threshold layer.

Note: To change the color or transparency of the binary layer, click on the 'Overlay' icon in the image window:
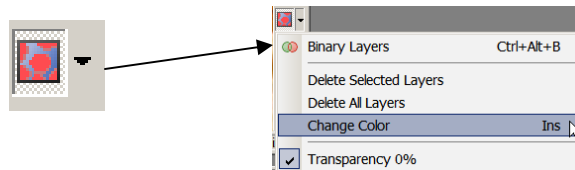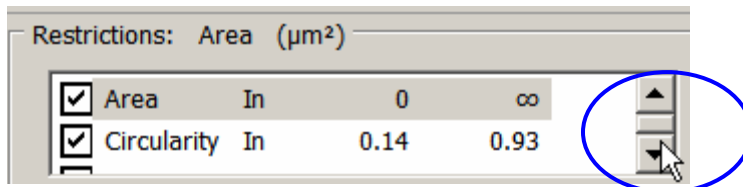
Object Count using Restrictions

Restrictions are used to specify certain object features to act as features for including or excluding objects within the image.

In the middle section of the Object Count dialog labeled 'Restrictions,' a list of features is available for creating a restriction(s). Right click on the list of features to see the available eleven measurement features. To select a feature (s), click on the feature and a blue check mark will display to the left of the feature.
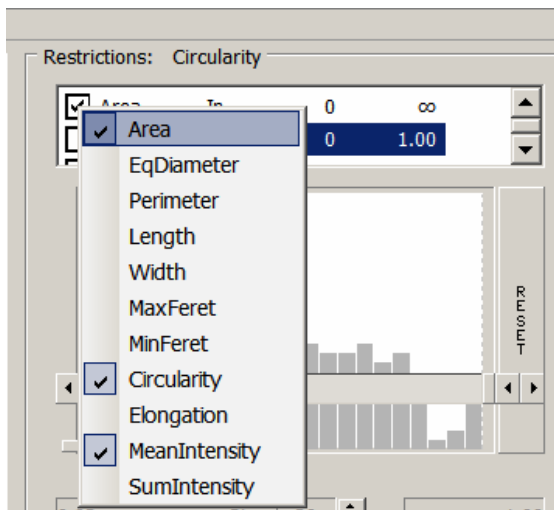

If more than two features are selected, the up and down arrows are available for scrolling through the list:

Here is an example of how to use the restrictions in the Object Count dialog:

1) Select 'Area' from the list of available restriction features.

2) Use the sliders in the histogram to specify the lower limit and the upper limit.
The lower and upper limit will update in the display. In this example, the lower limit is set to 13.29 and the upper limit is set to 38.11.
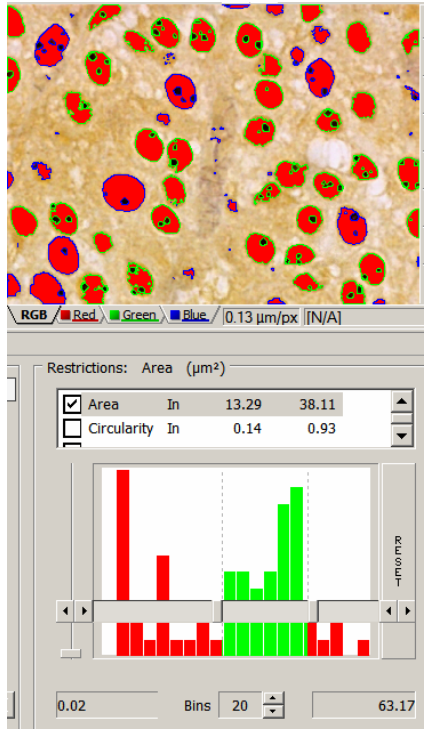
The green area of the histogram represents the "accepted" range areas. Any object with an area in the green range will be considered an object. Alternatively, any object with an area in the red ranges (s) will not be considered an object.

The objects on the image will result in differing outlines based on whether the object is restricted or accepted. In this case, accepted objects have a green outline and restricted objects have a blue outline.

Increasing/ decreasing the number of bins will modify the histogram. For example, this option for increasing can be useful for narrowing the sections of the histogram; this will allow for more sensitivity in isolating a specific section of the distribution. This helps to detect small differences between objects
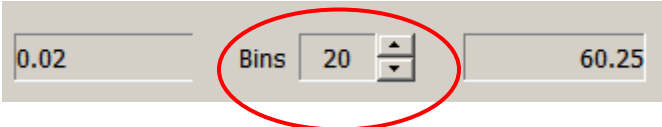
In this example, the value to the left (0.02) represent the lowest area value in the distribution of the objects in the image. The value on the right (60.25) represents the highest area value in the distribution of the objects in the image.

3) An additional method to use restrictions is to use the objects in the image as indictors to set the included/ excluded objects. Simply right click on a thresholded object. In this example, 'Area' is the highlighted restriction in the restriction section of the Object Count dialog. Right clicking on the object displays four options for including/ excluding objects with higher or lower areas than this object.
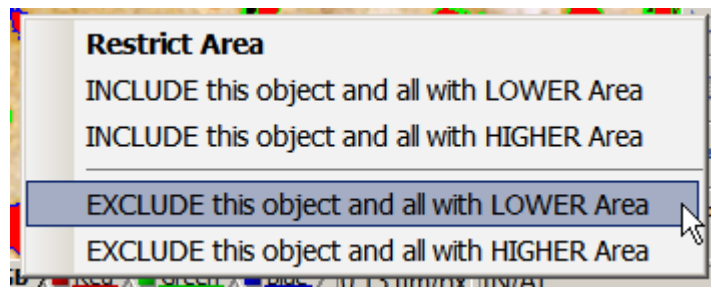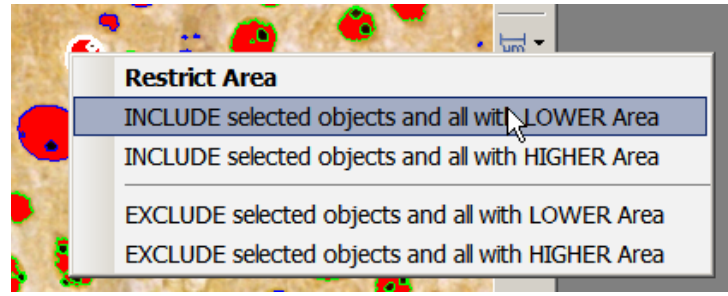

Note: Changing the active restriction feature in the restriction section in the Object Count dialog will display options for including/ excluding objects with higher or lower of the selected feature.

While using the restrictions either through the use of the histogram or right click on the image, the object count in the upper right hand of the dialog will update with the changes.

It is also possible to utilize more than one restriction at a time to assist in narrowing down the selected objects. For example, it is possible to use 'Area' and 'Mean Intensity' restrictions to find large objects with low intensity.

To ensure that that count is updating with any change, click this icon: .
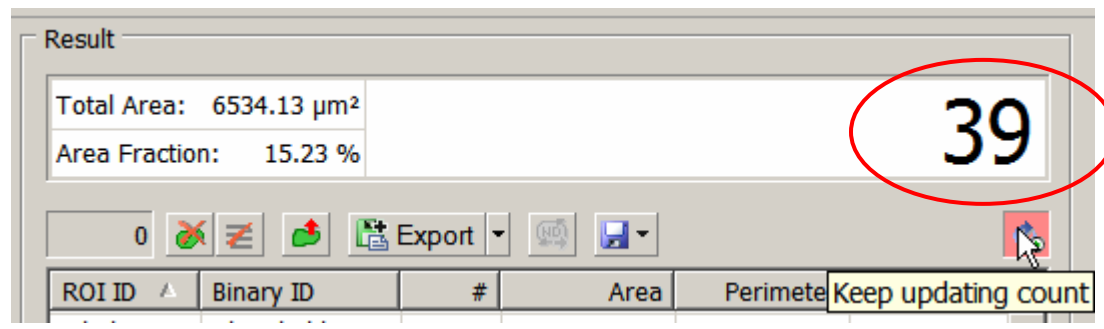
Result Section of the Object Count dialog

The Result section displays the measurement features, area of the image, the area fraction (% of image that the objects in the image occupy) and the current number of objects. The result section also includes export options to move the results to either a text file or an Excel spreadsheet.

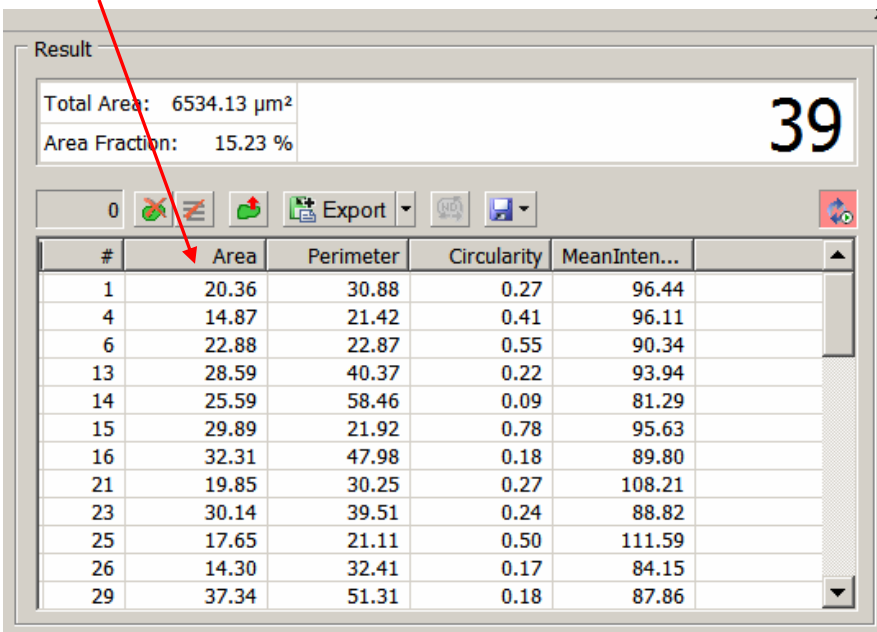### Removing/ Deleting an unwanted object

Delete an object from the object count by selecting the icon from the Result Section. The object will lose its thresholded overlay and also be ignored in the result spreadsheet view.
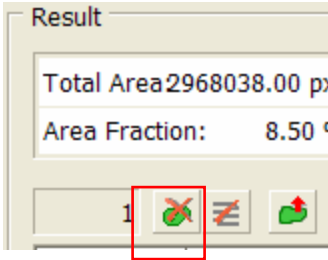
> *Note: You cannot restore this object by performing an 'Undo' function. To restore an object after performing an 'Undo,' repeat the original threshold procedure (or load a saved Object Count configuration (described on following page of this document)).*

Sorting the Values in the Result Section

The columns of the results section area also sortable. Click on the top of a column such as 'Area' and the data will sort in ascending or descending order (toggling/ clicking again will alternate the order).

Result

| Total Area: | 6534.13 µm² | | | **39** |
| Area Fraction: | 15.23 % | | | |

| #	Area	Perimeter	Circularity	MeanInten...	
1	20.36	30.88	0.27	96.44	
4	14.87	21.42	0.41	96.11	
6	22.88	22.87	0.55	90.34	
13	28.59	40.37	0.22	93.94	
14	25.59	58.46	0.09	81.29	
15	29.89	21.92	0.78	95.63	
16	32.31	47.98	0.18	89.80	
21	19.85	30.25	0.27	108.21	
23	30.14	39.51	0.24	88.82	
25	17.65	21.11	0.50	111.59	
26	14.30	32.41	0.17	84.15	
29	37.34	51.31	0.18	87.86	

> *Note: It is possible to add additional measurement features beyond those selected from the Restriction section. Right-click on the column headers and select from the available features.*
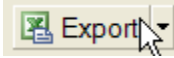
Exporting data to Excel spreadsheet or a text file:
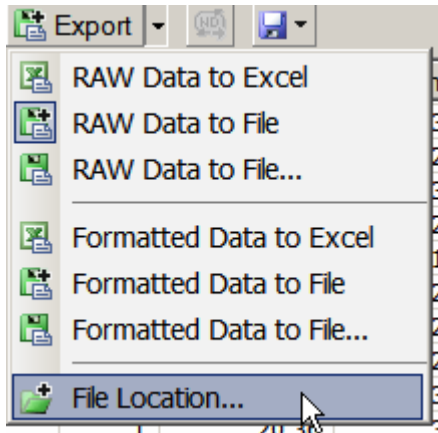Select the format to export:
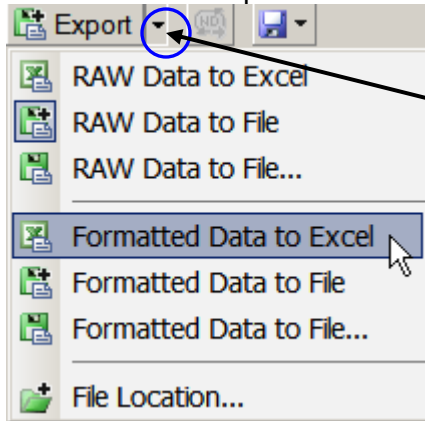
Click the upside down black arrow to view the drop down menu of export options.
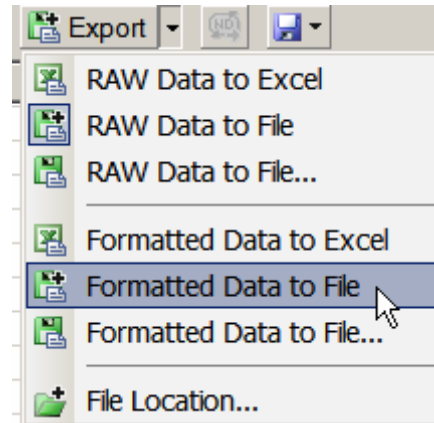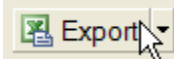
Click on the Export icon:

To export to a text file, first specify the file location:
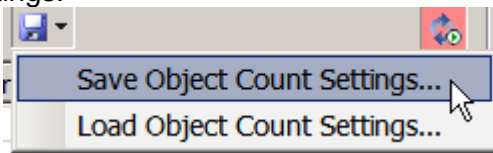

Then specify to export to a file:

Click on the Export icon:

Saving/ Loading the Object Count configurations:
It is possible to save or load the settings from the Object Count configuration to use at a later date or transfer to another system. This configuration includes the threshold and restrictions settings.
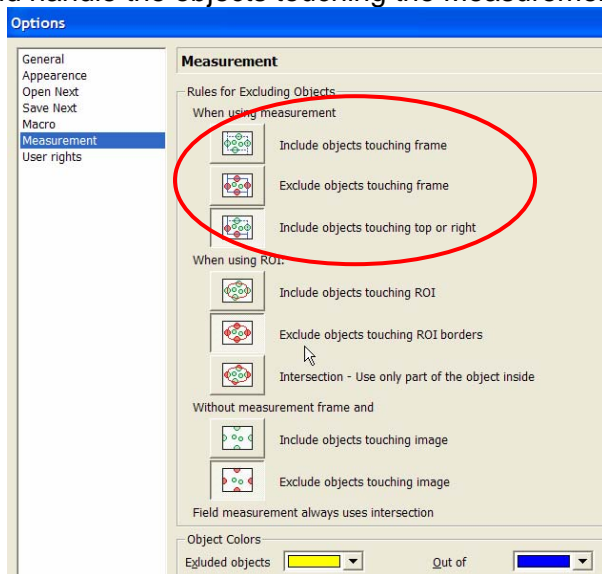
**Other tools that work in conjunction with the Object Count Dialog**

The Measurement Frame and Region(s) of Interest (ROIs) are also compatible with the Object Count dialog.

Using the Measurement Frame

In general options (Edit Menu-→ General Options), it can be can specified how NIS-Elements should handle the objects touching the Measurement Frame.

To use the Measurement Frame, access the frame from the Image Window.
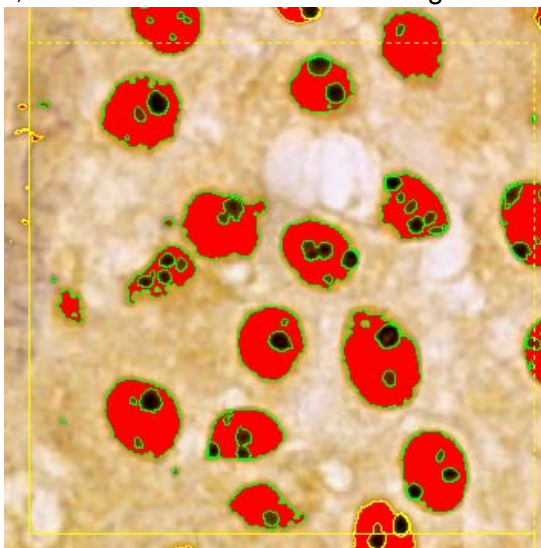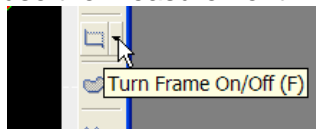
In this example above, green objects are accepted and yellow objects are excluded because 'Include objects touching top or right' is selected in the Measurement section of the General Options.
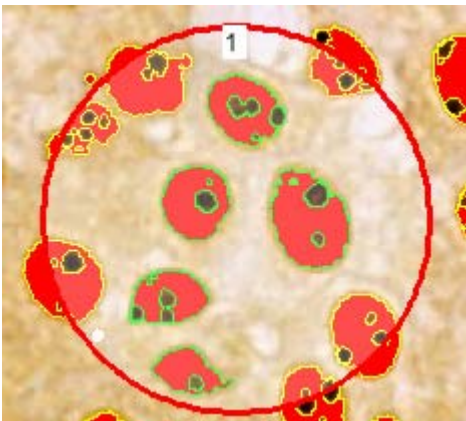
Using the ROIs[1]

In general options (Edit Menu-→ General Options), it can be can specified how NIS-Elements should handle the objects touching the ROIs in the image.

In this example above, green objects are accepted and yellow objects are excluded.

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[1] Please reference document 'How To' –Region of Interest_ROIs_0001.pdf for more detailed information and step by step instructions of using ROIs in NIS-Elements.