NIS-Elements: Binary Masks and Regions of Interest: The Pillars of Quantitative Imaging Analysis

Many basic quantitative imaging analysis questions can be addressed with two functions: the creation of a binary masks and regions of interest (ROIs). These operations are available in most quantitative imaging packages. Screenshots in this note are captured from Nikon's NIS-Elements Advanced Research package.

Note number: 0011
Date: 2/2010
Date Modified: 2/2010
Software Version: 3.1 SP2
Package: AR
Modules: 2D tracking

<u>What is the image analysis question?</u>
Common image analysis tasks that can be easily tackled with binary masks and ROIs are the following: What percentage of probe A is in the treated vs. control condition? What is the change in probe A overtime or due to an antagonist? What is the intensity of probe A in various cells that move and change shape over time? What is the percentage of three probes across the tissue sample? What is the percentage of image that contains both probe A and probe B?

In all of these cases, the binary mask and the ROI are the main tools for the solution.

<u>What is a binary layer or mask?</u>
Every digital image is a collection of pixels with varying level of gray values, representing the signal of the background and the signal of the sample.
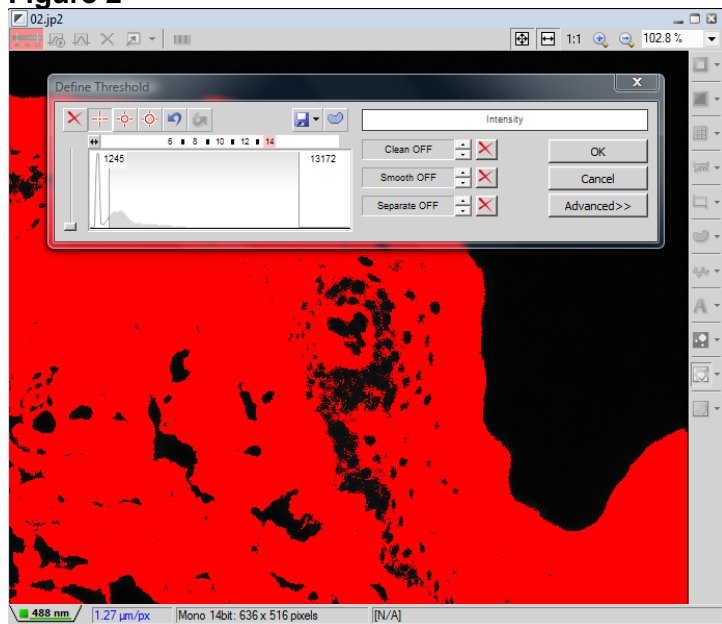
Figure 1
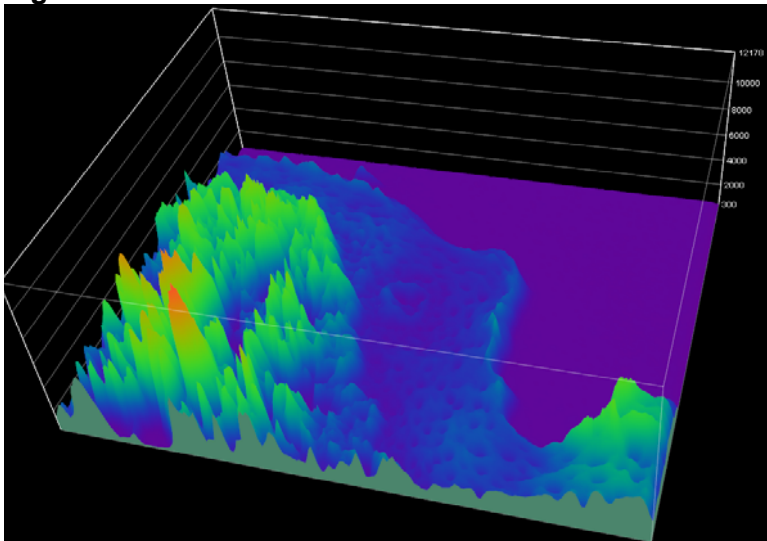
In Figure 1, the background intensity level is represented in purple and the signal is in the blue/ green/ red shades.

For image analysis, it is critical that the intensity of interest/ signal is delineated. In some cases, it may also be needed to delineate the background as well. In order to "mark" or

input to the software what portion of the intensity histogram is of interest, we will create a threshold, which will create our binary mask or layer.
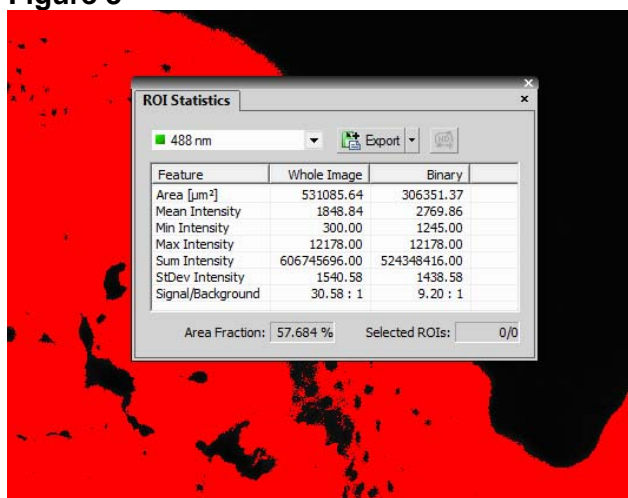
Figure 2

In Figure 2, the area of the histogram that is gray is the range of intensity values from the image that are determined to be of interest for the analysis question. Further image analysis steps will use this masked area.

Once a binary layer (mask) is created, several options "open" up in image analysis software. Now, it is easily to retrieve intensity statistics of the thresholded area. As seen in Figure 3, area, intensity measurements and area fraction (percent area) are all accessible and exportable. In addition, many measurement features can be calculated across the binary layers.
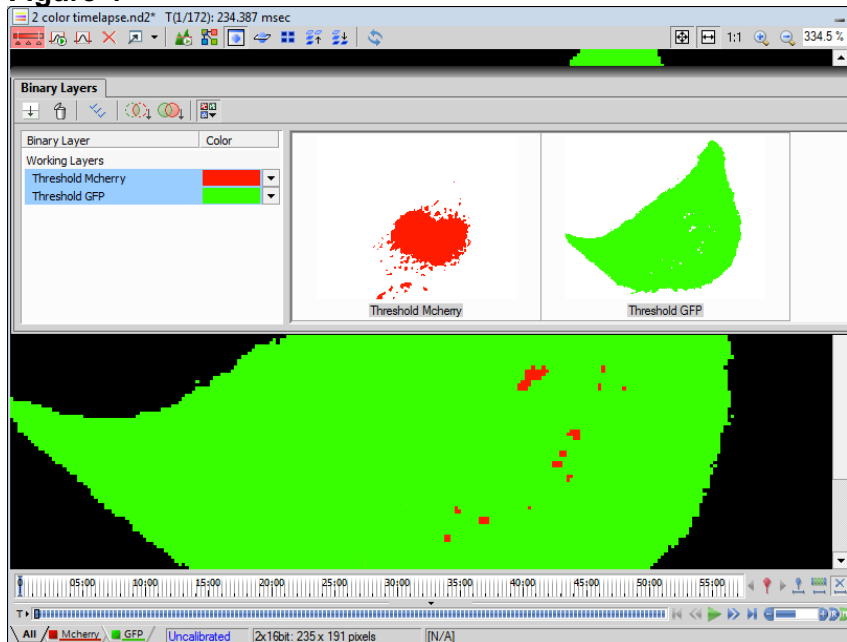
Figure 3

In the case of a dataset that has two channels overtime, creating multiple binary layers is also possible. In Figure 4, two differing binary layers were created over the timelapse dataset: one for the red channel and one for the green channel.

Figure 4

Once multiple binary layers are generated, the binary operations can also been added to the analysis. In this case, we will assume the question is 'What is the percentage of the image where red probe and green probe are both present?" The intersection of the red binary layer and the green binary layer will identify where a pixel is both in the red channel and the green channel. As shown in Figure 5, the intersection binary layer yields an AreaFraction of 10.5%. The intersection of the two original binaries can also be run over time to examine how this percent area changes over or across stage positions or Z positions.
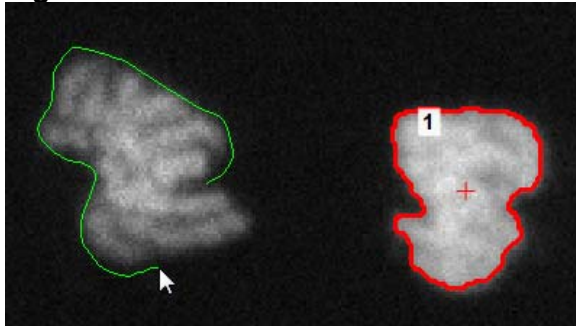
Figure 5
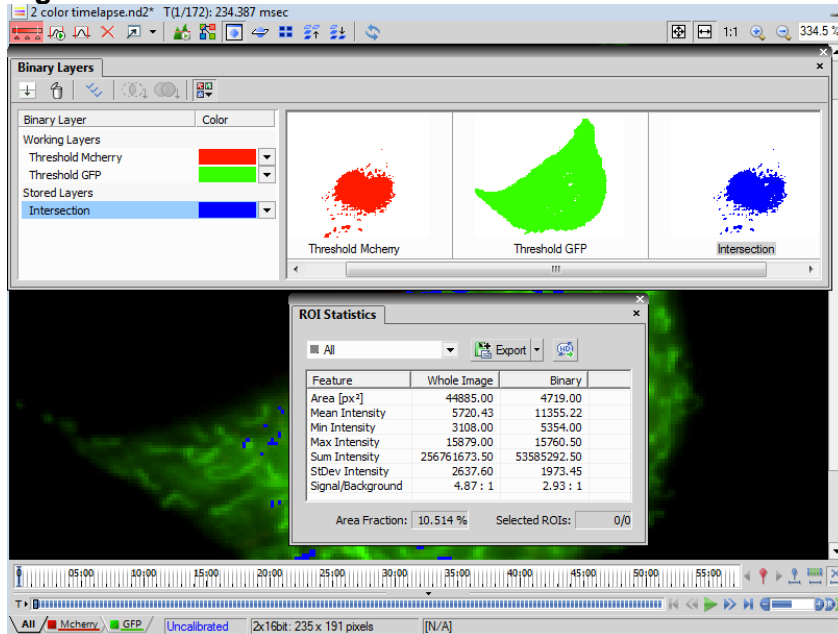
Adding ROIs into the image analysis solution

Building onto the binary layers, ROIs specify the specific area of pixels of interest from an image or a multi-dimensional dataset. In biology, many ROIs are hand drawn as opposed to very perfect circles or square shapes.
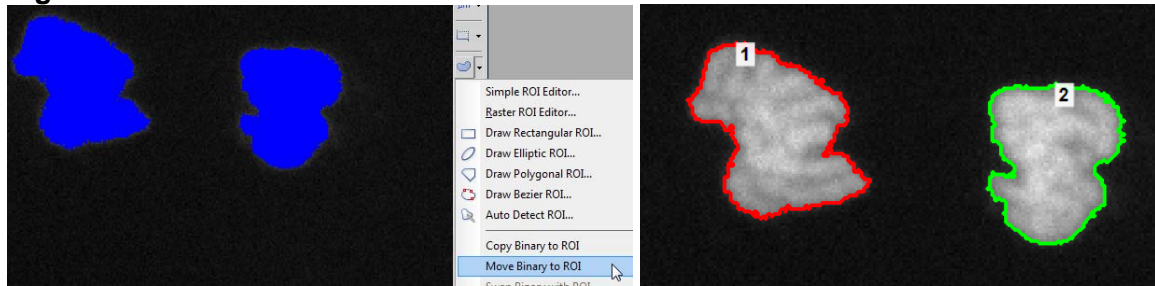
Figure 6

However, there are several tools that help in automating the ROI creation.
Binary layers and ROIs are linked very closely in some software. Within NIS-Elements, it is very convenient to move or copy the binary to ROI. This uses the thresholded area and creates the ROI around the object.

Figure 7

As before when the binary layer was created, the ROI creation also "opens" up several other automated measurement options. Revisiting a previous dataset, with an ROI created from the binary layer, it is possible to now answer "What is the percentage the cell has both the red and green probe at each pixel?" Using the both the binary layer and the ROI provide a method to determine that 25.3 % of the cell has pixels with both the red and green probe.
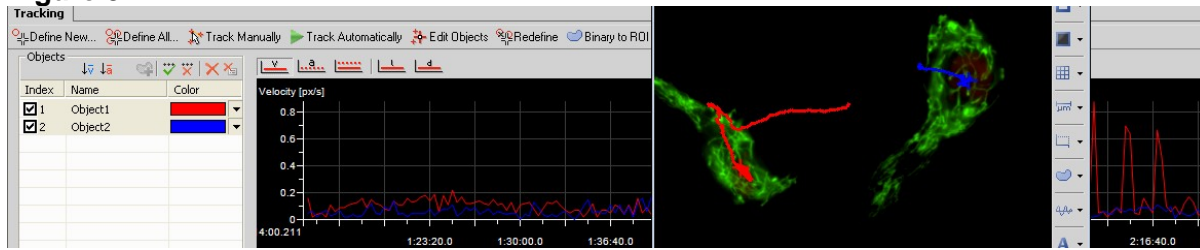
Figure 8
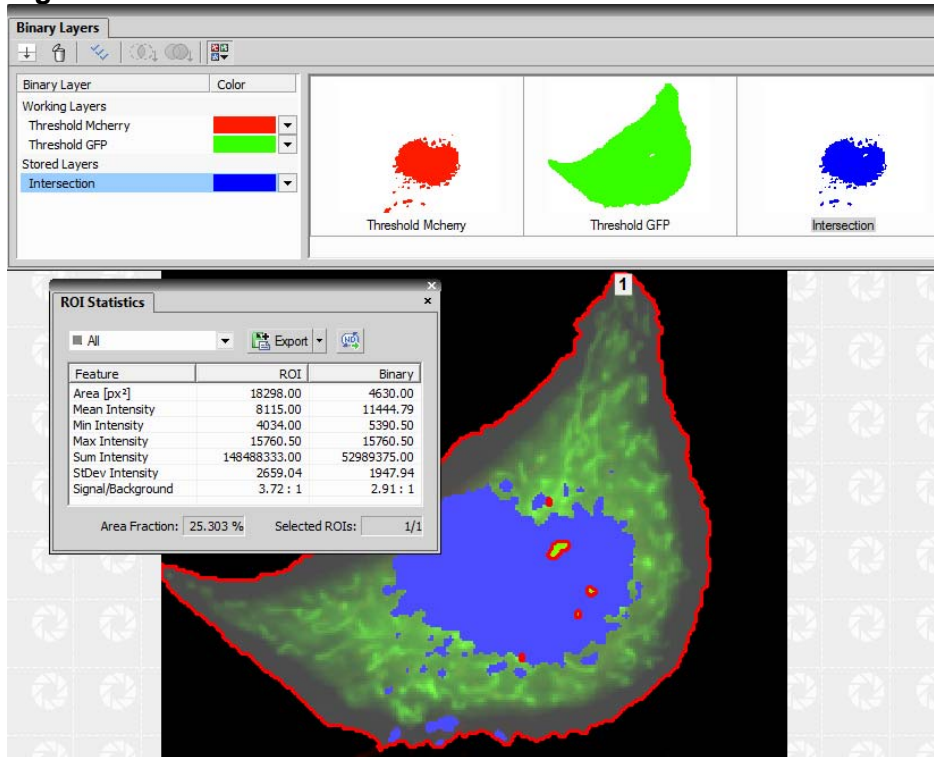
Many cells (or ROIs) naturally move, change shape, and change intensity over time. NIS-Elements software also has tools for linking ROIs with 2D Tracking.
NIS-Elements tracks the cells or objects. It is then possible to create a binary layer or ROIs using the tracked data as shown in Fig. 9. The ROIs will change shape and size accordingly over time in order to more accurate and efficiently capture the quantitative information.

Figure 9

More examples of the varied use of ROIs

In the case of a calcium wave across the cell over time, ROIs assist in intensity quantification over time.

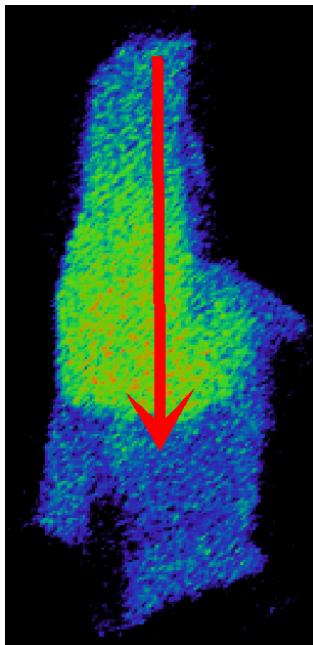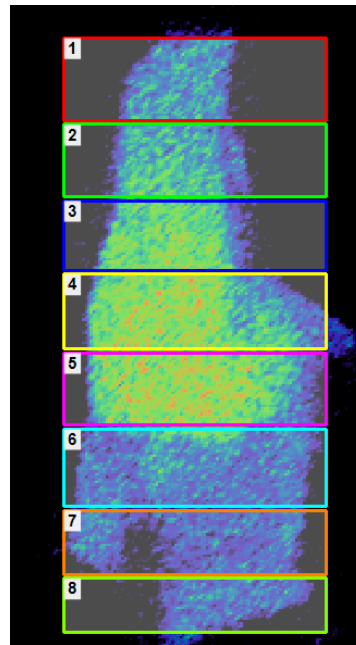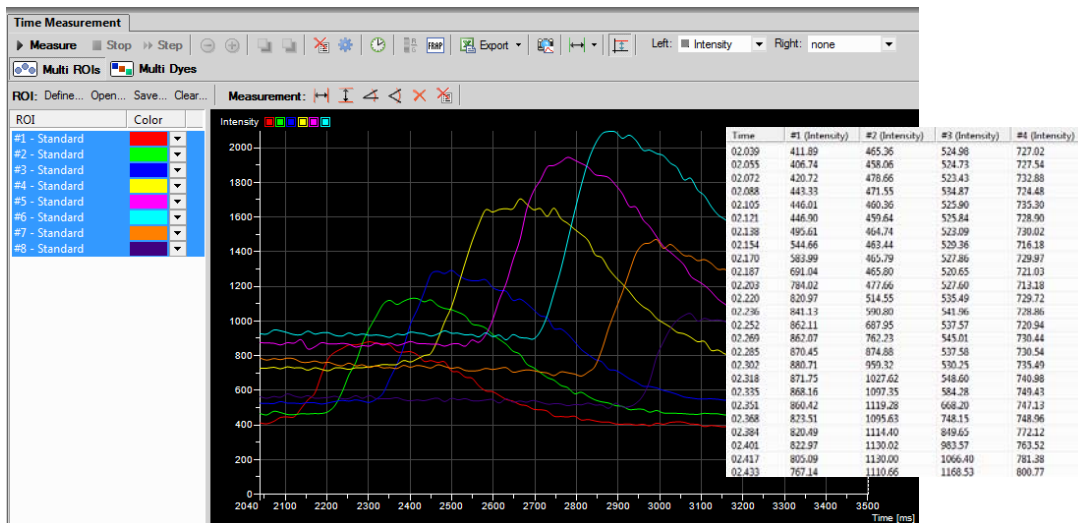**Figure 10a**   **Figure 10b**

When ROIs are drawn side by side on the dataset and analyzed over time, quantification of a fast, fleeting event across sections of the cell is possible.

Figure 11

In the case of a sample or colony that grows and expands over time, using concentric circular ROIs can help determine areas and intensity.
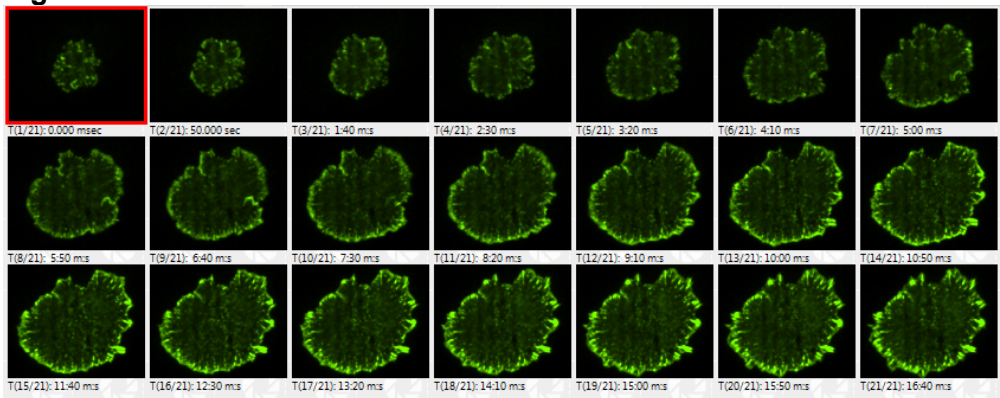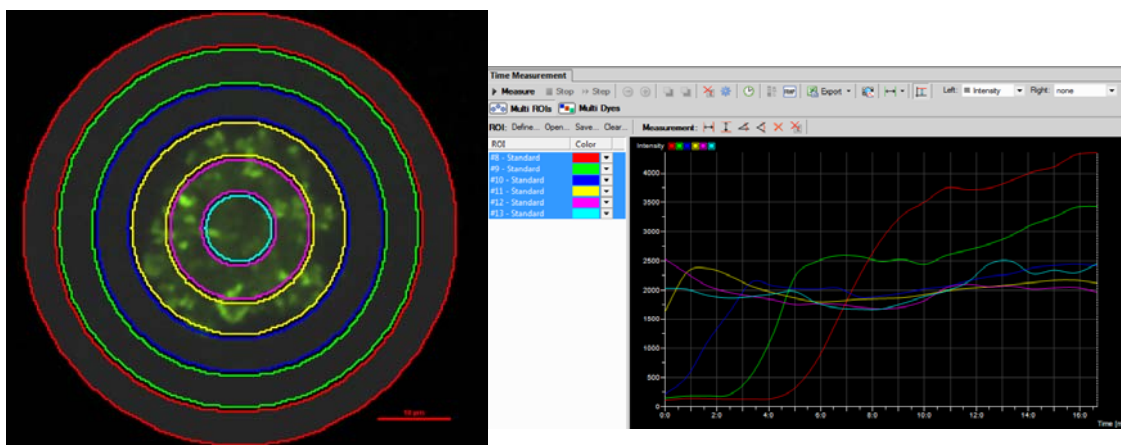
Figure 12

Figure 13

A variety of quantitative image analysis solutions utilize the binary mask and ROIs. For more details on either binary masks or ROIs and use with other software functions, there are several NIS-Elements 'How To' technical notes that are available.