

## ND Processing Tools in NIS-Elements

### Overview

This technical note describes basic uses of the **ND processing** tools available in NIS-Elements. These tools are specifically designed for arithmetic functions on multi-dimensional data sets. *All of the ND processing tools require an ND data set (more than 1 frame) to be used.*

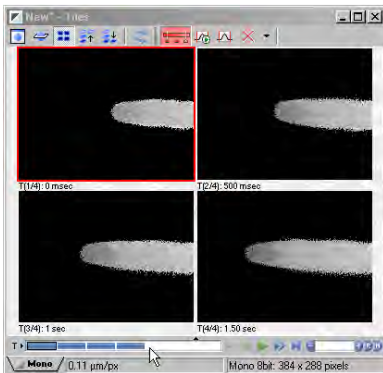
Note number: 0003  
 Date:8/2008  
 Date Modified: 8/2008  
 Software Version: 3.0 SP3  
 Package: AR Only

ND Processing tools can be found in the **Image** pull-down menu, in a subcategory labeled **ND Processing**.

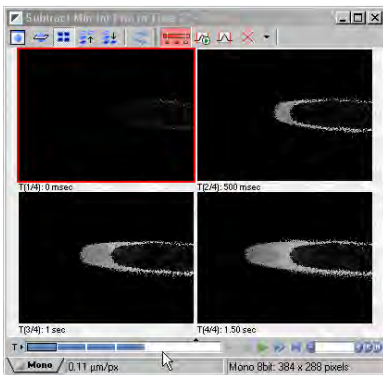
### ND Processing Tools – Subtracting from Min or Max projection in Time or Z

The first set of ND processing tools allows the user to process time or Z stacks by subtracting either the maximum intensity projection or minimum intensity projection from the stack.

A typical use-case scenario for subtracting projections in time would be to assess growth or movement:



In this example, a time lapse was acquired of a pollen tube growing over time. The Tile View was selected in order to see all 4 time points side-by-side.



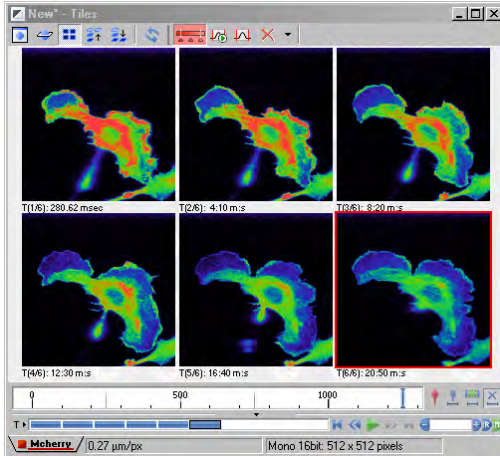
Applying ND Processing > Subtract minimum intensity projection in Time subtracted the minimum intensity projection of the dataset from each frame of the data set. The result, shown in Tile View, is only the area of growth remains.

Now the amount of growth per frame can easily be determined by thresholding and ROI statistics.

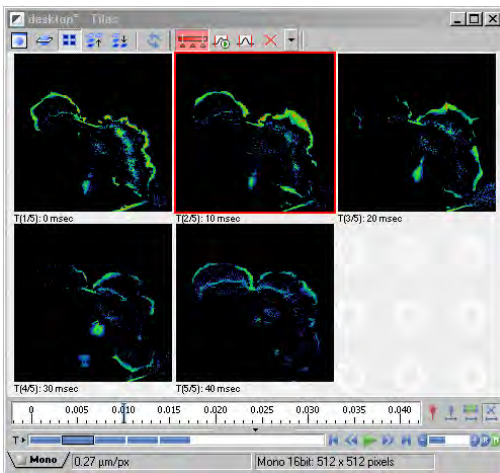


## ND Processing Tools – Difference of subsequent frames in Time or Z

This ND processing tool allows a user to visualize only the pixels that have changed between successive time or Z points, which makes this a useful function to assess morphological change, growth, or movement.



Here, a time lapse dataset is shown in Tile View and the LUT is pseudocolored to improve contrast.



Applying the ND Processing tool **Difference of subsequent frames in time** creates a new window with 1 less image than the original data set (because the function subtracts subsequent frames).

In Tile View, the time lapse is shown at left. The result images only show the parts of the image that changed from one frame to the next. A pseudocolor LUT was again used to enhance contrast in this example.



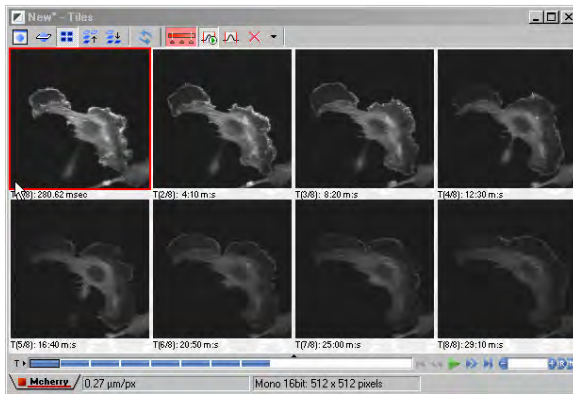
## ND Processing Tools – Equalize Intensity in Time or Z

These ND processing tools are provided to create a *qualitative* result data set from an input data set that may have fading, intensity change, flickering, or other illumination issues that make the data set difficult to visualize.

Equalize intensity calculates the intensity range and statistics of the entire data set, then *nonlinearly* adjusts the each frame of the data set to set a common level of intensity throughout the data. Typically, this is used for purposes of display or presentation.

### Equalize Intensity in Time

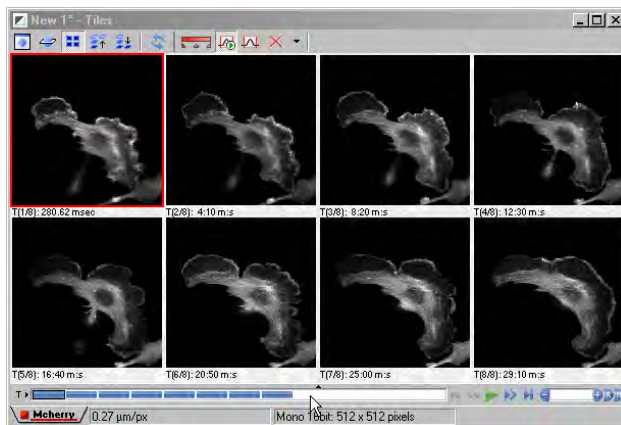
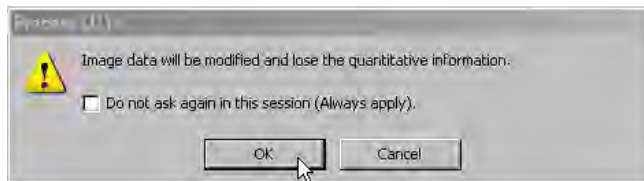
A common example is a rapidly fading specimen:



Here, a time lapse data set is displayed in Tile View so each frame can be visualized.

The specimen rapidly fades. However, our interest is the movement of the specimen rather than its intensity, so we would like to equalize the intensity of all frames.

Applying an intensity equalization is a nonlinear technique, so the user is prompted with a warning before proceeding:



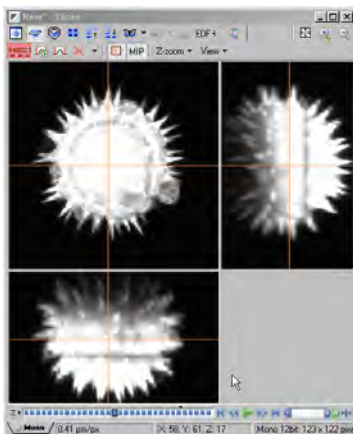
After equalize intensity in time, the data set has a constant intensity applied to it, so the fading is not apparent and the morphology is easy to distinguish throughout the time lapse.



## Equalize Intensity in Z

A common example is a confocal Z series where illumination is attenuated as the sectioning is deeper and deeper into the specimen.

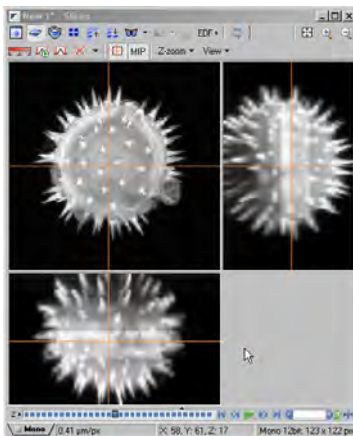
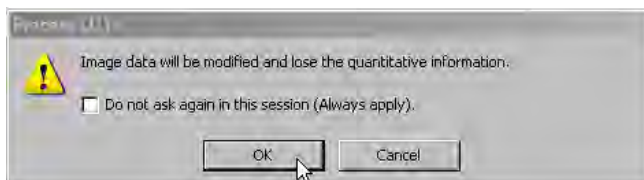
Equalize intensity calculates the intensity range and statistics of the entire data set, then *nonlinearly* adjusts the each frame of the data set to set a common level of intensity throughout the data. Typically, this is used for purposes of display or presentation.



Here, a Z series data set through a pollen grain is displayed in Slice View.

It can be seen that the section intensity decreases as the Z section goes deeper into the specimen.

Applying an intensity equalization is a nonlinear technique, so the user is prompted with a warning before proceeding:



After equalize intensity in Z, the data set has a more constant intensity applied to it, so the fading is not as apparent and the morphology is easier to distinguish throughout the Z series.

Some fading is still apparent, because there was simply no signal to collect at very deep parts of the specimen.

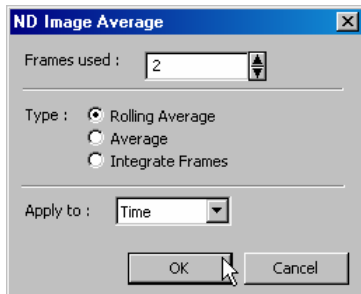
In this specimen, there are also positive results in equalizing the bright intensities – the data set is much sharper looking and more fine details are apparent.

**NOTE:** It is oftentimes useful to perform **Background Subtraction** on a data set *before* applying **Equalize Intensity** algorithms, so that background intensity is not included in the calculations.



## ND Image Average

This ND Processing tool allows the user to perform post-acquisition frame averaging or frame integration to a data set. This is oftentimes useful to suppress noise, create images to use for later background or shading correction, and various other uses.



**ND Image Average** Dialog Box options:

**Frames used** is the number of image frames to use in the average

### Type of Average:

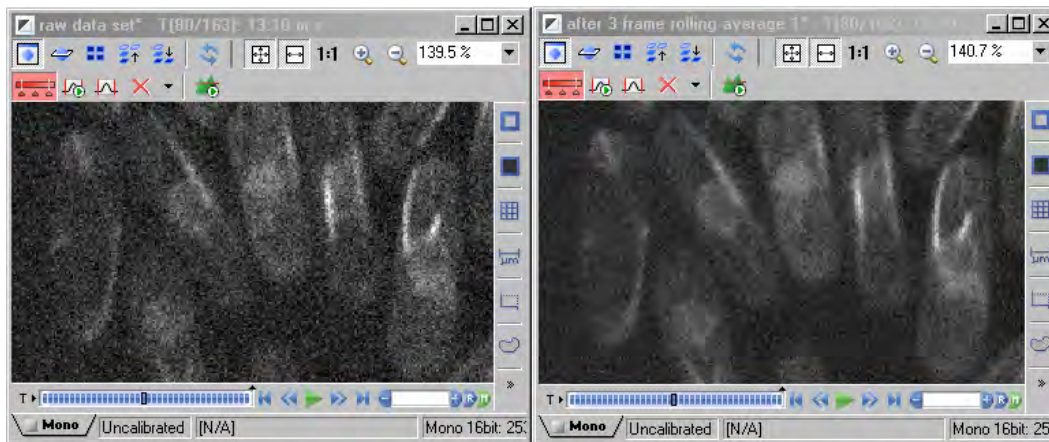
**Rolling average** – end result has same number of frames as the input data set. (Averages frame 1+2, 2+3, 3+4, etc.)

**Average** – end result has fewer frames than the input data set. Computes the mean value of all of the frames used. (e.g., frames used = 5; an input data set of 100 frames will result in an averaged data set with 20 frames). Averages frames 1-5, 6-10, 11-15, etc.

**Integrate Frames** – is not averaging at all, but rather sum of the input number of frames used. The end result has fewer frames than the input data set.

**Apply to** allows the user to choose which dimension of an ND data set to apply the calculations to (in the case of a data set with Z and time)

Example: Suppress noise in a low light time lapse. Using rolling average of 3 frames, quality of image is improved.



## ND Image Arithmetics

ND Image arithmetics perform the same arithmetic functions as *Image Arithmetics* (found in the References menu), only they allow the arithmetics to be performed frame-by-frame with ND data sets.

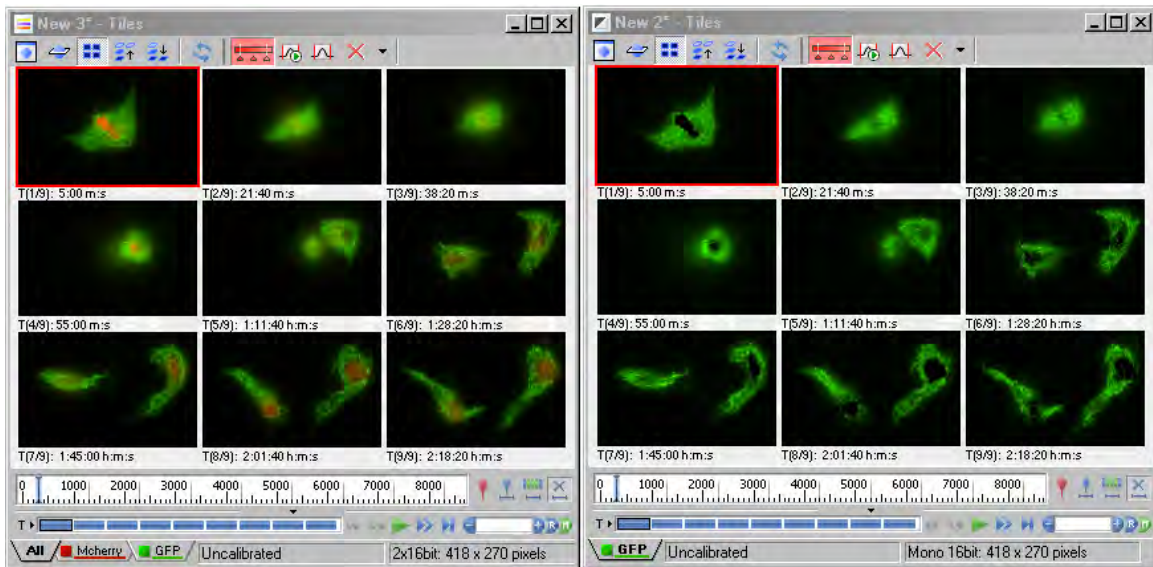
Simple image arithmetic allows a user to add/subtract intensity values taken from a single image on an ND data set. However, if a user wished to use a new set of intensity values for each frame of an ND data set, simple image arithmetic would not allow this.

**ND Image Arithmetics** does allow “updating” values from frame to frame in an ND data set. Some common examples of use would be:

- Acquisition of multichannel data in time or Z, then a user wishes to subtract the value of one channel from the other channel at each time point.
- Using two Z series data sets, and creating a result data set that displays the maximum intensity of the two input data sets at each plane.

Example: a user wishes to subtract the nuclear stain intensity (mCherry in the example below) from the cytoskeletal channel (GFP).

**ND Image Arithmetics** allows the subtraction of one channel from the other, but also updates this arrangement over time.



Original two channel data set

After creating a separate ND files for each Channel, ND Arithmetic was used to subtract the mCherry channel across the entire time lapse data set.

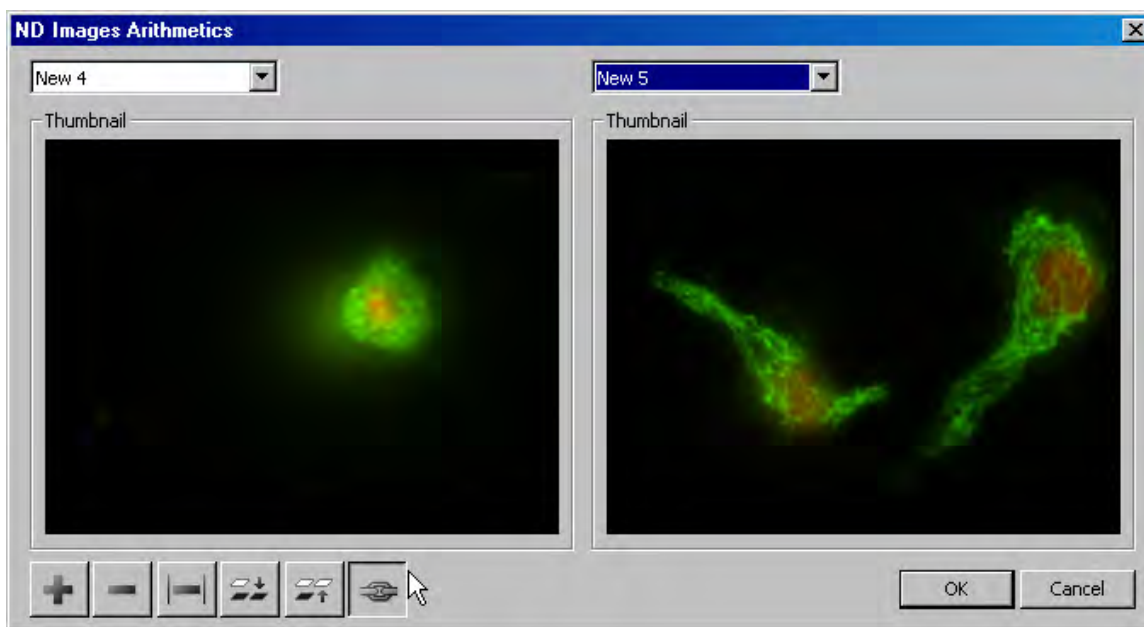


## ND Image Arithmetics, continued

Another function of ND image arithmetics is to concatenate (append) two ND stacks and produce a single result ND file.

This is commonly used to rebuild time lapse data sets, for example, those acquired by RAM capture, where the user has acquired 2 separate RAM capture timelapses in succession, and wants to later combine them into a single ND2 file.

2 input data sets at a time can be connected to equal a resultant ND data set.



Above is an example of using the ND Image Arithmetic dialog to combine (concatenate) two stacks together. The two component ND files must have the same dimensions in XY and Z in order to be successfully joined. Choosing the icon of the linked chain allows the two stacks to be combined.

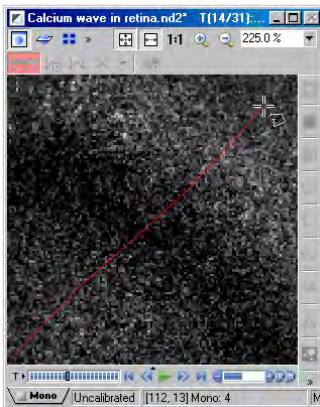
Note: If more than two stacks need to be combined, use the ND Image Arithmetic dialog multiple times ( $A+B = C$ , then  $C+D = E$ , then  $E+F = G...$ etc).

## ND Processing – Create Kymograph by Line

A **Kymograph** refers to an orthogonal (slice) view of a *time lapse* data set. This orthogonal view can represent one XY point vs. time, or changing XY positions vs. time. A common usage of kymograph images is to visualize the velocity of moving objects over time. Kymographs are particularly useful in determining velocities of objects that are difficult to track by automated tracking techniques.

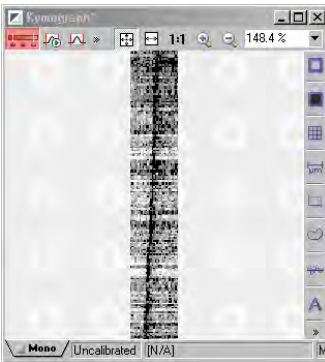
Kymographs created in NIS-Elements maintain full intensity bit depth, and can be created easily for multichannel images as well.

The function in ND Processing, **Create Kymograph by Line** converts the mouse pointer into a line drawing tool.



Using the line drawing tool, the user defines the line (XY positions) by which to create the orthogonal view of time.

Once the line is completed, a **right-click** on the mouse creates a new image window that displays the Kymograph.



Here, the kymograph displays the wave of calcium depolarization (black wave) as a line.

The axes of the Kymograph represent position and time.

X axis = time  
Y axis = XY position

From this, velocity can be calculated by slope ( $m$ ) of 2 points along the kymograph.

The function **Set Kymograph Line Thickness** selects the number of pixels around the kymograph line that is drawn. The mean value of all pixels at any position on the line is calculated and used in the kymograph when the line thickness is  $>1$ .

