



Technical Notes



NIS-Elements: Grid to ND Set Up Interface

This document specifies the set up details of the Grid to ND macro, which is included in material # 97157 *High Content Acq. Tools*.

This documentation assumes some familiarity with Nikon NIS-Elements software as well as general knowledge of PC Windows environment.

The Grid to ND interface is an NIS-Elements plug-in module that aids with setting up the multipoint dimension of an NSD acquisition conducted using rectangular Grids. The Grid to ND macro can also process a the ND file from the ND Acquisition by splitting the dataset into multiple ND files per grid position and/or to stitch rectangular grid images into a combined large image. This processing can be performed immediately after the ND acquisition is completed (online) or later (possibly on a different PC) as an offline process.

Notes: Motorized XY Stage is required. Motorized Z is optional.

'97157HCAInstall32.zip' and '97157HCAInstall64.zip' are available at:
<http://www.nissoftware.net/NikonSaleApplication/> in the
'NII Modules, Drivers and Macros' folder.

Note number: 0015
Creation Date: 7/2011
Date Modified:
Software Version: 3.2
Package: C, AR + 6D,
or Br + 4D + API
Required Module (s): 97157,
MQS41100 for non-Nikon
motorized X,Y stage



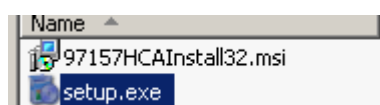
Installation and Camera/Objective Configuration

- **Installation:**

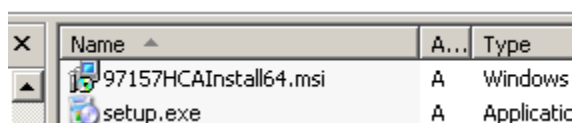
There are two install options for the Well Plate and Grid to ND macros '97157_HCATools:' 32-bit and 64-bit:



'97157HCAInstall32' folder will have the files used for 32-bit installation: 97157HCAInstall32.msi and setup.exe:



'97157HCAInstall64' folder will have the files used for 64-bit installation: 97157HCAInstall64.msi and setup.exe:



To install the macro module, select the appropriate installer, 32 bit or 64 bit, depending on the operating system. Double click and run the file 'setup.exe' from the corresponding folder. The installation will display a sequence of dialogs. Click the 'Close' button on the last dialog to complete the installation.

To verify the installation has been completed successfully, open the Windows Control Panel->Add or Remove Programs. The macro module will be listed under 'Currently installed programs:' as shown below:

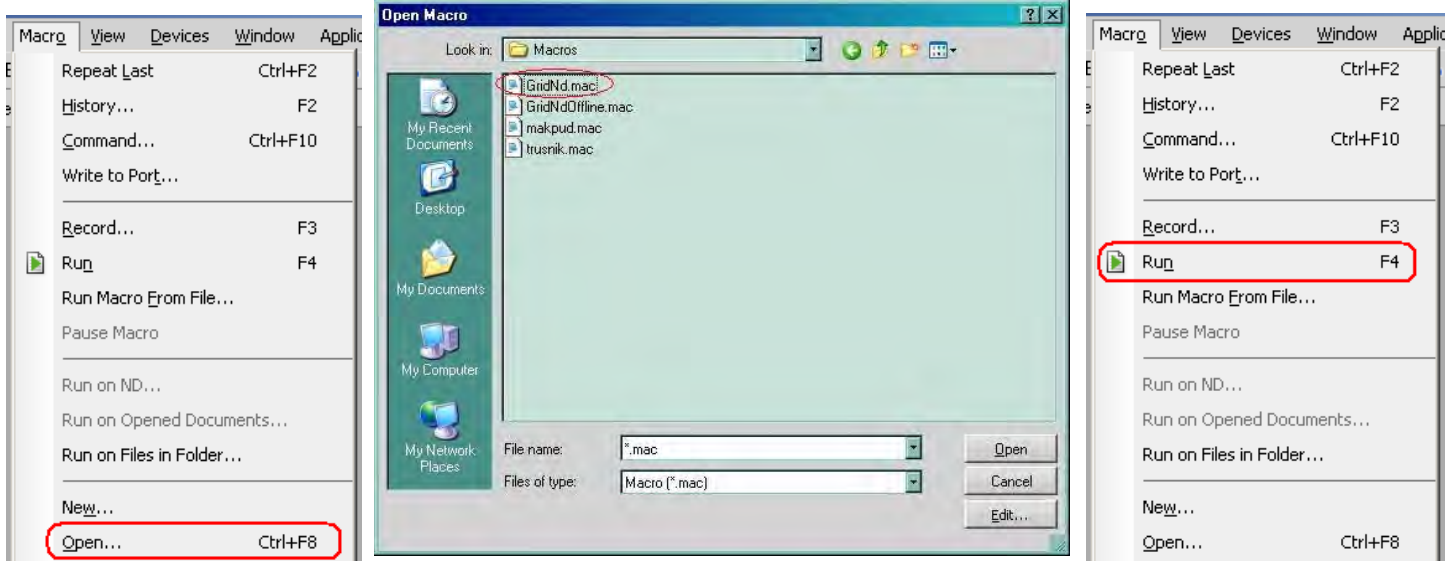


- **NIS-Elements Configuration**

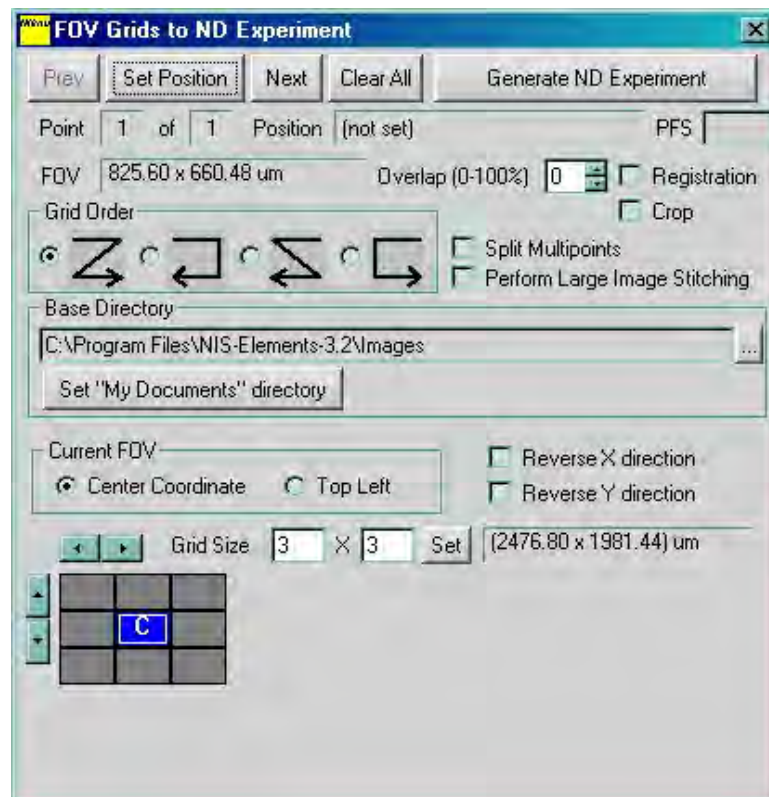
It is required to calibrate optical configurations for combinations of objectives and cameras, which will be used for acquisition. Since the camera's relative orientation (camera angle) to the stage is important, use the automatic or 4 point manual calibration

Opening and Running Grid to ND Macro in NIS-Elements

To launch the Grid to ND macro for the first time, load the GridNd.mac by opening the Macro Menu → and select the 'Open' option. Next browse for the GridNd.mac located in "C:\Program Files\Nikon\Shared\Macros" directory, and then open the Macro Menu → and select the 'Run' option.



To open the Grid to ND macro, after this first session, simply launch by pressing 'F4' 'or opening the Macro Menu → and select the 'Run' option. The Grid to ND window will display:





Overview of Grid to ND Macro and Grid to ND Acquisition

The Grid to ND macro assists multipoint data acquisition by preparing the list of multipoints (to be acquired) and transfers it to the 'XY Pos' tab of standard NIS-Elements ND Acquisition window where the points can be seamlessly integrated into a higher dimensions experiment with time-lapse, wavelength channels, Z-stack, etc through the ND Acquisition dialog. These multipoints consist of one or more independent grid locations (or physical fields of view) where each grid could be considered a large virtual field of view.

Terminology:

Here are the terms used in this document and the macro dialog:

- *Stage Position*
 - the XYZ coordinates of the stage
- *FOV*
 - Field of View of the image centered about the current stage position
- *Grids*
 - area defined by a specific number of columns and rows
 - For example, a 'grid' may be composed of 3 columns and 3 rows of grid elements (or FOVs). In this case, the grid is composed of 9 stage positions.
- *Grid Element*
 - the stage location of a physical FOV that is part of a bigger grid
- *Center Grid*
 - the geographical center of the grids
 - The grids' column/row numbers are always odd numbers so there always exists a single center grid field of view.

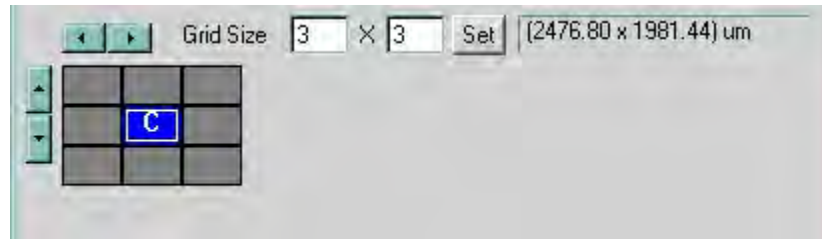
The main workflow steps are:

1. Specify the column / row number of stage positions that make up the grid
2. Move the stage with the joystick to the desired center of the virtual field of view.
3. Register that stage location as the grid location
4. Repeat the above steps (1-3) for desired number of grids
5. Setup options.
6. Transfer the grid positions to ND Experiment.
7. Run experiment and acquire data.
8. Offline post-processing of ND datasets (optional).

Specifying the column / row number of grids

Specify the number of columns and rows for the current grid by either typing in the numbers and clicking 'Set' button or incrementing/decrementing them with arrow buttons.

Note: The grids are placed around the center grid position, which is interpreted as a default FOV center for a given point. For this reason, only odd numbers of columns and rows are supported. The center grid is marked with a letter "C."

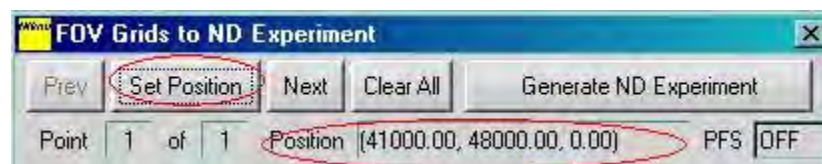


Specifying the grid elements to be acquired

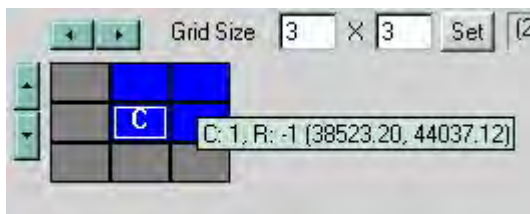
Individual grid elements can be specified to be acquired or not acquired. The grid elements in blue designate acquisition whereas the grid elements in gray designate non-acquisition. Click on the grid to toggle the state to be acquired or not acquired.

Specifying the stage position for the grid

Move the XY stage to the desired location, then click 'Set Position' button. The current stage position will be assigned to the point and shown in an area next to 'Position.'



Once the stage position for the grid is set, the tooltip for each grid element shows the grid element's stage position along with column/row indices. Double-clicking the grid element moves the stage to that position.



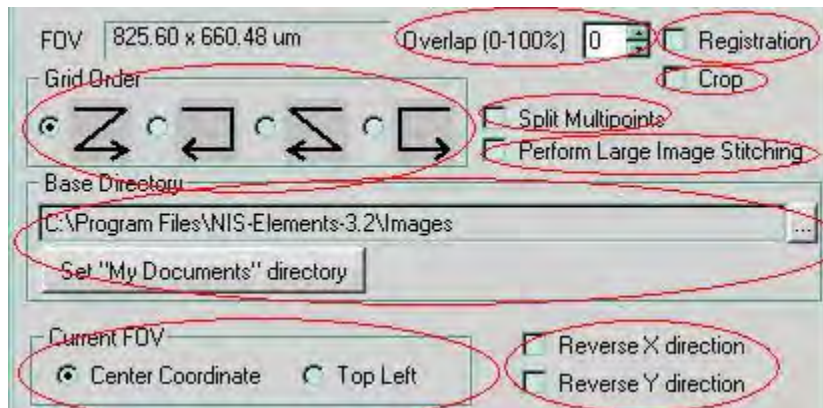
Adding a new Grid

The 'Next' button works in two ways. When the current point is the last, it adds a new one and set it as current. Otherwise it makes the next point as current. 'Previous' and 'Next' buttons are used to browse points. The current point number and total number of points are shown below 'Prev,' 'Set Position,' and 'Next' buttons.



Setting up options

The Grid to ND macro has several options for flexibility.



- **Overlap**
 - Specifies overlap between two grid elements in percentage of grid size.
- **Registration**
 - Specifies if image registration will be performed for large image stitching
- **Crop**
 - Specifies if image cropping will be performed for large image stitching
- **Grid Order**
 - Specifies the order in which grid elements are added to ND Experiment XY tab in the "multipoint" tab
- **Split Multipoints**
 - Specifies if 'Split Multipoints' operation is performed immediately after ND experiment is completed
 - 'Split Multipoints' operation generates separate ND files for each grid defined in the macro.

Note: To perform offline 'Split Multipoints' operation, please do NOT enable this option.



- *Perform Large Image Stitching*
 - 'Perform Large Image Stitching' operation stitches the grid images for a given grid and generate a single large image.
 - Specifies if 'Perform Large Image Stitching' operation is executed immediately after ND experiment is completed

Note: To perform offline 'Perform Large Image Stitching' operation, please do NOT enable this option.

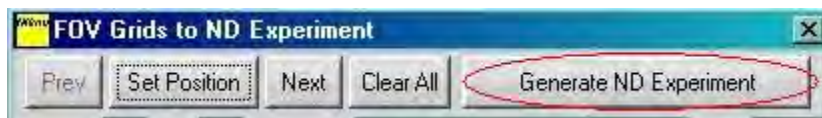
- *Base Directory*
 - Specifies the directory where temporary/resultant image/ND files are output

Note: This is used only when "Split Multipoints" and/or "Perform Large Image Stitching" options are enabled.

- *Current FOV*
 - Specifies which grid element is associated with the stage position as a current FOV
- *Reverse X/Y direction*
 - Used to compensate for various stage configurations with different axis direction

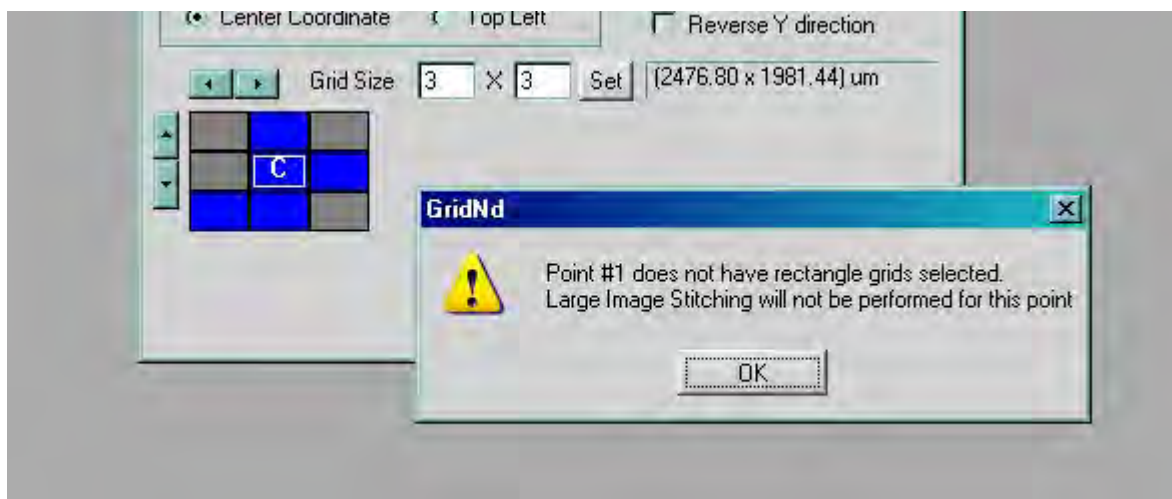
Transferring the grid positions to ND Experiment

Once all the grids are added and the options are selected, clicking 'Generate ND Experiment' button transfers grid positions to ND Acquisition window's 'XY Pos' tab.



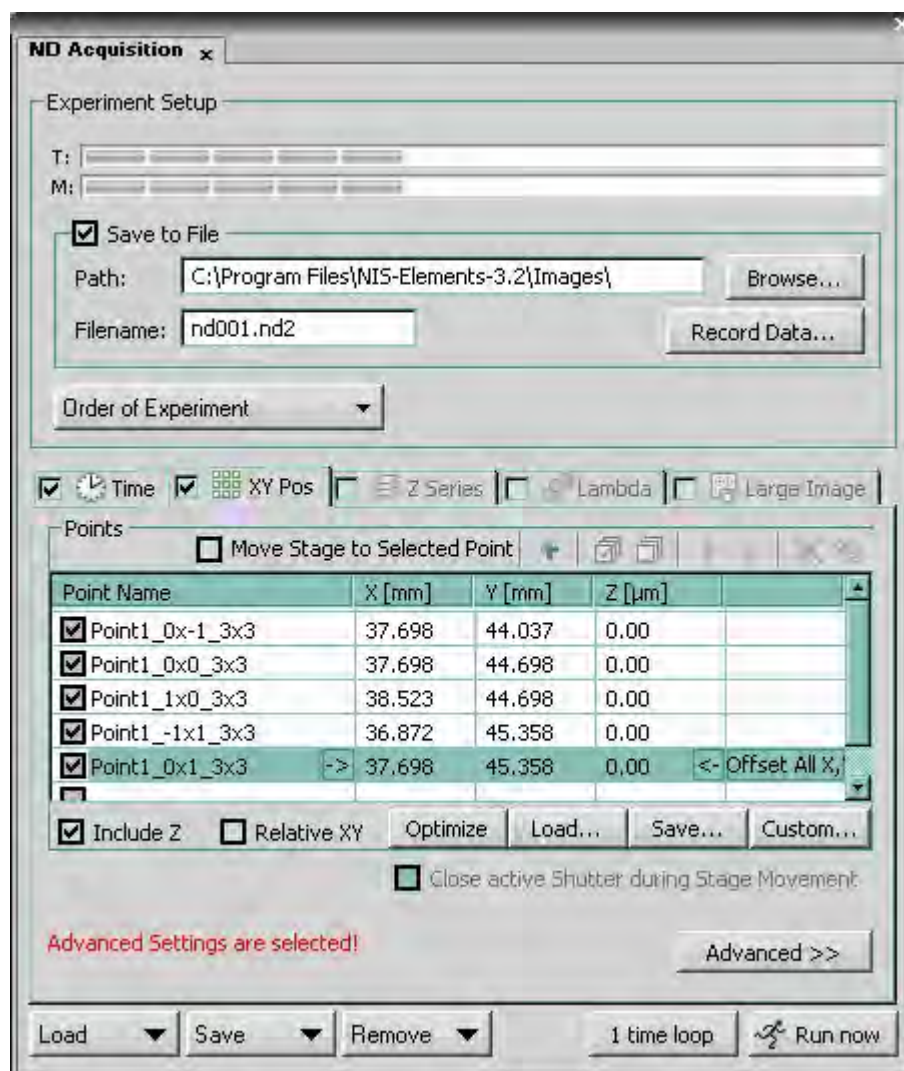
Note:

- If multipoints already exist in the tab, they will be deleted.
- If 'Perform Large Image Stitching' option is selected and the grid elements are selected such that selected grid elements do not form a contiguous rectangular area, the warning message below will be displayed. This is because NIS-Elements Large Image Stitching requires contiguous rectangular grid images. All the multipoints are still added to the ND Acquisition but the large image stitching for the stage point(s) which grids do not form a contiguous rectangular area will not be performed.



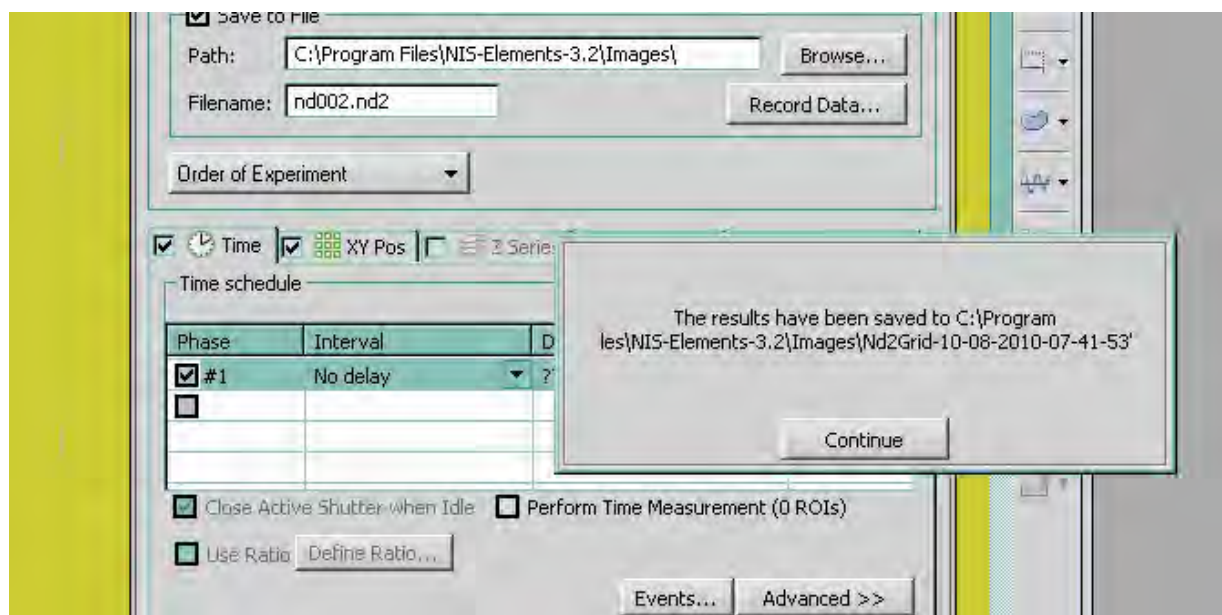
Running experiment and acquire data

Open ND Acquisition window (NIS-Elements Menu: Applications | Define/Run Experiment).



Configure other dimensions such as Time and Z Series tabs if necessary. Click 'Run now' to run the experiment.

If either one of 'Split Multipoints' or 'Perform Large Image Stitching' is selected, then post-processing of the experiment result starts immediately after the experiment completes. At the end of the post-processing, the message similar to the one shown below will be displayed:

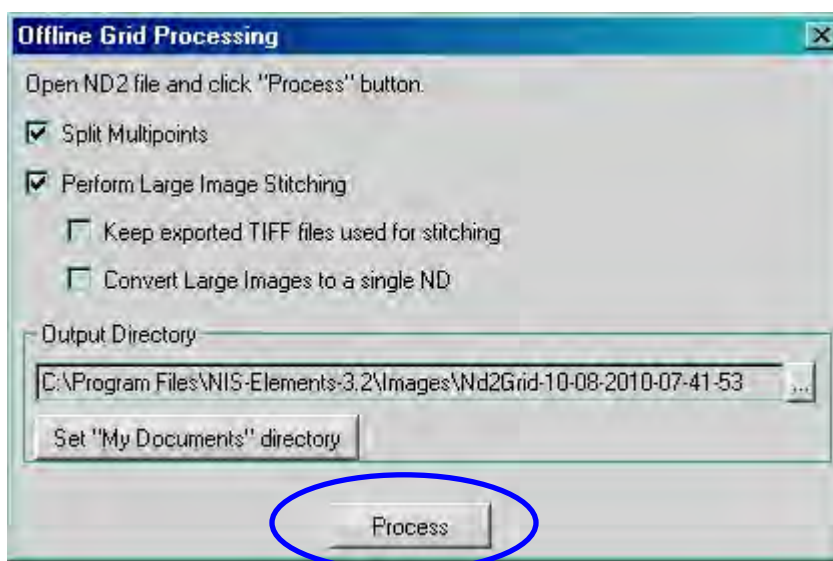


Note: This post-processing will be disabled after the first run. Therefore, running the same experiment for the second time will not run the post-processing again.

Offline post-processing of ND datasets

Typically post-processing of ND datasets takes a long time. In some cases, it is convenient to setup a PC dedicated for the post-processing rather than tying up the PC setup for the acquisition. An offline version of Grid to ND macro is available. This macro takes ND files generated by ND Acquisition and performs Split Multipoint and/or Large Image Stitching operations.

To launch the macro, open the NIS-Elements Menu [Macro | Open], then select 'GridNdOffline.mac' from 'Macros' directory. Then open the NIS-Elements Menu [Macro | Run]. The window below will be displayed:



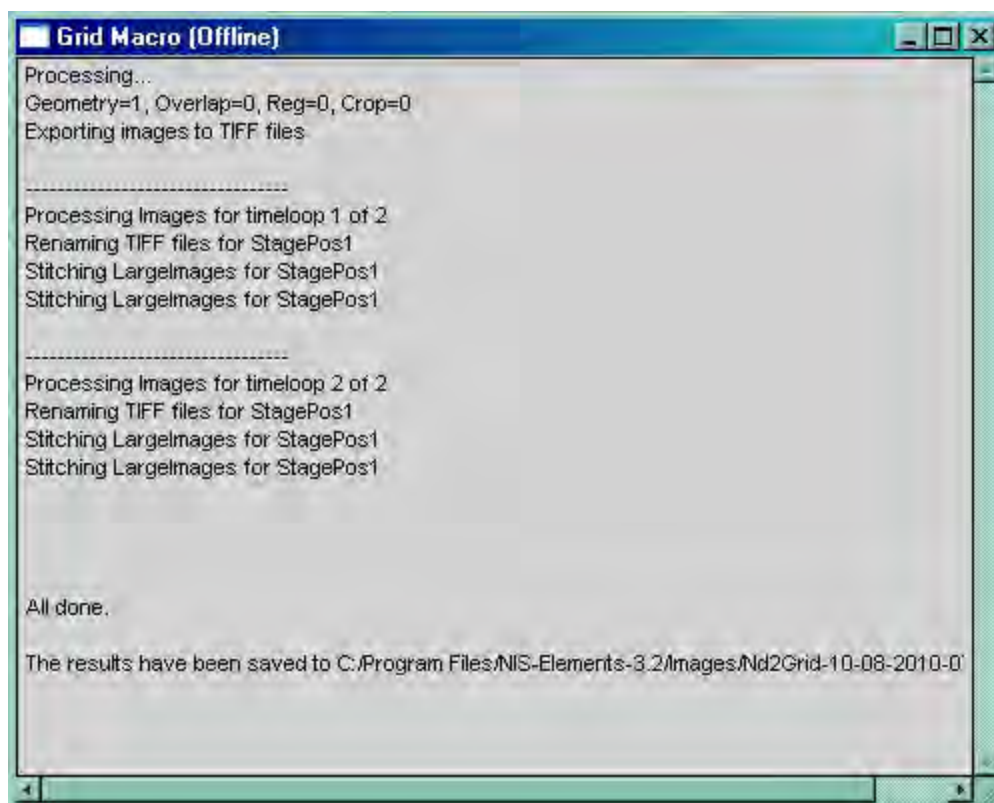
Open an ND file (the result of Grid to ND Acquisition) in NIS-Elements (Menu: File | Open) and click 'Process' button in Offline Grid Processing window (above).

Note:

- If multiple ND files are open, the current ND file will be processed.
- The ND files must be the results of the experiments whose multipoints were generated by online version of Grid to ND macro (GridNd.mac). Otherwise the message below will be displayed and the processing will be aborted.



During the processing, a status window similar to the following will be displayed:



Offline post-processing options

Similar to online post-processing, offline post-processing has some options.

- *Split Multipoints*
 - specifies if 'Split Multipoints' operation will be performed
 - 'Split Multipoints' operation generates separate ND files for each stage point defined in the online macro.
- *Perform Large Image Stitching*
 - specifies if 'Large Image Stitching' operation is performed immediately after ND experiment is completed
 - 'Large Image Stitching' operation stitches the grid images for a given stage point and generate a single large image.
- *Keep exported TIFF files used for stitching*
 - a Large Image Stitching option
 - the intermediate TIFF files generated for Large Image Stitching operation will not be deleted
- *Convert large images to a single ND*
 - an Image Stitching option
 - stitched large image files will be combined to a single ND file.

Note: If 'Registration' and/or 'Crop' options were selected in the online Grid to ND macro prior to generating multipoints for ND Acquisition, then selecting this option may cause an error. This is because the registration and crop functions tend to generate large images with slightly different XY dimensions and NIS-Elements will not allow combining different size images into a single ND.

- *Output Directory*
 - specifies the directory where temporary/resultant image/ND files are output

Note: Other options selected in online Grid to ND macro are saved to the ND file and read by offline macro. It is not possible to overriding these saved settings.