



## NIS-Elements: Well Plate Set Up Interface

This document specifies the set up details of the Well Plate 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 Well Plate interface is an NIS-Elements plug-in module that aids with setting up the multipoint dimension of ND experiments conducted using rectangular well plates. Templates for several commonly used standard well plates<sup>1</sup> are available for creating well plate map files. Custom plates can be configured and stored as well.

Note number: 0014  
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

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.

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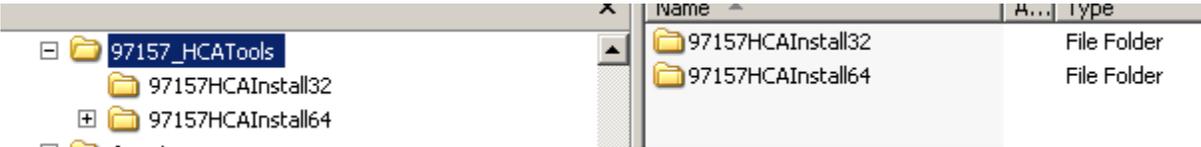
<sup>1</sup> A set of standard well plate dimensions. It is possible to create a custom XML file based on a particular manufacturer and model. This XML file can be added to the library of possible templates. This XML builder is available on the NIS-Elements Download Site (<http://www.nissoftware.net/NikonSaleApplication/>).



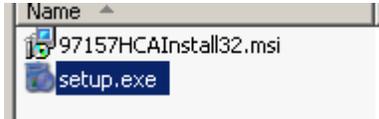
**Installation and Camera/ Objective Configuration**

- Installation:**

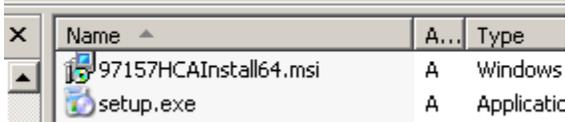
There are two install options for the Well Plate and Grid to ND macros '97157\_HCATools:' 32-bit and 64-bit:



'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:



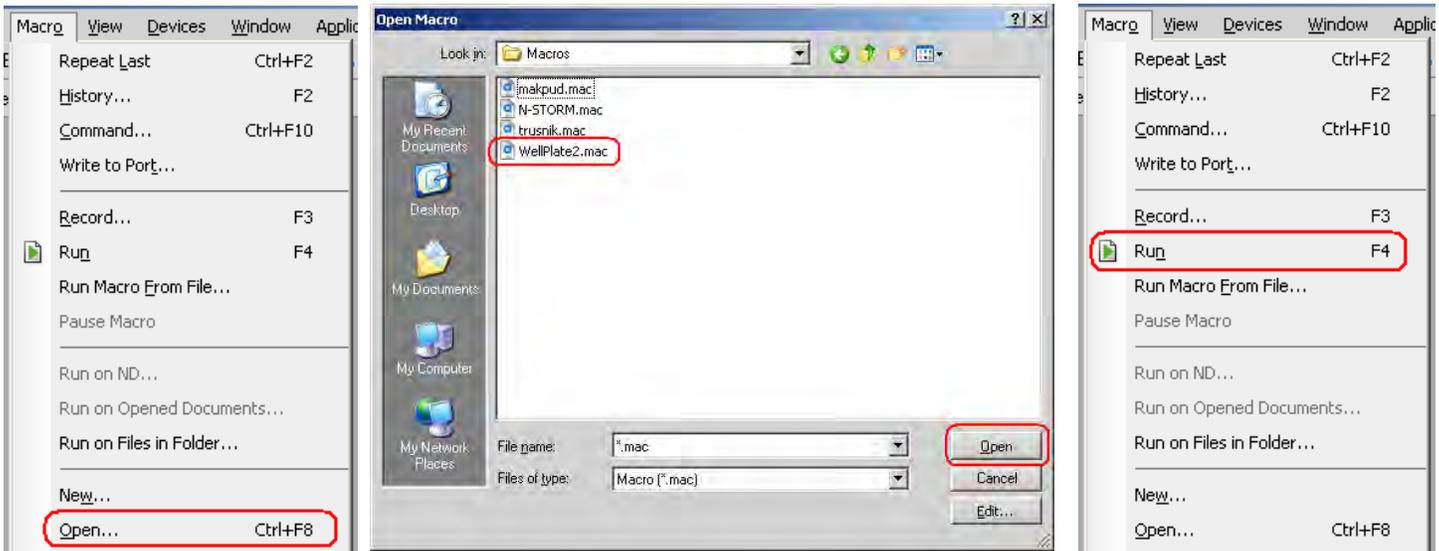
- NIS-Elements Configuration**

It is required to calibrate optical configurations (OC) 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.

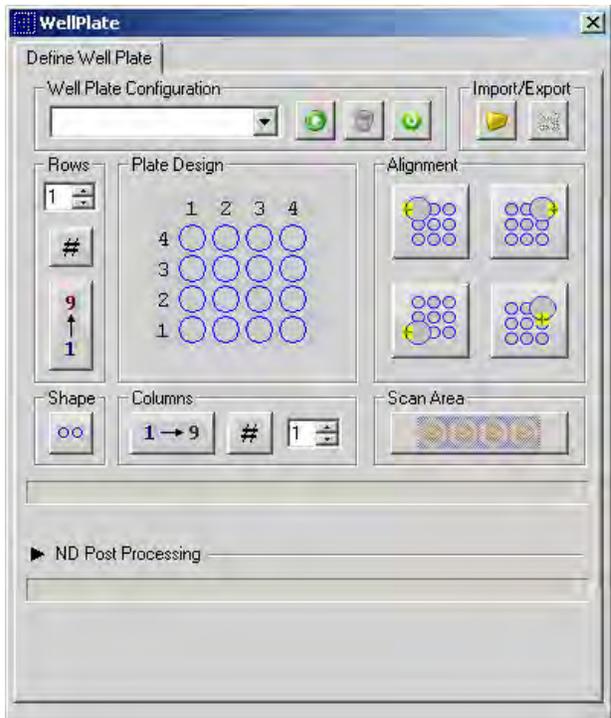


## Opening and Running Well Plate Macro in NIS-Elements

To launch the Well Plate macro for the first time, load the WellPlate2.mac by opening the Macro Menu → and select the 'Open' option. Next browse for the WellPlate2.mac located in "C:\Program Files\Nikon\Shared\Macros" directory, and then open the Macro Menu → and select the 'Run' option.



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



*Note:* It is possible to combine the 'Open' and the 'Run' steps by selecting Macro Menu -> 'Run Macro From File...'



## Overview of Well Plate Macro and Well Plate Acquisition

The Well Plate macro assists with Well Plate data acquisition by preparing the list of stage points (to be acquired) and transfers it to XY multipoint tab of standard NIS-Elements ND Acquisition window. This allows multiple well locations to be seamlessly integrated into a higher dimensions experiment with time-lapse, wavelength channels, Z-stack, etc through the ND dialog.

The main workflow steps are:

1. Create and edit well plate map (# and labeling of rows, columns)
2. Align the well plate.
3. Save format and alignment for future use (optional) \*
4. Create and edit well selection map and Field of View (FOV) map.
5. Save format, alignment and selected positions for future use (optional) \*
6. Transfer the points to ND Acquisition dialog.
7. Run experiment and acquire data.
8. Post-processing of ND datasets (optional).

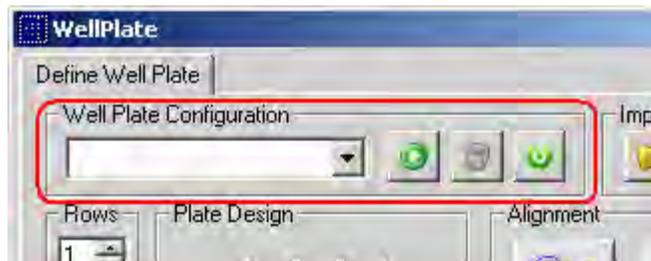
\* Once a well plate configuration is saved, it can be loaded from the drop down list. There are different types of configurations that can be saved, which is explained in detail later in the document.

## Creating Well Plate Maps

A Well Plate Map can be created in one of three basic ways:

1. Load from standard or previously configured template
2. Load from previously saved plate map
3. Create a 'New' (custom) plate map without using a predefined template

The Well Plate macro maintains a list of well plate configurations. Each configuration entry can contain several pieces of information about the physical well plate it pertains to:



- a. Well plate template (well shape, well size, distances between wells, numbers of rows and columns and their labeling options)
- b. Alignment information (placement orientation and position) of plate
- c. Wells to visit during acquisition
- d. Number and positioning of FOV(s) (multiposition(s)) within the well



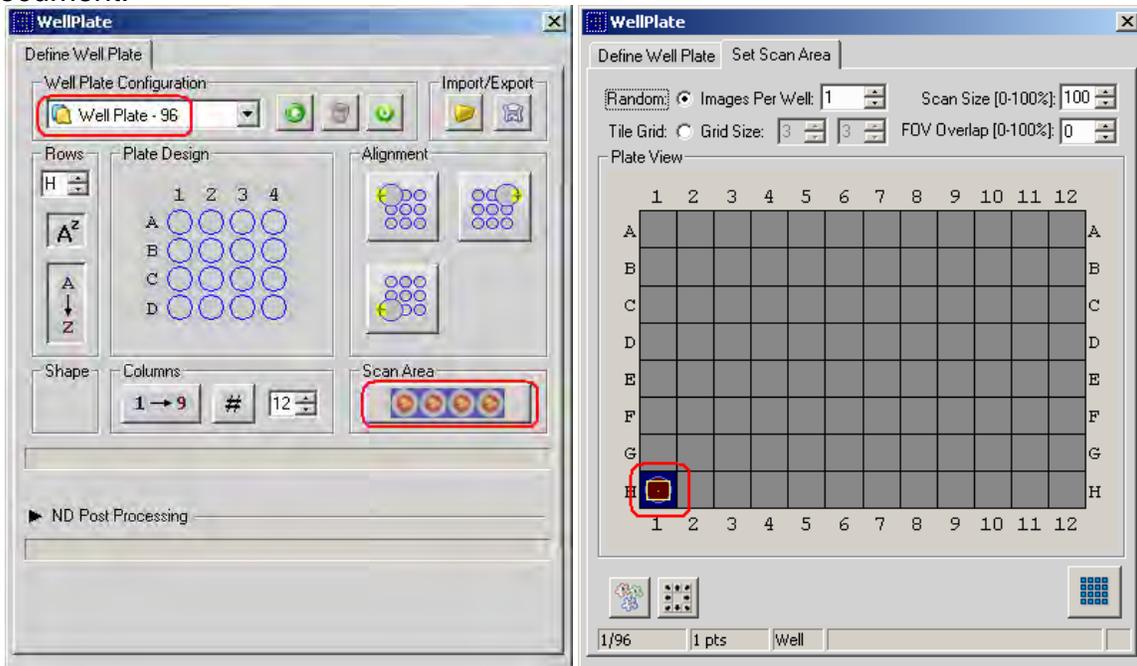
**There are four types of well plate configuration types:**

(Note: Different types are marked with corresponding icons)

1. **Well plate configuration template**
  - Stores the well plate format (# of rows & columns, well labels, shape of wells)
  - Well Plate Macro installs a set of commonly used formats
  - 'New' (and custom) Templates can be created
2. **Aligned well plate configuration template**
  - Stores the well plate format (# of rows & columns, well labels, shape of wells)
  - Stores the plate alignment
3. **Regular well plate configuration** (blank white icon)
  - Stores the well plate format (# of rows & columns, well labels, shape of wells)
  - Well selections and multi-positions to add to the ND acquisition
4. **Aligned well plate map file** (letter "a" on white background)
  - Stores the well plate format (# of rows & columns, well labels, shape of wells)
  - Stores the plate alignment
  - Well selections and multi-positions to add to the ND acquisition

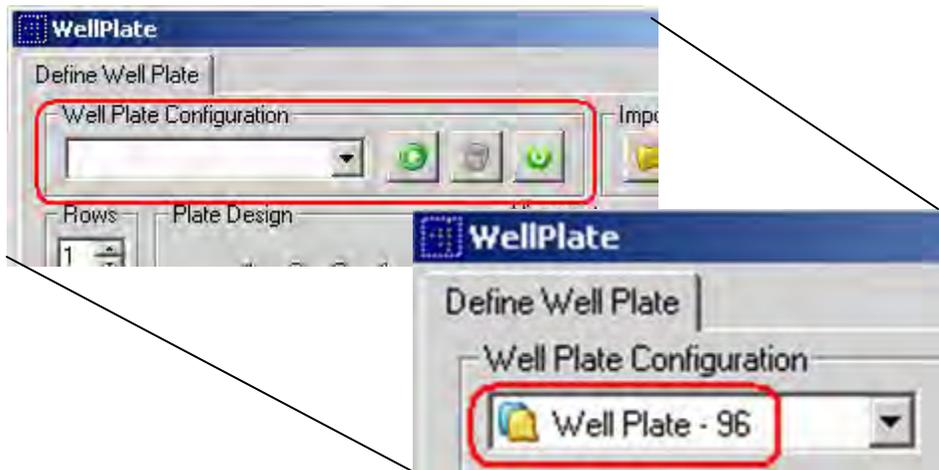
Templates contain the well plate format information, but do not include the alignment and selected wells and FOV(s) locations for acquisition. Templates can then be reused to quickly create specific well plate configurations by adding alignment and well selections and FOV maps to them.

As an example, choose one of the standard templates (i.e "Well Plate – 96") and press "Define Scan Area" button. This opens the "Set Scan Area" tab with Well Map view. Setting FOV(s) and creating specific templates will be discussed later in the document.



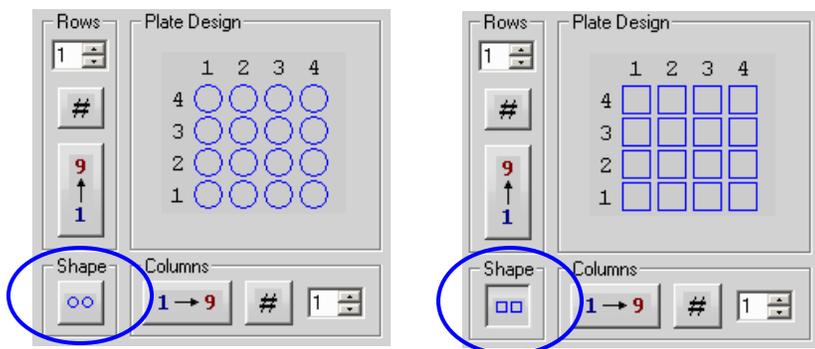
**Example Workflow**

1. **Select a template** from the drop- down list. For example, select 'Well Plate -96.'

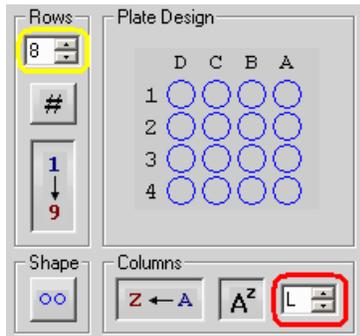


2. **Configure Plate Format**

The 'Well Plate – 96' template will automatically load in the well plate format (# of rows, columns and labeling). If there are changes to the pre-defined template, use the groups of controls below Well Plate Configuration group for configuring the numbers or well rows, columns, their labeling and the shapes of the wells.



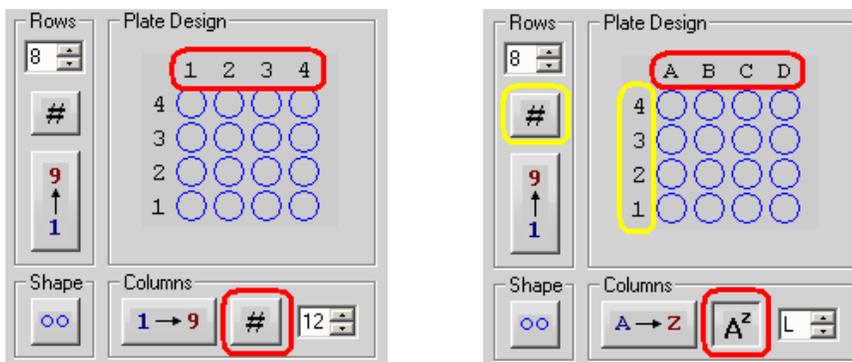
The button in the bottom left corner switches the shape of the wells between round and square. The controls in the top left and bottom right corners set the numbers of rows and columns.



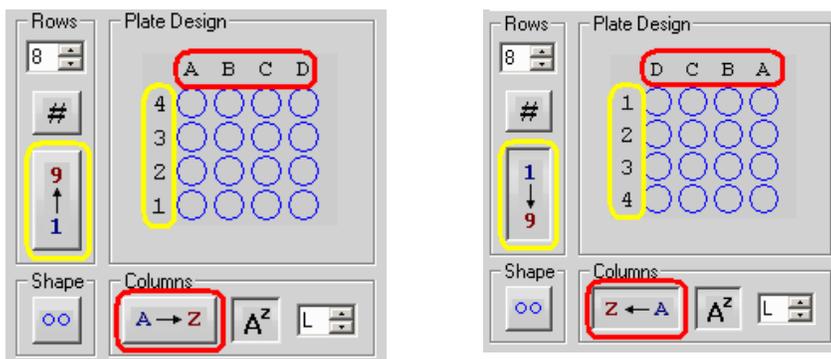
Note: The Plate Design display is an interactive preview of the subset of the plat, which displays well shapes as well as the rows and columns labeling. The graphic preview updates and changes according to the label settings.



The square buttons with '#' switch labeling between numbers and letters independently for rows and columns. When letters are chosen, the corresponding button icon switches to 'A.'



The elongated direction buttons toggle between ascending and descending labeling order for rows and columns.



### 3. Plate Alignment

Plate alignment is required to create a custom well plate configuration and for generating stage locations for the ND Acquisition. Alignment is a procedure that measures the well plate stage placement. This is accomplished by moving the stage to points called Alignment Points.

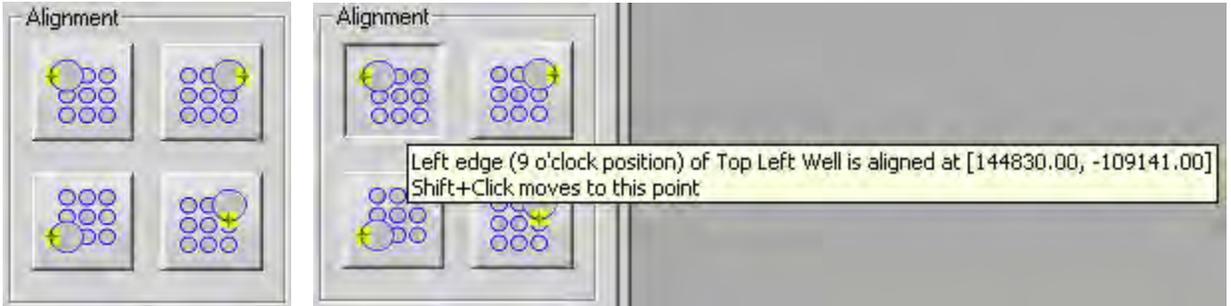
In order to align a well plate, 3 distinct alignment points are required. In some cases, 4 distinct alignment points are required.

Once a point is aligned, the corresponding button remains pressed and the tooltip shows the actual stage position of the alignment point. Use these tooltips to confirm that the positions for different alignment points are not the same, which could happen if there is a problem with XY stage device configuration.



The alignment procedure for each point is the following:

1. Using motorized stage joystick, navigate the stage and align the required point on well plate against the crosshair graticule in live window.
2. Press the corresponding alignment button in the Alignment program group. The button icons and tooltips should help determining button designations.

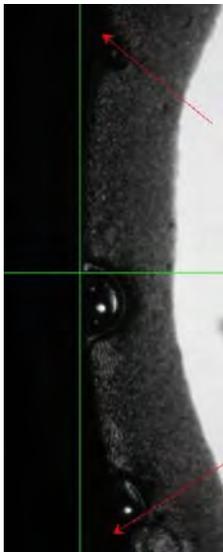


*Notes on realigning a point:*

- *Reposition the stage point using joystick and click the corresponding alignment button twice (first to un-press it and clear the old alignment position and second time to record the new position).*
- *If desired, once aligned, click the pressed alignment button while holding the Shift key down to move the stage to the corresponding alignment position.*

*Notes on general alignment:*

- *With a low magnification objective, it will be possible to view more of the curvature of the well when imaging the edge.*



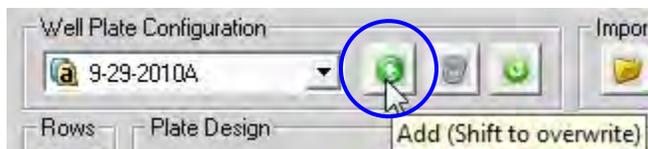
- *Placing the crosshairs on the center outside edge of the wells as seen to the left is a recommended alignment placement.*
- *With a lower magnification lens, it is easier to view the center the edge by comparing the size of the areas indicated by the two red arrows in the screenshot to the left.*
- *A lower magnification calibrated objective will yield fewer possible FOVs (FOV placement is covered in Step 6.) It is possible to align at a lower magnification and set FOVs with a higher magnification. In order to do this, simply change and select the calibrated objective and the macro will update the FOV size in the interface.*



#### 4. Saving the template/ aligned template

Once the custom well plate is aligned and the well plate organization (well shape, labeling for rows, columns, etc) is defined, but before any well selections are made, the configuration should be saved. Saving at this step is optional, but it will prevent the loss of the alignment points that have been input into the dialog.

To save an aligned template, simply type in a unique name in Well Plate Configuration edit box and press 'Add' button or select 'Save As...' option from drop down list.



Notes:

- Aligned templates are marked with icon.
- Unaligned templates are marked with icon.
- Loading unaligned template later will require a 3 point alignment before ND experiment XY points can be generated.
- Un-pressing alignment buttons irreversibly clears the alignment information.
- It is a good idea to save the aligned template first and then clear the alignment and save unaligned template. The aligned template can always be deleted later if it is not needed.

#### 5. Using 'Scan Area' - Well Selection and # of Positions Per Well (FOVs)

The Scan Area Tab becomes available as a result of either one of these actions:

- Loading a previously saved template
- Defining custom well plate and alignment is complete

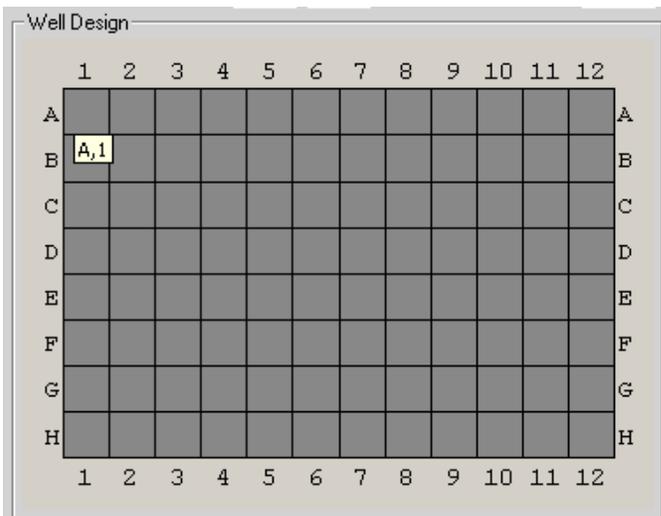


Pressing the 'Define Scan Area' button enables well selection and the # of positions per well (the FOV map) to current configuration and converts it from template to plate map.

Note: If this type of configuration is now saved, its icon background will be blank white rather than . Pressing 'Define Scan Area' button adds 'Set Scan Area' tab at the top and automatically opens the tab:



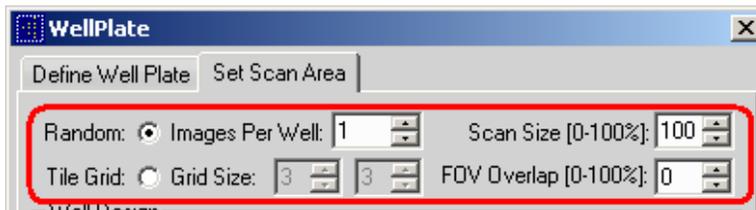
The Well Plate view is displayed:



**6. Set Scan Size % and # of Positions Per Well (FOVs)**

Scan Size % (Well %):

At times, it may be desired to avoid edges of the well for acquisition due to intensity or cell density differences. The FOV map will interactively change with the selected Scan Size % settings.



# of Positions Per Well (FOVs)

There are two options for adding number of positions per well:

- a. Random : for a random position and/or manual positioning of FOV(s)
- b. Tile: for creating a grid of adjacent FOV(s) in each well

Steps for setting # of Positions Per Well (FOVs):

1. Select Random or Tile Grid option
2. If selecting Random, set Scan Size, then number of images per well
3. If selecting Tile Grid, select FOV Overlap and Grid Size (i.e. 3x3)

Notes:

- FOV overlap affects both 'Random' and 'Tile Grid,' but most useful with grid for post processing stitching.
- If it is desired to keep the Scan Size at a certain % and the number of FOV a certain number, then hold the 'Shift' key down while changing the Scan Size %. This will force the number of images not to decrease as the Scan Size % is decreased.
- As mentioned in Step 3 for Plate Alignment, is it possible to align with a lower magnification objective OC. After alignment, selecting a higher magnification objective OC is optional to select a higher number of FOVs.

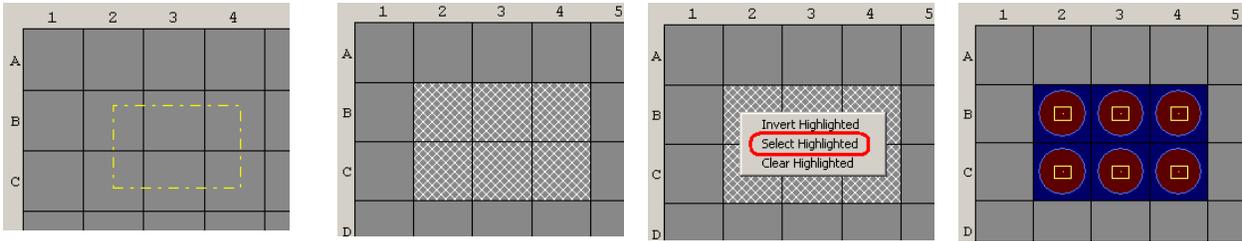


## 7. Well Selection

One of the final step in creating the well plate map is selecting the wells to include in the acquisition.

### Well Selection Tools:

- Hovering mouse cursor over wells displays the tooltip with row and column label.
- Wells can be selected and deselected by a single mouse click.
- Rectangular groups of wells can be highlighted for group selection by dragging the mouse cursor across several wells.



- Once a group of wells is highlighted, right click menu shows group selections options.
- All highlighted wells can be selected, deselected (or cleared), or their individual selection status can be toggled (or Inverted).
- Same group selection choices can also be applied to all wells, or a single row, or a single column by right clicking in the Well Plate view while none of the wells are highlighted (left image below).

## 8. Saving the Well Selection and # of Positions Per Well (FOVs) to the well plate map

1) If it is desired to save the selected wells and number of images (positions, FOVs) per well to the well plate map, click back to the 'Define Well Plate' tab.



2) Type the text of the name into the field.

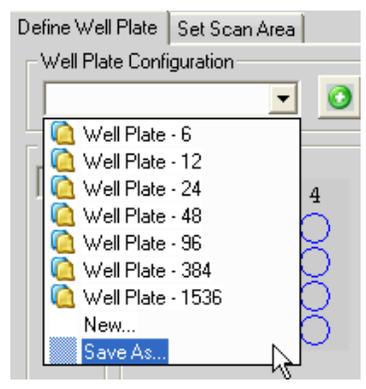


3) Click the 'Add' icon  to add the name to the list of available configurations in the configuration drop down.

OR



1) Alternatively, it is possible to select 'Save As' from the drop down list:



2) Type the text of the name into the field.

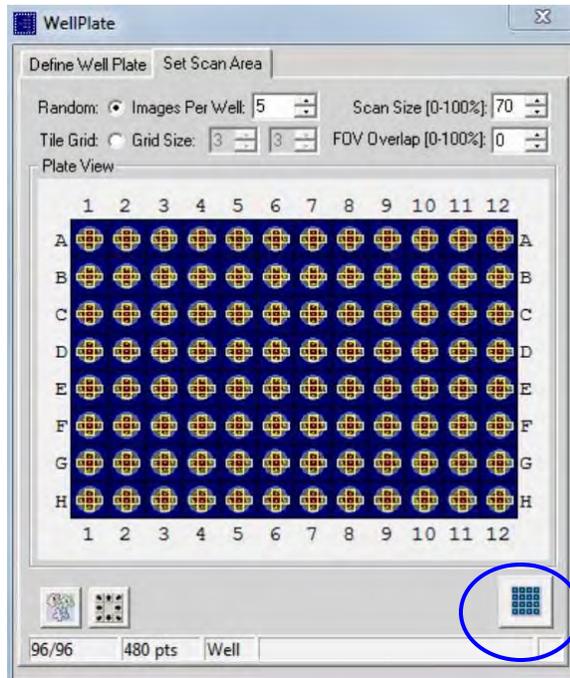


3) Click the 'Add' icon  to add the name to the list of available configurations in the configuration drop down.

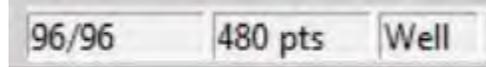


## Importing the well plate stage points to the ND Acquisition dialog

- Click on the grid icon  on the bottom right hand corner of the dialog to transfer the selected points to the ND dialog:



- Progress of the transfer will display on the screen.
- The list of points transferred to the ND dialog will over-write the existing stage points (i.e. it will not append the points).
- Notes:
  - On the status bar of the 'Set Scan Area' tab, at any time before or after the transfer of points, there is text that indicates the number of selected wells and the number of points.
- Once the ND Acquisition is running, it is possible to view the current/ active well in the well plate macro interface. The active well will be highlighted. This is a useful visual cue of the progress of the well plate acquisition.

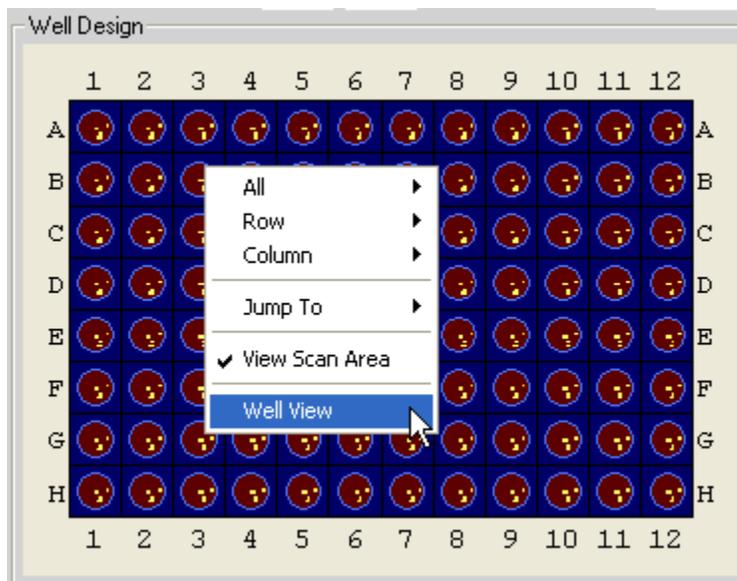


**General Notes about Well Plate Macro Navigation and Options**

**Viewing Options**

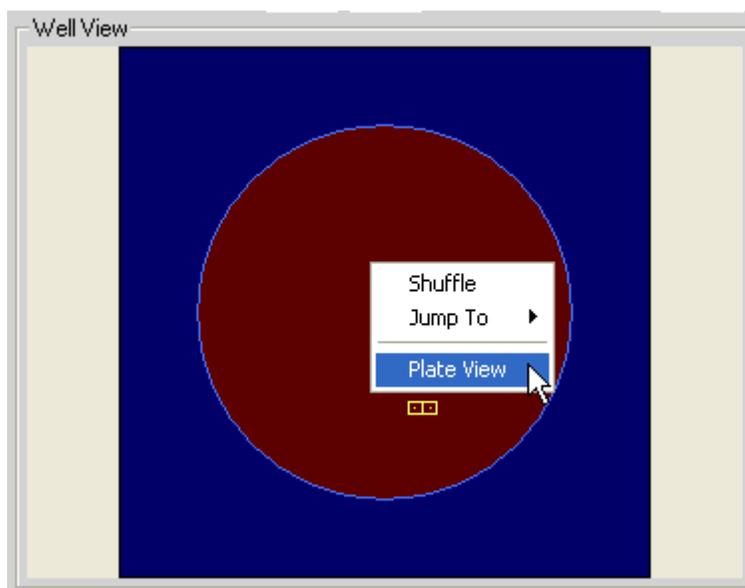
**Plate View**

- When in Plate View, it is possible to view all wells (selected and non-selected)
- It is possible to switch to Plate View by right clicking and selecting 'Well View:'



**Well View**

- When in Well View, it is possible to view and manually select FOVs
- It is possible to switch to Plate View by right clicking and selecting 'Plate View:'



## Stage Navigation

### Moving Stage Positions through Well Plate Macro Interface

#### General Notes

- The current well is outlined (after jump and during ND acquisition)
- To view current well location during acquisition, keep well plate macro open.

#### 'Jumping' Options:

- 'Jumping' moves the stage from one well position to another well position
- Double clicking on a well in Plate View will move the stage to the selected location
  - Available jump modes are:
    - *Well Center* – will move the stage to the selected well's center
    - *FOV Center*- will move the stage to the relative FOV in the selected 'move to' well
    - *Cursor*- will move the stage as close as possible to the current cursor position

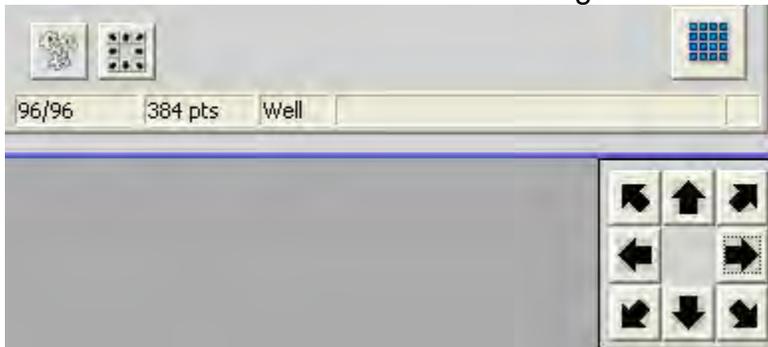


- A virtual joystick is also available for moving well to well.

- Click on the virtual joystick icon



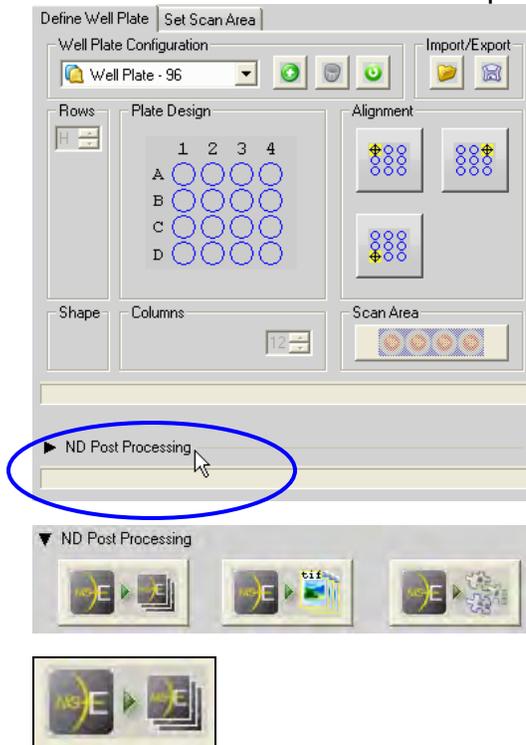
- The virtual joystick will appear on the bottom right below the dialog.
- Click on arrows will move the current stage location from well to well.



## Post Processing Options

- Post Processing for splitting and stitching datasets is available post-acquisition
- Due to how the well plate macro interacts with adding information to the image header, It is important to keep the Well plate macro running during ND Acquisition. This is especially important if Post Processing is desired.

Click on the 'ND Post Processing' text  ND Post Processing to expand the interface to show the available options.



- Converts an opened multipoint ND dataset to separate ND files per well
  - For example, if converting a 96 well plate with three positions per well, 96 separate ND files each having three positions will be created.
- Destination folder location will be the same location as the dataset.
  - The output will be an ND file if the multi-position dataset has a Z or T dimension.
  - The output will be a TIFF if the dataset is Multipoint and Channel only.
- The order of dimensions of the exported file is :
  - time, well, z, lambda channel  
(for example: filenameet01Well[B,3]c1.nd)
- A progress message will appear during the conversion.



- Converts an opened multipoint ND dataset to TIFFs
- Destination folder location will be the same location as the dataset
- The order of dimensions of the exported file is :
  - time, xy, z, lambda channel
 (for example: filenameet2xy10c1.tif)
- A progress message will appear during the conversion.



- Stitches an opened multipoint ND(s) with tile grid into a large image TIFF(s)
- Each channel is separated into a separate large image TIFF
- Destination folder location will be the same location as the dataset.
- The order of dimensions of the exported file is :
  - time, xy, z, lambda channel
 (for example: filename011c1Well[A,1].tif, filename011c2Well[B,1])
- A progress message will appear during the conversion
- Note: Currently, this function is only compatible with XY only datasets. (Datasets with Time and Channel components should not be used with this function.)

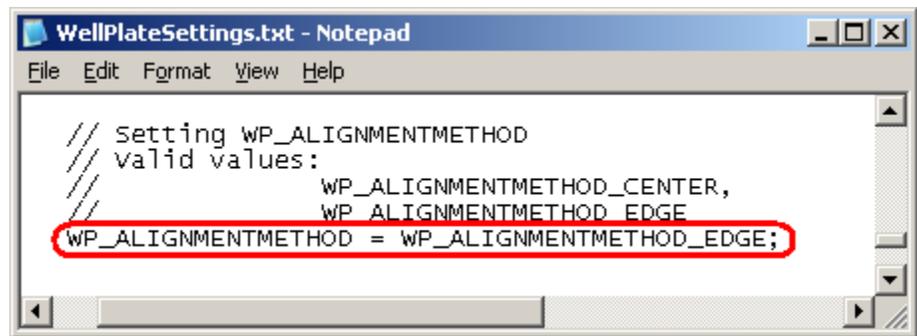
**Advanced Notes for Alignment and Stage Direction**

**Alignment**

There are two alignment modes that can be used with this Well Plate macro:

1. Well Center Alignment
2. Well Edge Alignment (this is the default setting)

*Advanced Note: The alignment mode can be set by editing WellPlateSettings.txt file in NIS-Elements\macros directory. Change the WP\_ALIGNMENTMETHOD value to either WP\_ALIGNMENTMETHOD\_CENTER or WP\_ALIGNMENTMETHOD\_EDGE. Please preserve the semicolon at the end of the line during editing. Macro and NIS-Elements have to be restarted in order for new setting to go in effect.*

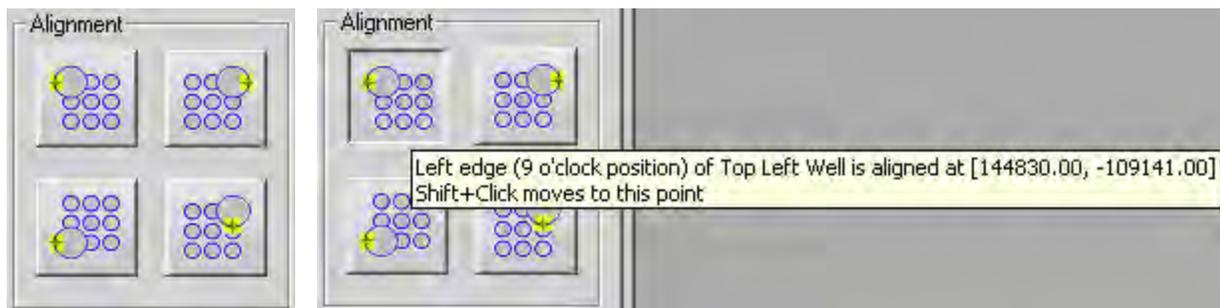


## Well Edge Alignment

Well edge alignment was designed to simplify the alignment because well centers are not marked and therefore can be difficult to align against with higher magnifications. Well edge alignment expects 3 alignment points to be set: Left edge of the Bottom Left corner well, Left edge of the Top Left corner well, and the Right edge of the Top Right corner well.

For unknown geometry plates, this alignment mode also requires a 4<sup>th</sup> alignment point, which is the bottom edge of the Top Right corner well. The alignment procedure is the same as in center mode. The button icons and tooltips are tailored to each mode.

*Note: When the well plate with known geometry is loaded the fourth alignment button is hidden.*



*Note: The order of aligning is arbitrary as long as eventually all required points are aligned.*

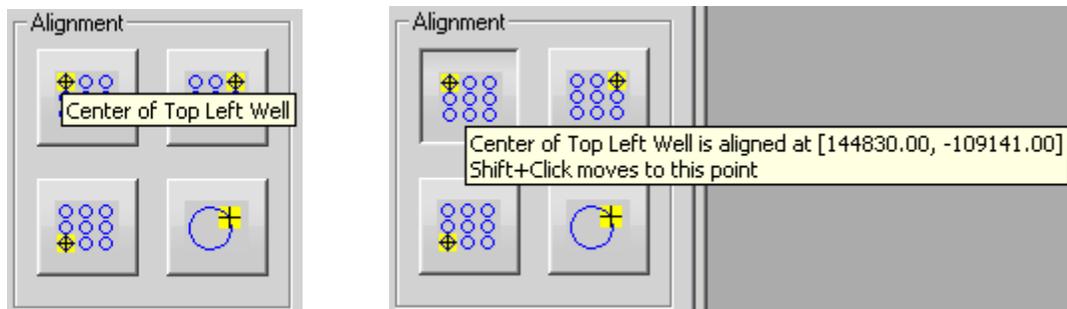
## Well Center Alignment

Well center alignment requires to align input for 3 points: center points of Bottom Left, Top Left and Top Right corner wells. For unknown geometry plates, this alignment mode also needs to align a 4<sup>th</sup> point, which can be any point on the edge of any well.

*Note: Just as in Well Edge Alignment mode, when the well plate with known geometry is loaded the fourth alignment button is hidden.*

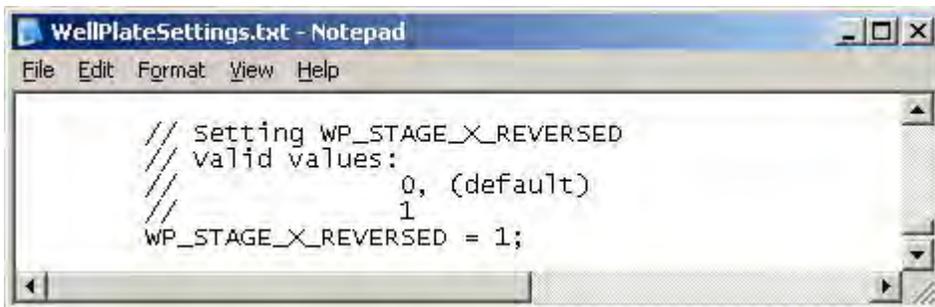
The alignment procedure for each point is the following:

1. Using motorized stage joystick, navigate the stage and align the required point on well plate against the crosshair in live window.
2. Press the corresponding alignment button in the Alignment program group. The button icons and tooltips should help determining button designations.



### Stage Direction

WP\_STAGE\_X\_REVERSED can be set to 0 (false) or 1 (true). When the stage X axis is reversed moving the stage left to right across the sample results in the decrease of the numeric value of the X coordinate.



```
WellPlateSettings.txt - Notepad
File Edit Format View Help

// Setting WP_STAGE_X_REVERSED
// valid values:
//           0, (default)
//           1
WP_STAGE_X_REVERSED = 1;
```