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Multiple Colour Combinations Well-plate Imaging

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Protocol status: Working

We use this protocol and it's working

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Abstract

The following JOB will guide the user through how to align a well-plate on a Nikon Ti2, find the focus and then setup up multiple imaging channels for imaging different color combinations in different wells on a well-plate. The JOB can be easily expanded to add further colors and is annotated within to describe how this can be done.

Materials



Diff_Colour_Rows.bin 25.3KB

The file above is the .bin file containing the JOB.

Nikon Ti2-E microscope

Imaging well-plate (e.g. 96 well-plate)

Fluorescent sample

Imaging light source with multiple colours (e.g. laser bed or LED light source).

PC running NIS elements (NIS5 or 6 recommended).

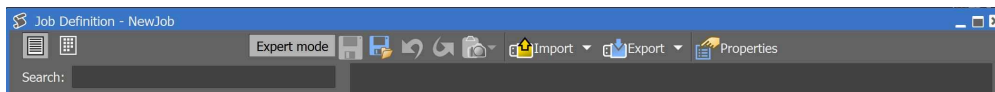
NIS elements JOBs module for automated imaging.

Start-up

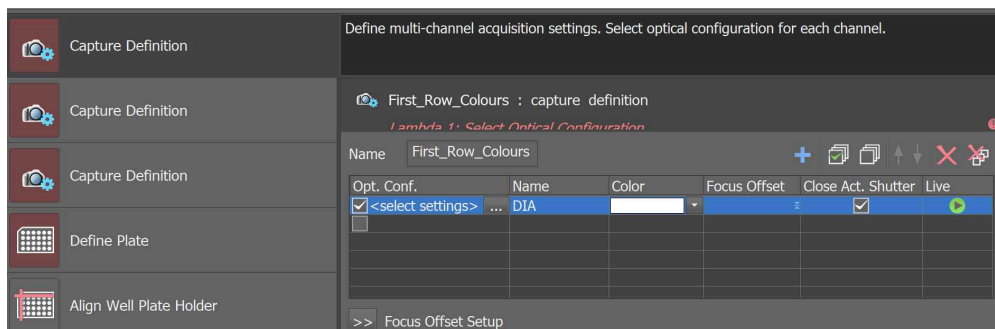
- 1 Power the microscope and open NIS elements software
- 2 Place the well-plate onto the stage holder, select an objective and focus on the first well to be image with the required magnification (e.g. 4x objective).
Note: for scanning large numbers of wells an air objective or water objective with water immersion dispenser is advised.

Running the JOB

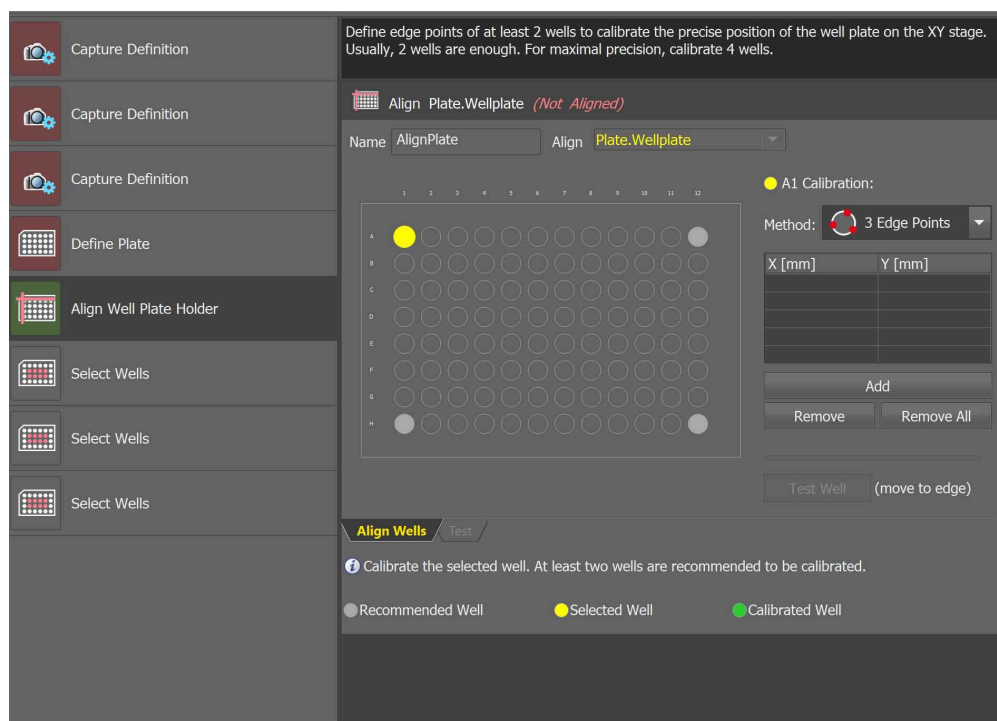
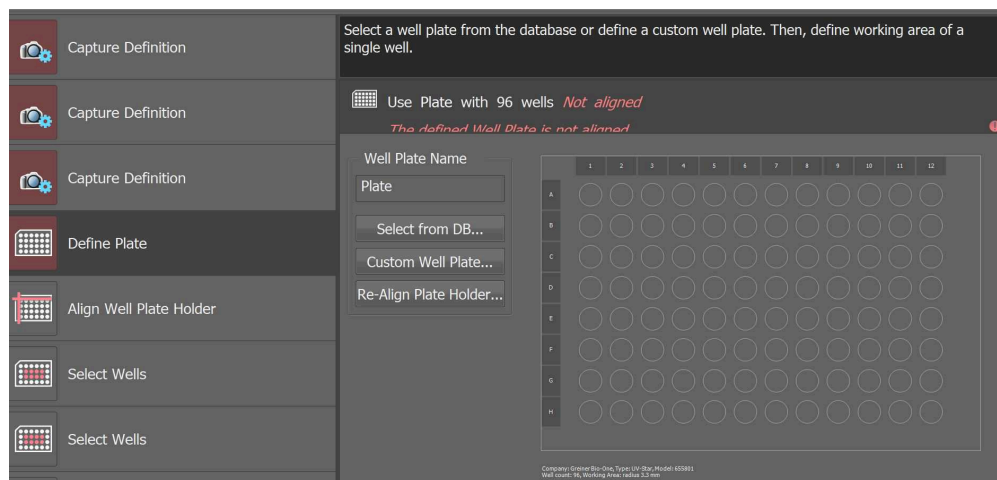
- 3 open 'JOBS' explorer, click 'new' and then 'import'. Import the JOB (in material section of this protocol) from wherever you have saved the .bin file.



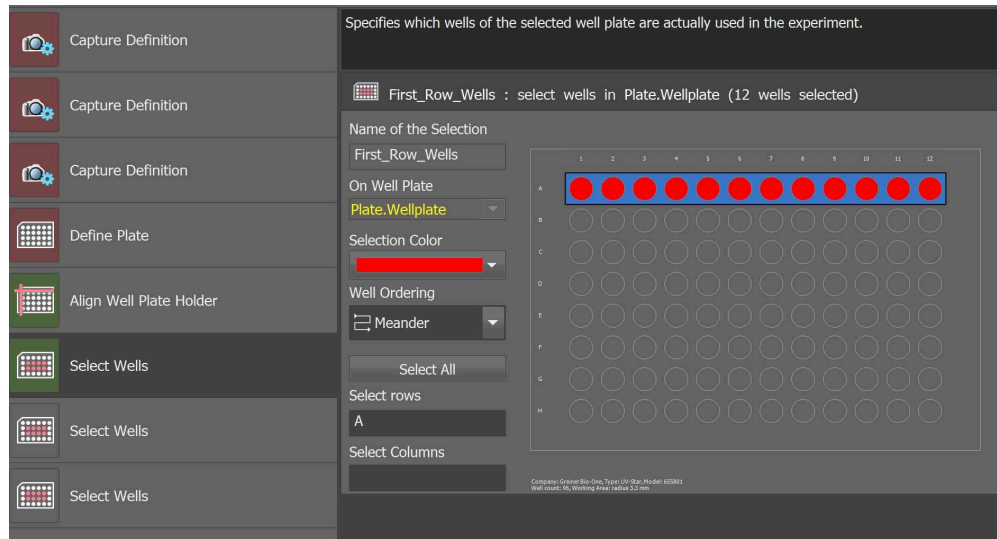
- 4 Run the JOB by clicking the 'play' icon next to the JOB in the JOB explorer window. This will open the JOB GUI and guide you through setting up the colors and rows to be imaged.
- 5 Select the color combinations required for each row of the well-plate by selecting combinations of optical configurations. Note that the first capture definition (contain multiple or single OCs) will be used on the first row and so on.



- 6 Define the well-plate being used by selecting from the database in NIS and align the well-plate by following the guidance in the alignment GUI.



- 7 Select the rows or columns of wells to be imaged with each successive capture definition.



8 Click 'run'

Note by clicking 'edit' on the JOB prior to running, annotations on what each step contains and how these can be duplicated to add >3 color combination and >3 rows / columns can be found:

First_Row_Colours : capture definition
 ! ★

Lambda 1: Select Optical Configuration.

Second_Row_Colours : capture definition
 ! ★

Lambda 1: Select Optical Configuration.

Third_Row_Colours : capture definition
 ! ★

Lambda 1: Select Optical Configuration.

If an already aligned and saved plate (found in the database under custom) is selected then the 'use plate' command will use that plate and further alignment is not needed.

If a new type of plate is being used that was not yet aligned, it can be aligned in the subsequent step.

Use Plate with 96 wells *Not aligned*
! ★

The defined Well Plate is not aligned.

Align Plate.Wellplate (*Not Aligned*)
 ★

Below you can select the required optical configuration(s) (OCs) needed for reach row. If more than three rows are being imaged simply add more:

- capture definitions (1 per row, each can contain one or more OCs as needed)
- well selections (1 per row)
- well loops (1 per row)

First_Row_Wells : select wells in Plate.Wellplate (12 wells selected)
 ★

Second_Row_Wells : select wells in Plate.Wellplate (96 wells selected)
 ★

Third_Row_Wells : select wells in Plate.Wellplate (96 wells selected)
 ★

WELL LOOP ▼

Wells : for each well in First_Row_Wells.Selection

Capture Capture using First_Row_Colours.Definition

WELL LOOP ▼

Wells1 : for each well in First_Row_Wells.Selection

Capture Capture1 using Second_Row_Colours.Definition

WELL LOOP ▼

Wells2 : for each well in First_Row_Wells.Selection

Capture Capture2 using Third_Row_Colours.Definition