

Prerequisites

You are familiar with NIS-elements – use help tool within software and in the manual

Note this script is based on NIS-elements template JOBS scripts

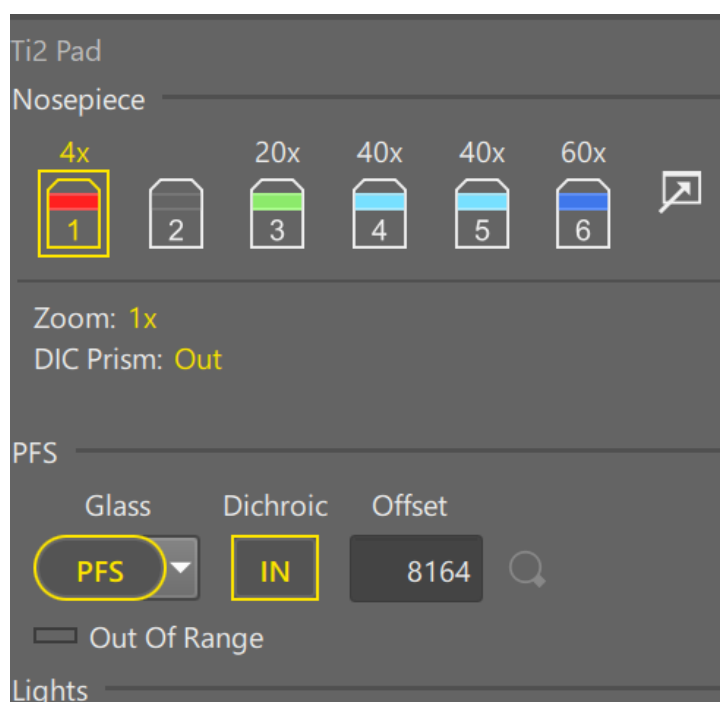
All of the scripts use a 'Wizard' that guides the user to define important parameters before the scripts purpose is carried out.

Use the NIS-Elements help tool to work out how and why you would want to use JOBS scripts.

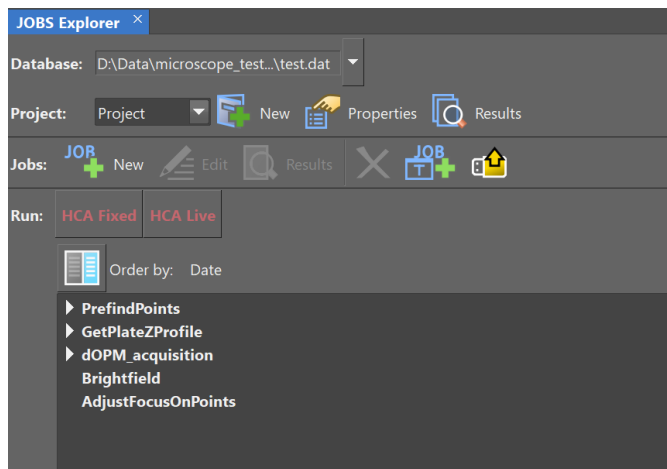
Introduction

The goal of this script is to acquire an image with Nikon's 'Perfect Focus' module engaged with the right-hand port (RHP) wide-field camera's field of view in the centre of every well in the plate. The goal is not to collect images but to visualise the metadata associated with each image – we want the 'Z' value for every well with the perfect focus engaged and for a fixed perfect focus offset. We can use JOBS results viewing functionality to navigate through the metadata and visualise the z value per well on top of a picture of the plate map. We can use this to check if the plate insert is flat, if the plate is flat, if the plate is tilted etc. We can also use this to see if the wells are curved – the profiles of the plate bottoms can be concave across each wells surface. How warped the plate is important for whether we can image with the short working distance 60x objective with the dOPM optical configuration.

Select a long working distance objective x4-20x that works well with perfect focus

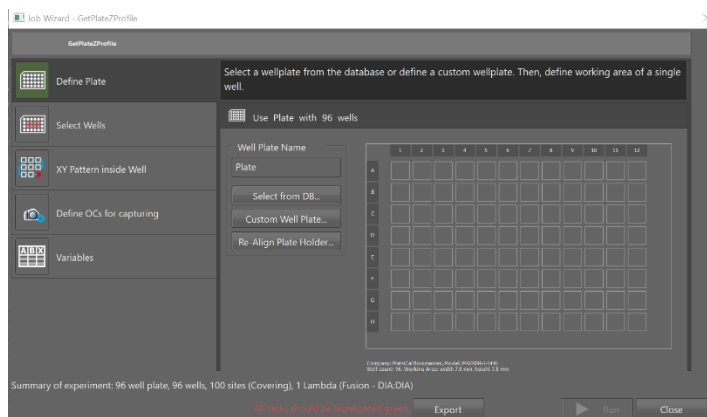


Run GetPlateZProfile JOBS script



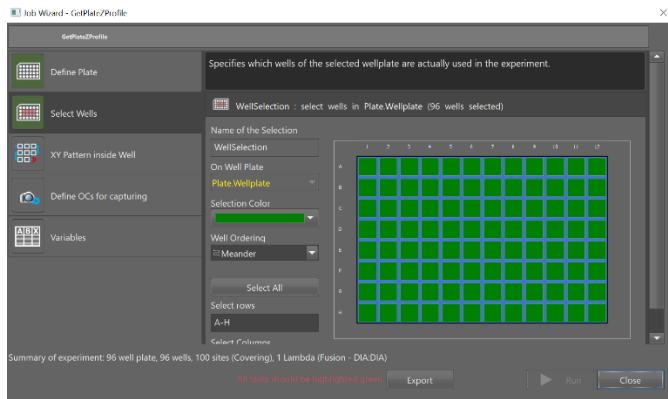
- Select the 'GetPlateZProfile' JOBS script from within the JOBS explorer
- Use the NIS-Elements help tool to work out how and why you would want to:
 - Use JOBS explorer

Select multi-well plate



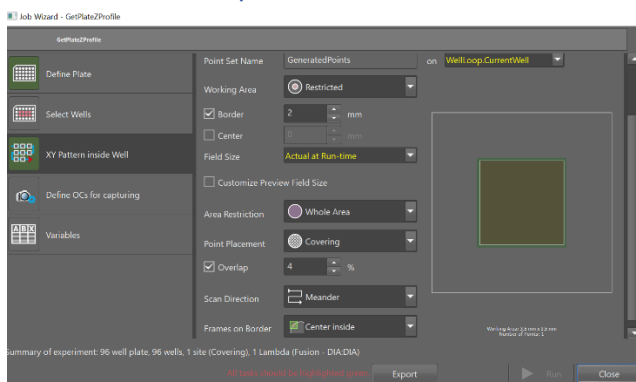
- Assuming the stage is initiated, and the plates have been calibrated with the stage
- Selected the plate you are working with or create a custom design if it is not in the NIS-Elements database
- Use the NIS-Elements help tool to work out how and why you would want to:
 - Initiate stage
 - Calibrate plate
 - Create custom plate design

Select wells



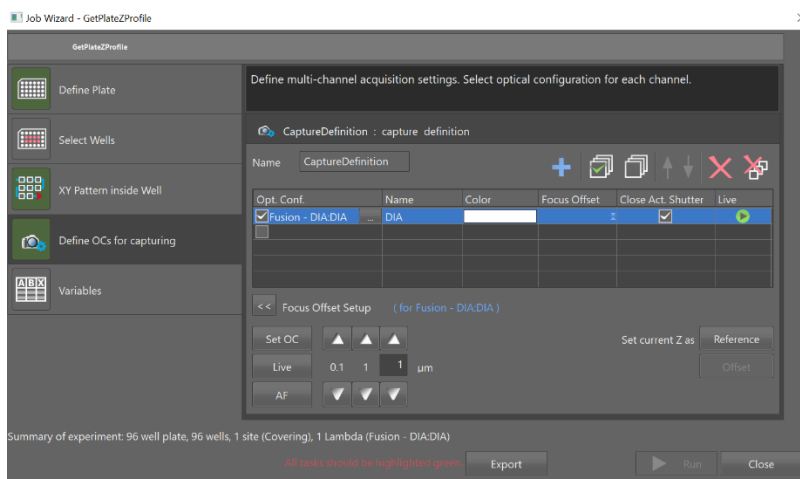
- We want a plate height map – select all the wells in the plate

Set well scan pattern



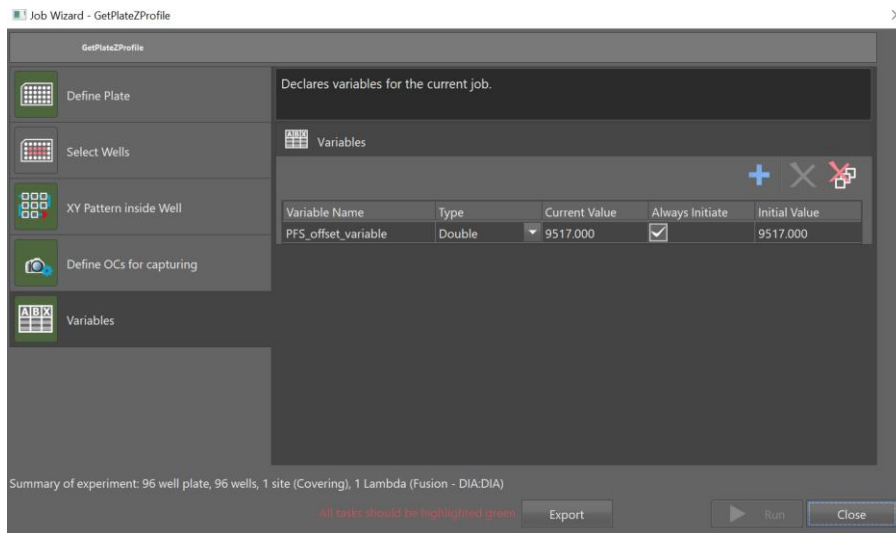
- Select the tile scan pattern to apply to each well.
- Use the NIS-Elements help tool to work out how and why you would want to:
 - Understand how the different parameters affect the 'GeneratedPoints' function in the above screenshot.
 - Essentially this function generates a list of relative points per well for tile scanning based on the plate you are using

Set widefield epi/trans-illumination mode



- Choose the type of wide-field imaging mode you want to use using NIS-Elements JOBS function 'CaptureDefinition'
- Use the NIS-Elements help tool to work out how and why you would want to:
 - Understand how 'CaptureDefinition' function works
- For the purpose of this script it does not matter - choose short exposure time brightfield image for fast acquisition

Set PFS offset

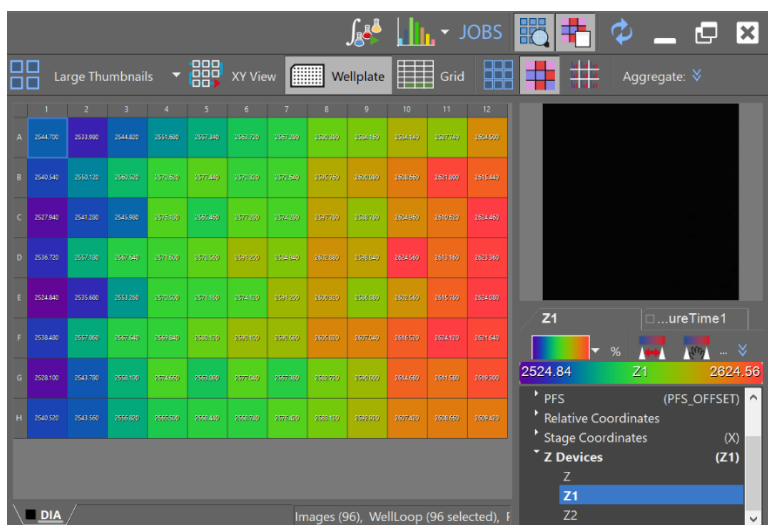


- This JOBS script uses Perfect Focus to acquire z-stacks relative to the plate bottom
- Make sure the Perfect Focus Offset is set to the bottom of the

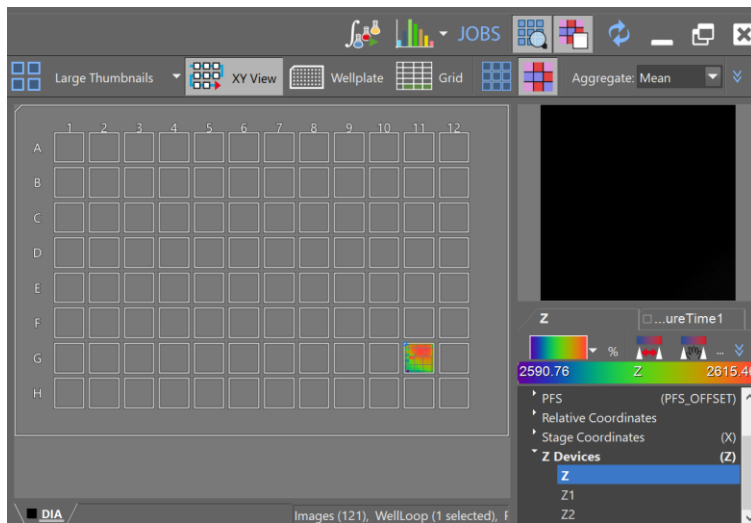
Use the NIS-Elements help tool to work out how and why you would want to:

- Perfect Focus and Perfect Focus Offset

Run acquisition



- Example of JOBS results viewing tools to get plate height map



- Example of JOBS results viewing tools to get plate height map for single well
- In this case we only select one well in the well selection step above
- Then for the 'set well scan pattern' section above we choose an array of points across a single well to get a profile of the well. The heatmap shows the well is concave.

Assumptions

- The 'Perfect Focus Offset' is set to a value that is coincident with the sample side surface of the bottom of the plate's wells.
- Using a x4 to 20x air objective, not a short working distance high NA objective like a water immersion 60x
- The script needs to start with perfect focus in range i.e. perfect focus can engage and track from the start of the script