

User guide- Prefind JOBS Script

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v1.00

25/05/2023

Prerequisites

- You are familiar with NIS-elements – use the help tool within NIS-elements and the NIS-Elements manual.

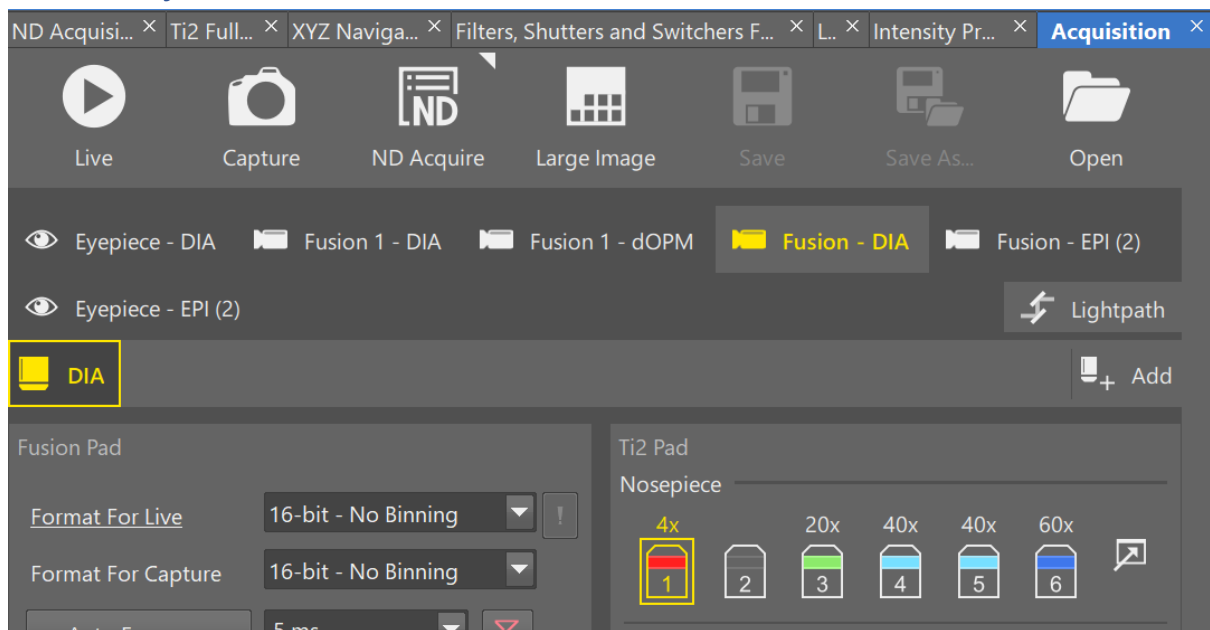
Notes

- This script is based on a NIS-Elements template JOBS script.
- The NIS-Elements help tool helps explain how you can use JOBS scripts and gives examples of why they are useful.

Introduction

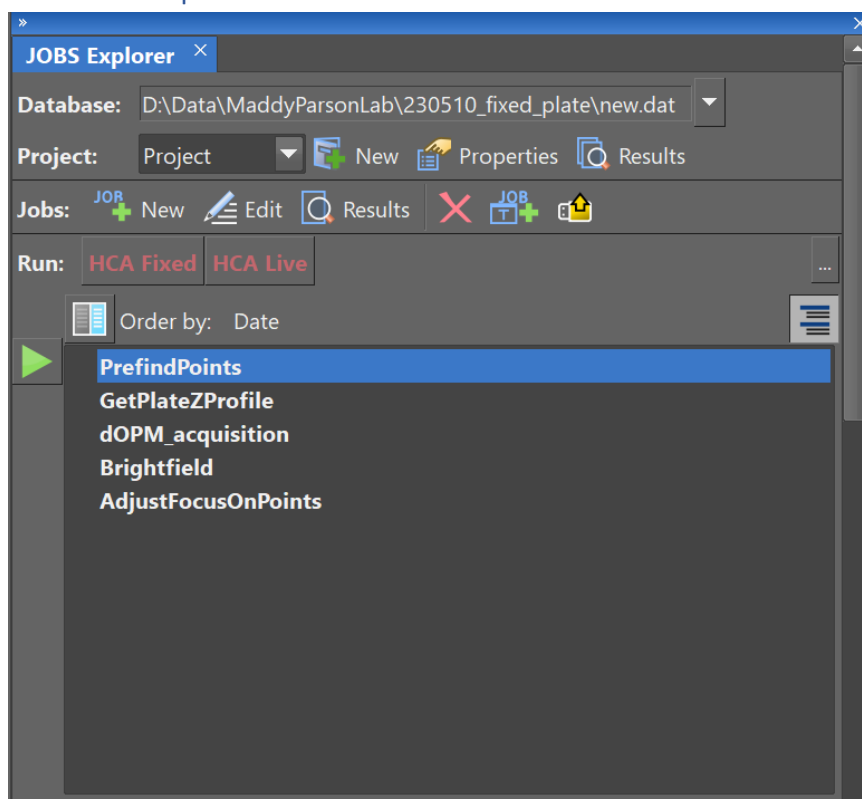
- The purpose of this JOBS script is to scan a multiwell plate with a low magnification air objective such as a 4x/10x/20x.
- Typically, the area of interest across each well is larger than the field of view of the chosen objective, so we need to do a tile-scan.
- Brightfield imaging or epifluorescence z-stacks are acquired with the right-hand port wide-field camera. Using brightfield is desirable as it is fast and low light dose to the sample. Epifluorescence may be required depending on what you are looking for.
- This script uses Nikon's Perfect Focus System (PFS) module to lock the microscope objective's z-position relative to the top surface of the coverslip that forms the base of the plate. This provides a reliable way to acquire z-stacks relative to these surfaces and can be used ensure that the regions of interest generated are within the working distance of the dOPM 60x objective.
- If regions of interest found with the air objective are beyond the working distance of the dOPM water immersion 60x objective then the dOPM 60x objective will hit the plate.

Select objective



- Go to acquisition tab in NIS-Elements and select a suitable low magnification air objective considering the field of view and resolution requirements for the prefind. The higher the magnification, the smaller the field of view and the longer it will take to acquire the prefind.

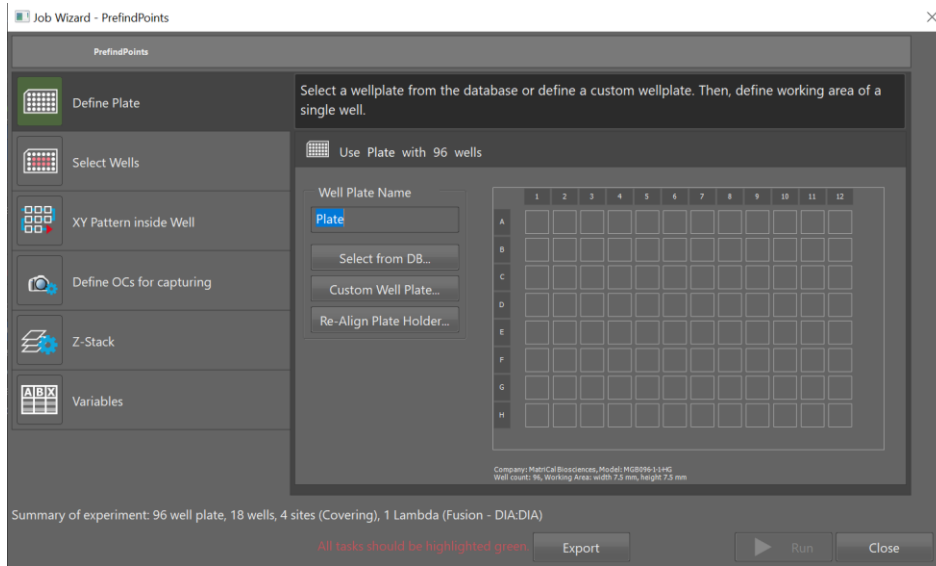
Select script



- Select the 'PrefindPoints' JOBS script from within the JOBS explorer.
- Use the NIS-Elements help tool for:
 - Help using JOBS explorer.

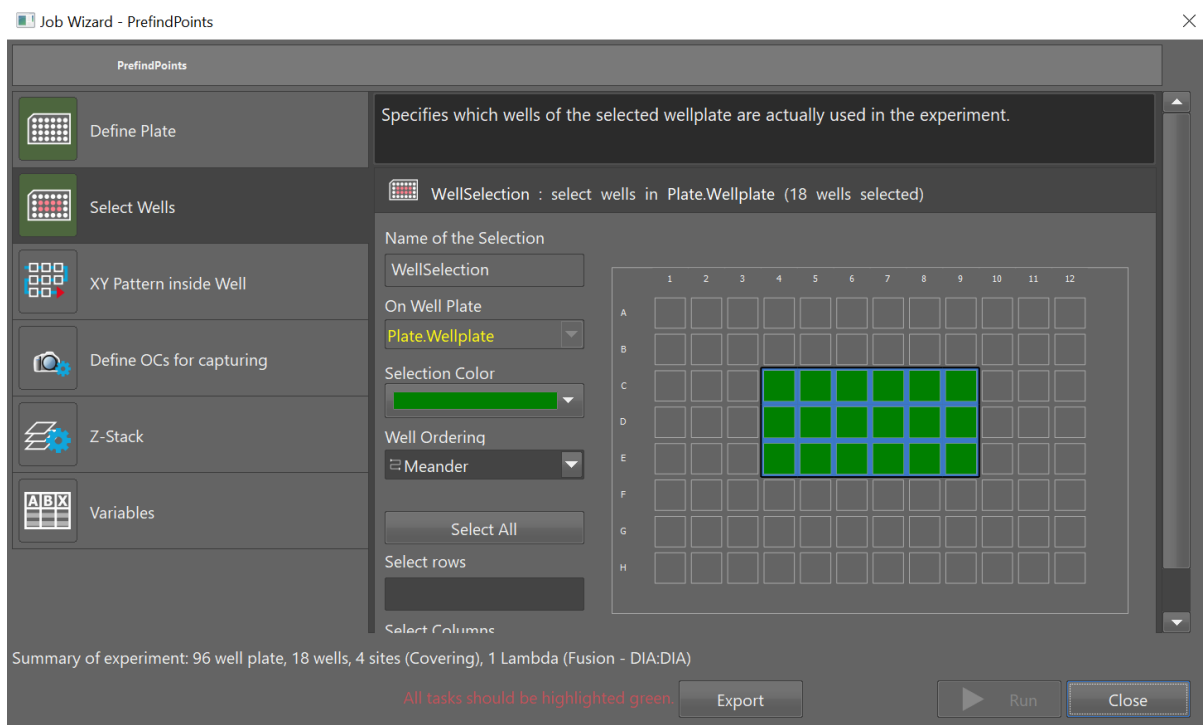
- Using JOBS scripts.

Select plate type



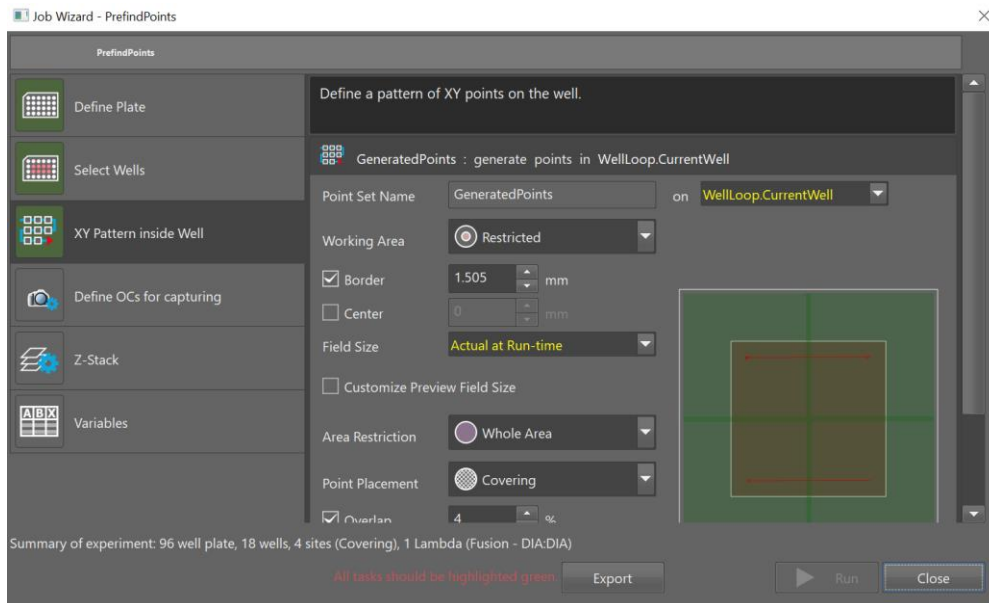
- Select the plate type you are working with, or create a custom design if it is not in the NIS-Elements plate database.
- Check that the Märzhäuser x-y stage has been initialised.
- Check that the plate has been calibrated with the stage.
- Use the NIS-Elements help tool for help on:
 - Initialising the stage.
 - Calibrating the plate.
 - Creating custom plate designs.

Select wells



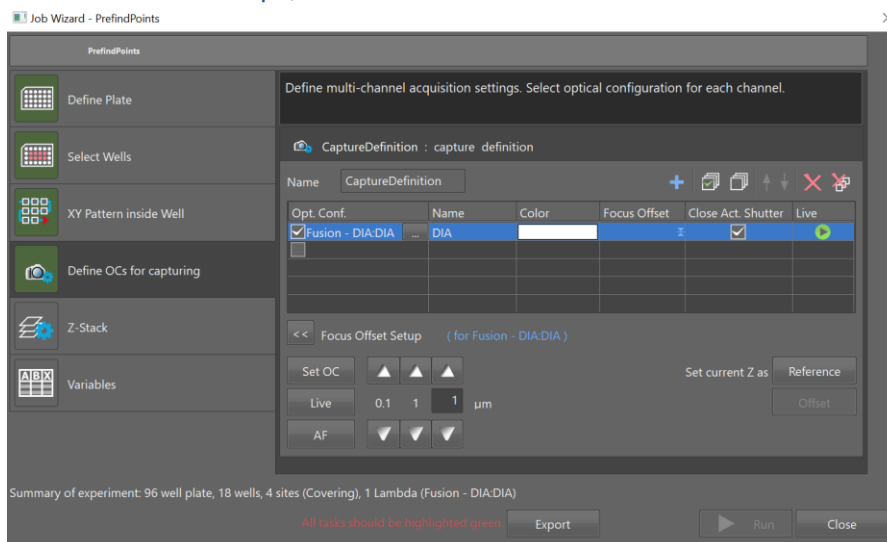
- Select the wells you plan to image, e.g. you may have a plate-map of the wells you seeded, you might want to select all of these wells for prefind.

Set well scan pattern



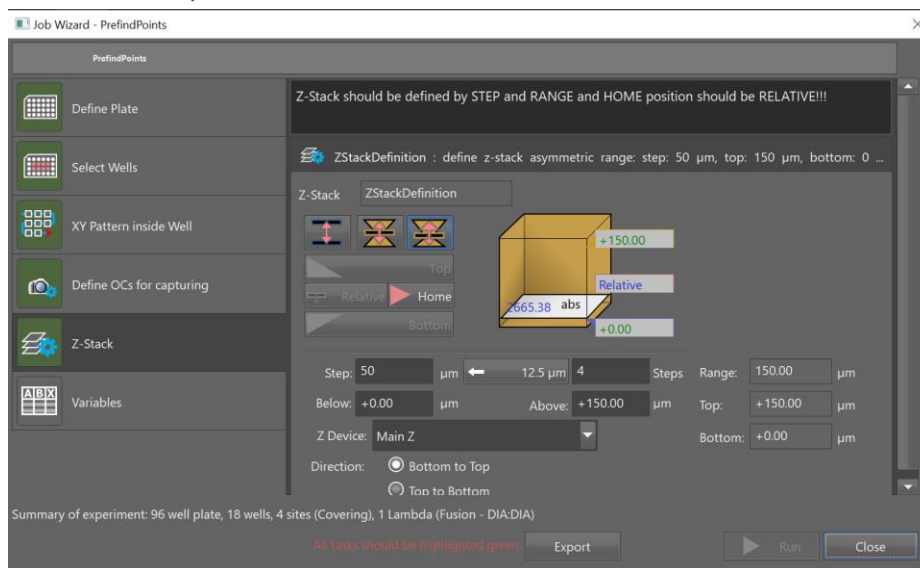
- Select the tile scan pattern to apply to each well.
- Use the NIS-Elements help tool to:
 - Understand how the different parameters affect the 'GeneratedPoints' function in the above screenshot.
- Essentially this function generates a list of relative points per well for tile scanning based on the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

Set widefield epi/trans-illumination mode



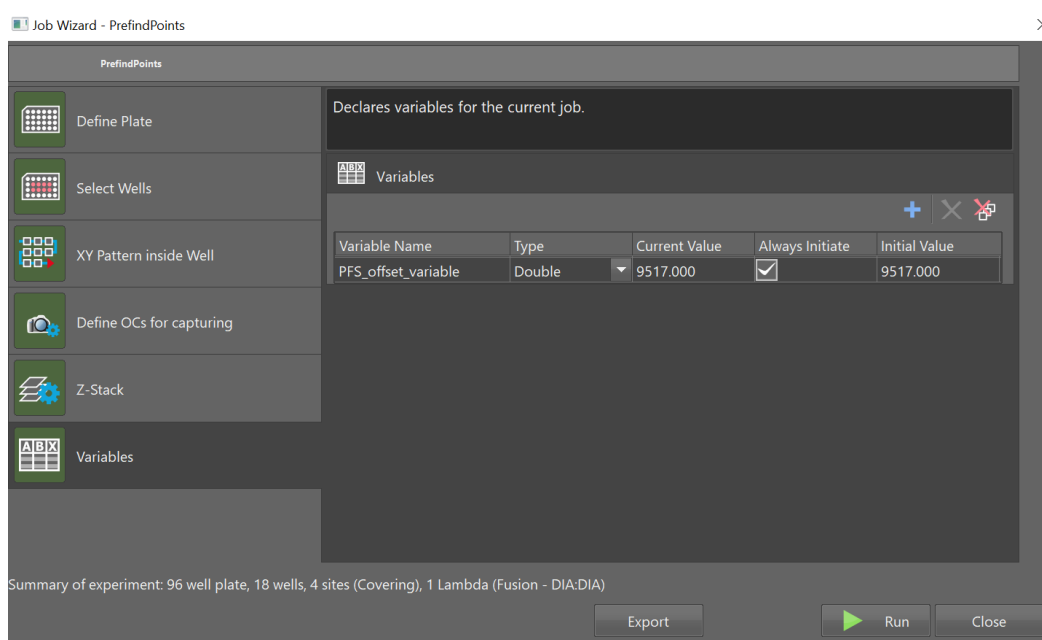
- Choose the type of wide-field imaging mode you want to use using the NIS-Elements JOBS function 'CaptureDefinition'.
- Use the NIS-Elements help tool to understand how the 'CaptureDefinition' function works.

Set z-scan pattern



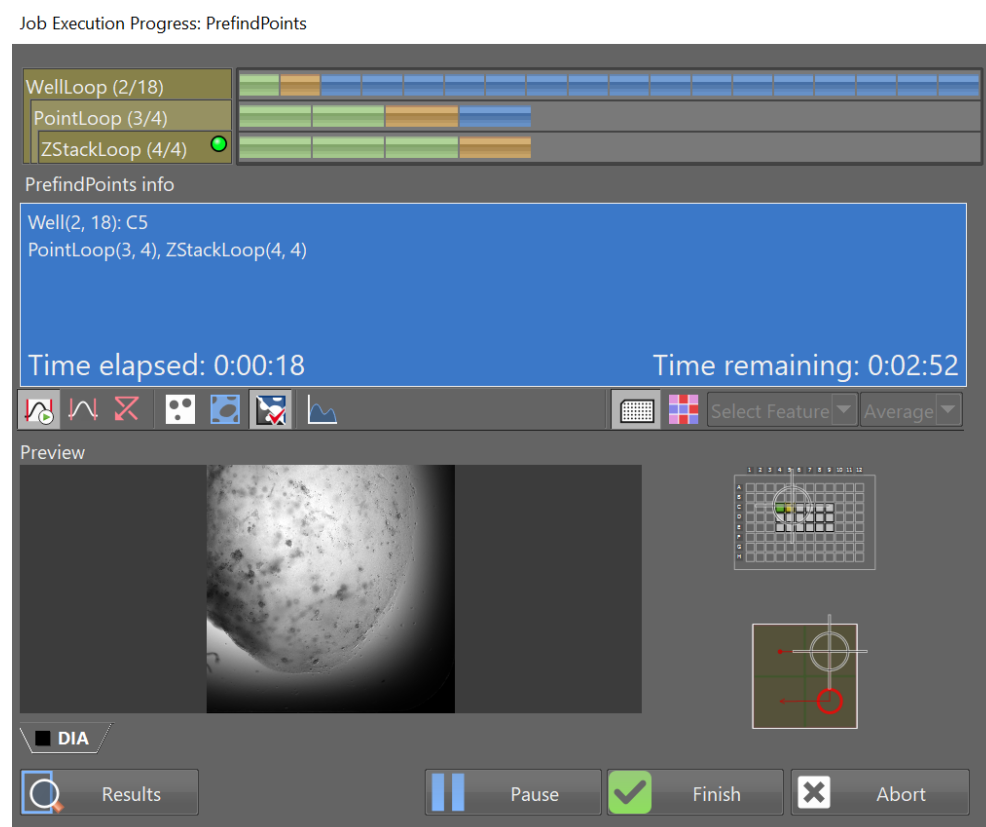
- Choose the z-scan pattern – typically fewer than 10 planes ranging from 0 µm to less than 280 µm relative to the coverslip-sample interface (280 µm is the working distance of the 60x objective currently used in the dOPM system).
- Note – for this prefind, we use the Nikon Perfect Focus System to collect z-stacks relative to the interface between the top of the coverslip and the sample. This allows the user to ensure that the prefind will only scan up into the sample by a user-defined distance. This can be used to ensure that prefind data is restricted to depths less than the 280 µm working distance of the 60x water immersion microscope objective used during dOPM acquisitions.**
- Use the NIS-Elements help tool to help understand:
 - How the 'ZstackDefinition' function works.
 - How the Nikon Perfect Focus System works.

Set PFS offset



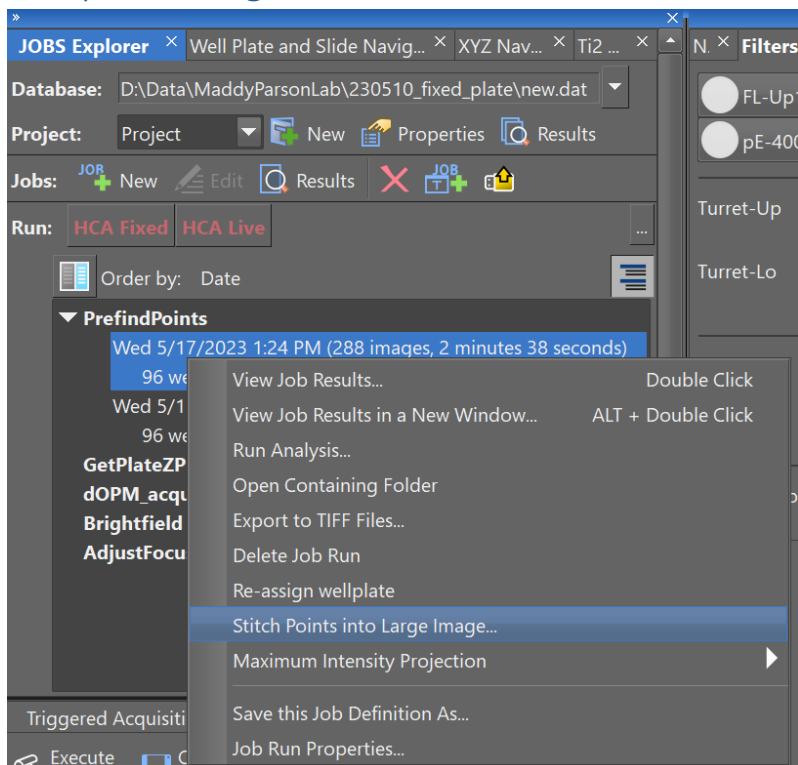
- The Prefind_JOBS_script uses the Perfect Focus System to acquire z-stacks relative to the top of the coverslip.
- Make sure the Perfect Focus Offset is set to the top of the coverslip.
- Use the NIS-Elements help tool to help understand:
 - Perfect Focus and Perfect Focus Offset.

Run acquisition

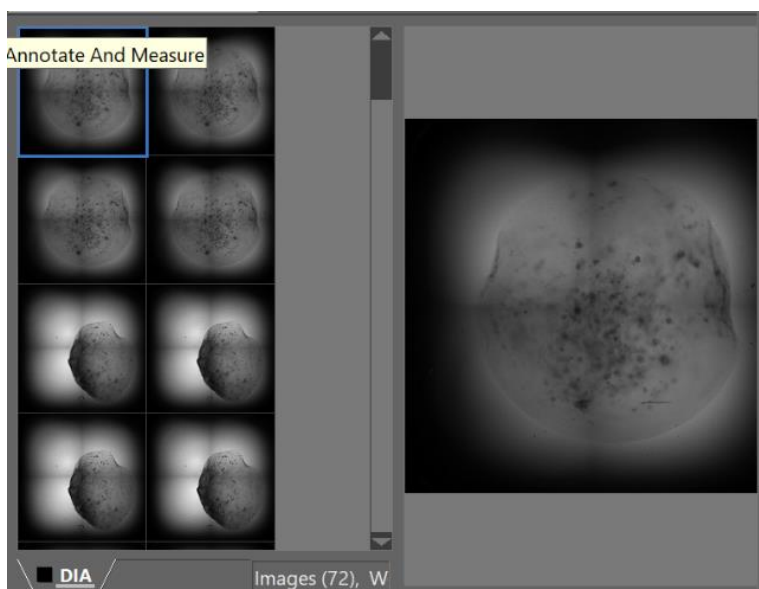


- Run the acquisition. Depending on the number of tiles, z-planes and spectral channels the acquisition will take some time. Fast brightfield imaging with 6 or fewer z-planes (e.g. 0 to 150 μm in steps of 30 μm) and 20x or 4x magnification objective lenses is recommended.

Postprocessing – stitch tiles



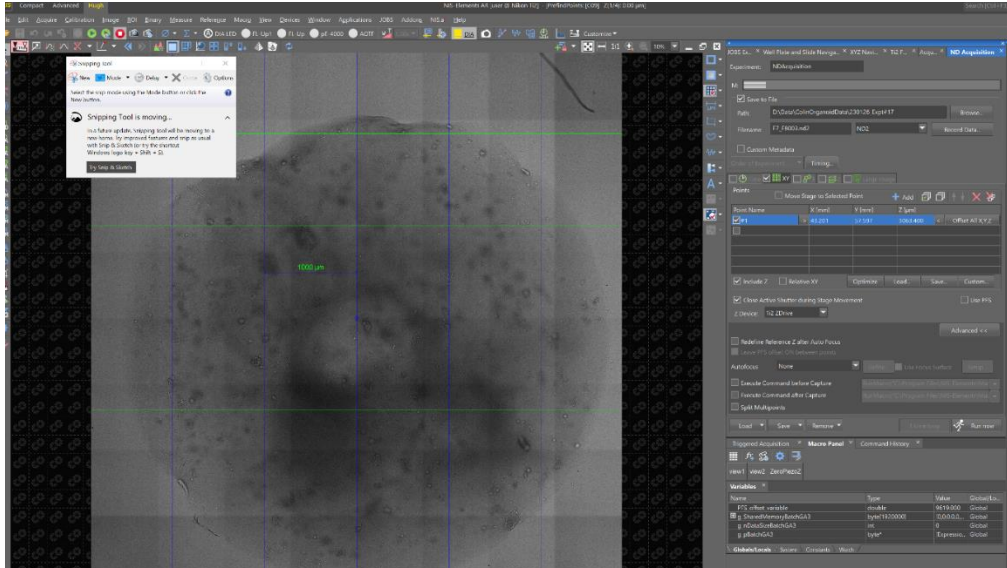
- Once the data is acquired, right-click on the JOBS Explorer event description and select the 'Stitch Points into Large Image...' option.
- Use the NIS-Elements help tool to:
 - Provide information about JOBS explorer.
 - Find out about the stitching and other postprocessing options.



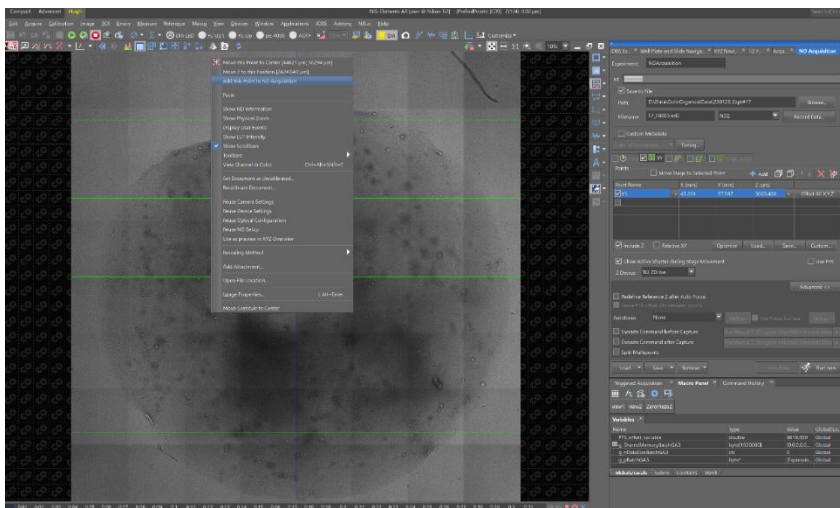
- Above is an example of the output of some stitched brightfield data showing a blob of gel with organoids embedded. This shows data acquired with a 4x objective, with a 2x2 grid of tiles stitched together resulting in a final image field of view of $\sim 5 \times 5 \text{ mm}^2$.

- Use the NIS-Elements help tool for help on:
 - The functionality of JOBS Results

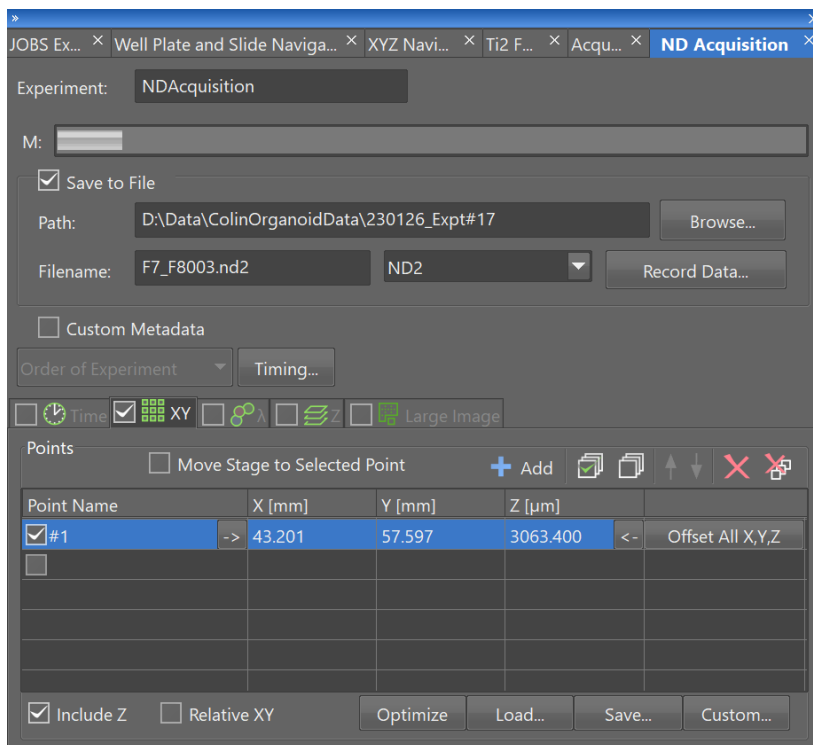
Navigate prefind data for manual selection of positions for subsequent dOPM imaging



- Once the prefind data has been acquired and stitched, you can manually locate objects in xyz using the stitched data.
- In the image above, a grid has been placed on the image to help keep track of sampling. This can be added using the 'show graticule' option on the live image toolbar in NIS-Elements.
- On the righthand side a position list can be populated. This position list is part of the 'NDAcquisition' tab – xyz position lists can be generated, imported and, saved from this tab.
- Use the NIS-Elements help tool to provide more information on:
 - NDAcquisition.

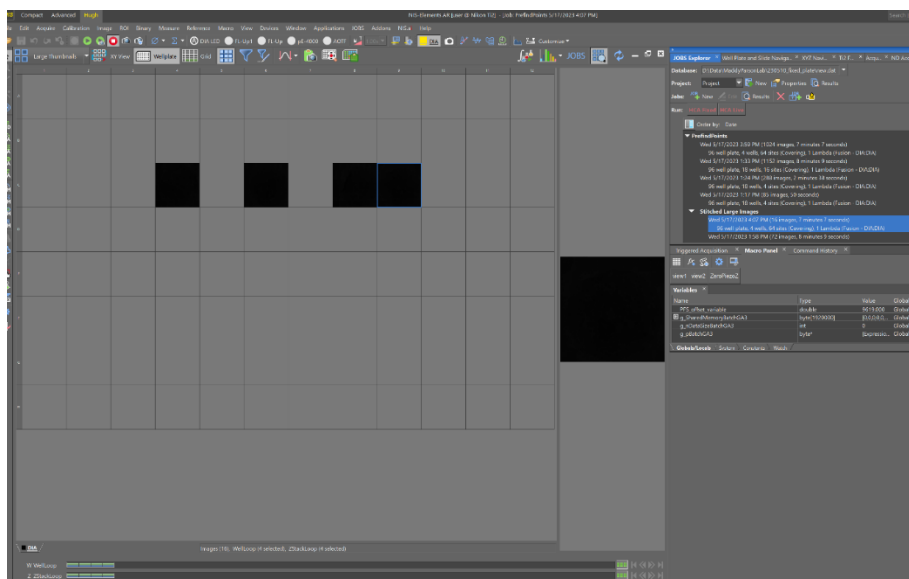


- Right-click on the stitched image data and then select the option to 'Add this point to ND Acquisition'.
- Repeat this step to populate the 'NDAcquisition' tab position list on the righthand side of the screen for all of the positions that you wish to image with dOPM.

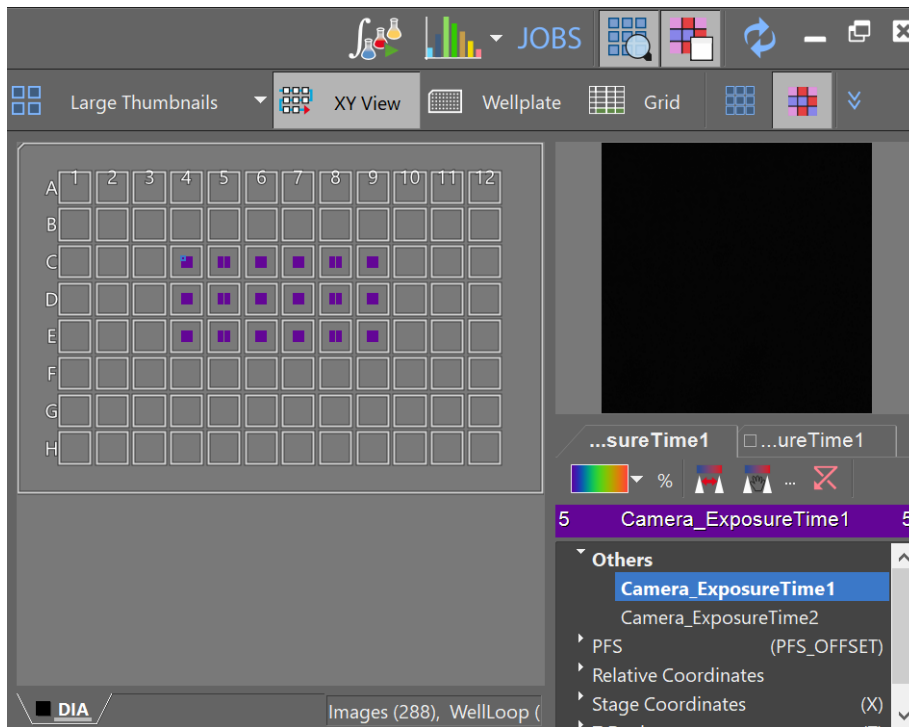


- The image above shows the 'NDAcquisition' tabs position list with one point in list.
- You can load and save position lists as .xml or .csv files.
- In particular, .csv files can be used to generate position lists from third-party software tools, which could be useful for custom image acquisition routines.

Use JOBS Results Viewer to navigate data



- JOBS Results Viewer can be used to present the acquired data in a number of useful ways that can aid sample navigation.



- JOBS Results Viewer can be used to browse the image data and to see image metadata, which can be useful for navigating and getting summary information about an acquisition.

Use the NIS-Elements help tool to:

- Learn about the functionality of the JOBS Results Viewer

Assumptions and notes

- The 'Perfect Focus Offset' is set to a value that is coincident with the top of the coverslip that forms the base of the plate's wells.
- The user accounts for well surface curvature and plate curvature when working out how deep it is possible to image beyond the top surface of the coverslip without the 60X objective hitting the plate. For example, if the coverslip is curved within each well – i.e. the bottom of the plate appears 'dimpled' when the reflection of light from a window or lamp off the bottom of the plate is viewed by eye – then as the front surface of the 60x dOPM objective is comparable in size to a well, it may not be possible to focus as far into the centre of the well as the edge of the well without the 60x objective hitting the bottom of the coverslip.
- The script needs to be started with the Perfect Focus System in range, i.e. that the perfect focus system can engage and track the top surface of the coverslip from the start of the script.