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.

Contents

Introduction

This script loops over a position list that you intend to use with a dOPM acquisition and refines the xyz values so that the region of interest (ROI) is centred in xyz on the right-hand port (RHP) wide-field camera. The dOPM optical path on the left hand port (LHP) is aligned with the RHP – zero remote refocus with dOPM images a plane rotated about the plane imaged by the RHP. This way if centred an object in xyz with the RHP it would also be centred on both views of dOPM on the LHP.

- The main purpose of this script is to refine the xyz values in a position list when switching from a course prefind method to the high resolution dOPM optical setup.
- For example, switching from manually finding things with a low-resolution air objective
- This list could have been generated with the 'PrefindPoints' list – see other guide on this topic.
- The user manually adjusts the focus for each point in the list.
- The script outputs a position list to the 'NDacquisition' modules point list tab
- The list is then manually saved as an xml or csv file

Select 60x objective for dOPM acquisition
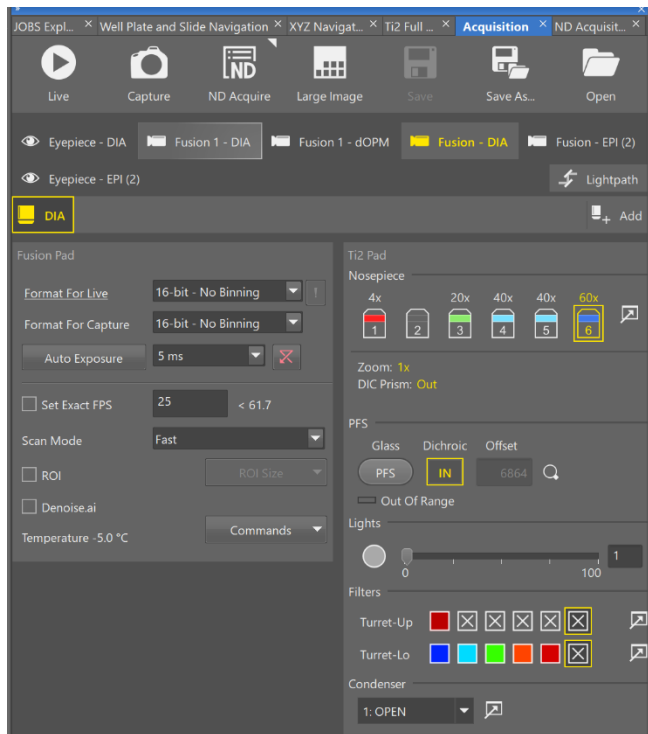
- At this point it is assumed you have completed setting up the acquisition in terms of using dry objectives i.e. prefind is done, plate is calibrated to stage
- Now change the objective to the 60x water immersion objective used in the dOPM optical configuration.

## Run AdjustFocusOnPoints JOBS script
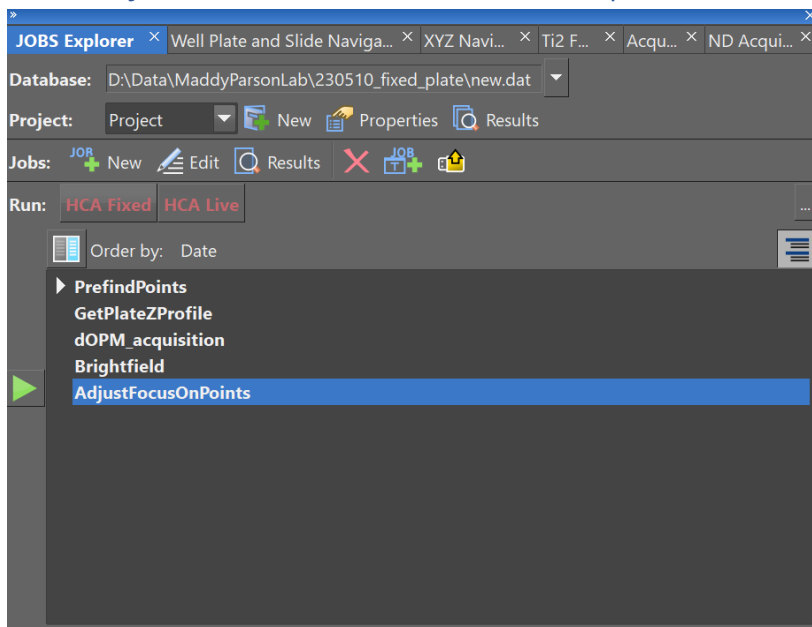
- Run the 'AdjustFocusOnPoints' JOBS script
- Use the NIS-Elements help tool to work out how and why you would want to:
 - Use JOBS explorer

Set z_retract parameter
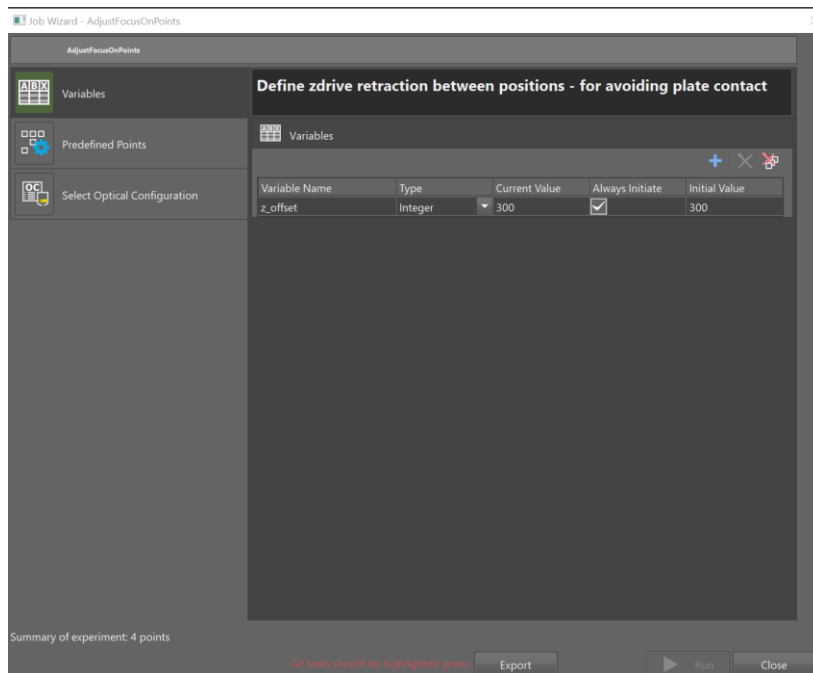
- Set the z_retract variable to a number that avoids the chance of the objective colliding with the plate during stage movements.
- Here a conservative 300µm is used but depends on the plate being used – see JOBS script 'GetPlateZProfile' for a way to measure plate flatness.

Load position list to iterate over
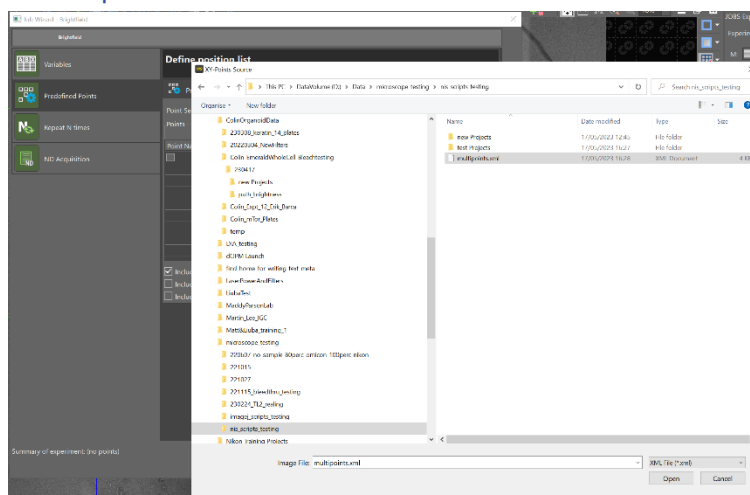
- The script assumes the user has already generated an xyz position list. Load this list in the next step of the wizard.
- Use the NIS-Elements help tool to work out how and why you would want to use a:
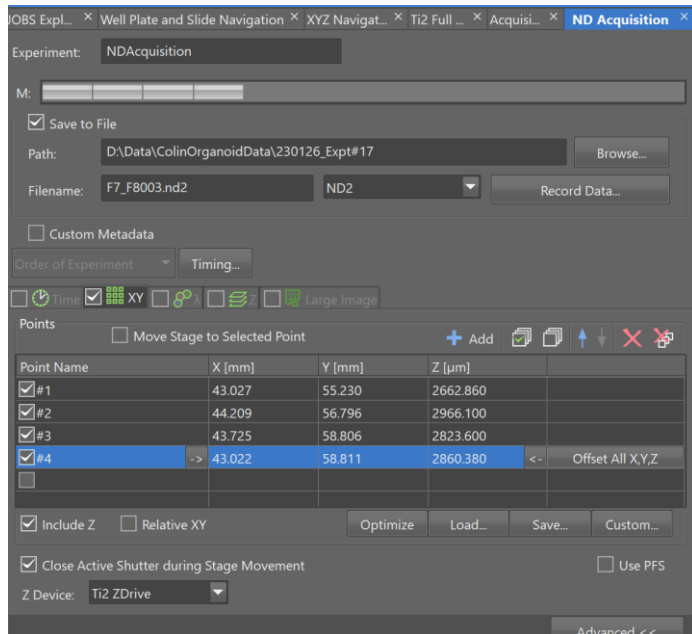 o position list

Define wide-field imaging
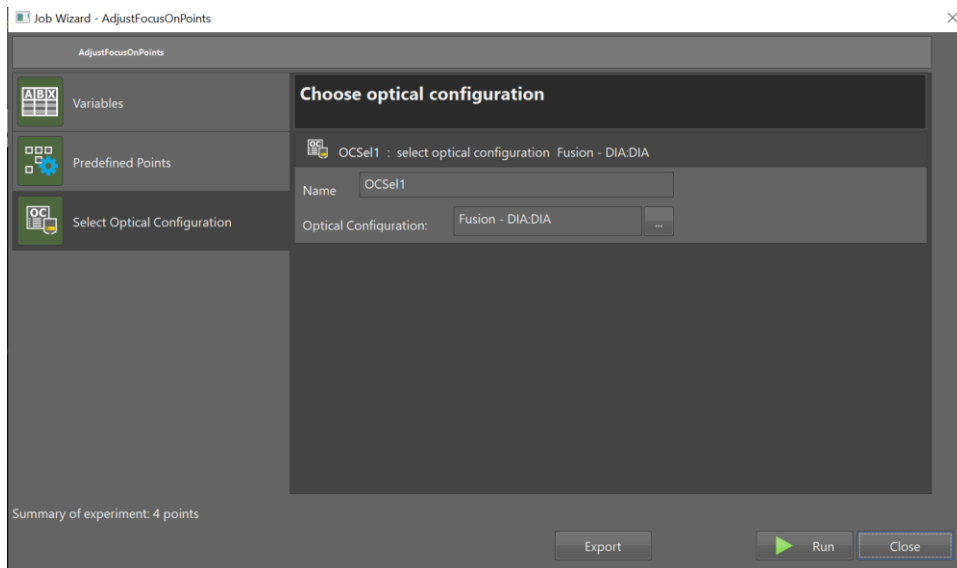
- Choose the wide-field imaging mode – in this case it is brightfield transillumination (diascopic)

Adjust focus with 60x dOPM objective
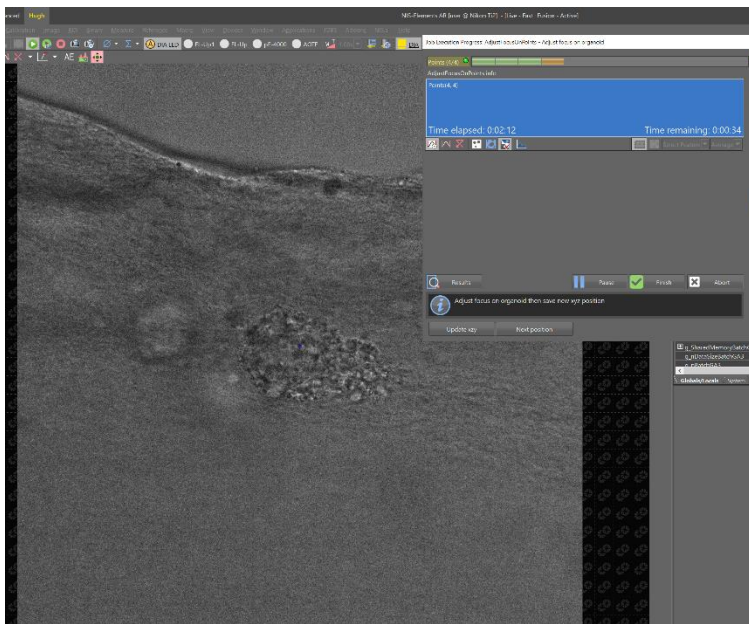
- Run the script. There are two buttons involved in adjusting the positions as you iterate through the list.
- Button => 'update xyz' – to update a position first recentre in xyz the view using the live image then press this button. The script then applies the new position values to the current position in the list and moves onto the next entry.
- The second is 'next position' – you'd press this button if the existing entry is adequate. In this case the entry remains unchanged, and we move to the next position

Manual saving of generated 'NDAcquisition' tab position list values

- Once you have iterated through the list the script exports the updated position list to the 'NDAcquisition' modules point list
- This list can be saved for later use by using the 'save button' in the module
- Use the NIS-Elements help tool to work out how and why you would want to find out about:
  - NDAcquisition

Script Assumptions

- **The system is thermally stable during the position list refinement**
- **You are happy to manually find and refine the position list – this solution is not scalable! Can work for a few hundred points…**
- **Positions to be updated are reasonably close to ideal otherwise you may end up refining the position on a different region of interest**