

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

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

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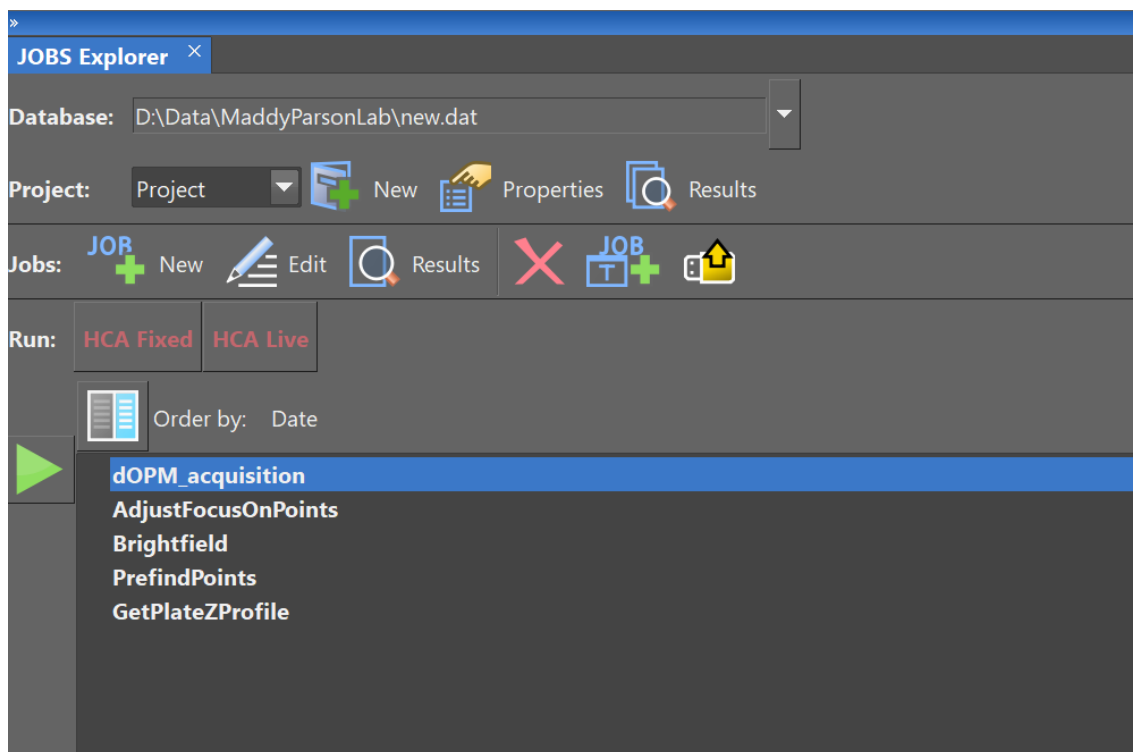
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Introduction

NOTE: this script makes use of a logical device called z-piezo in NIS-elements but not for its intended purpose. Normally this device is attached to the microscope objective for fast z-positioning. Here we use it for remote refocussing within dOPM's external optics on the left-hand port of the microscope frame. With this in mind we only use z-piezo when doing dOPM volumes scans.

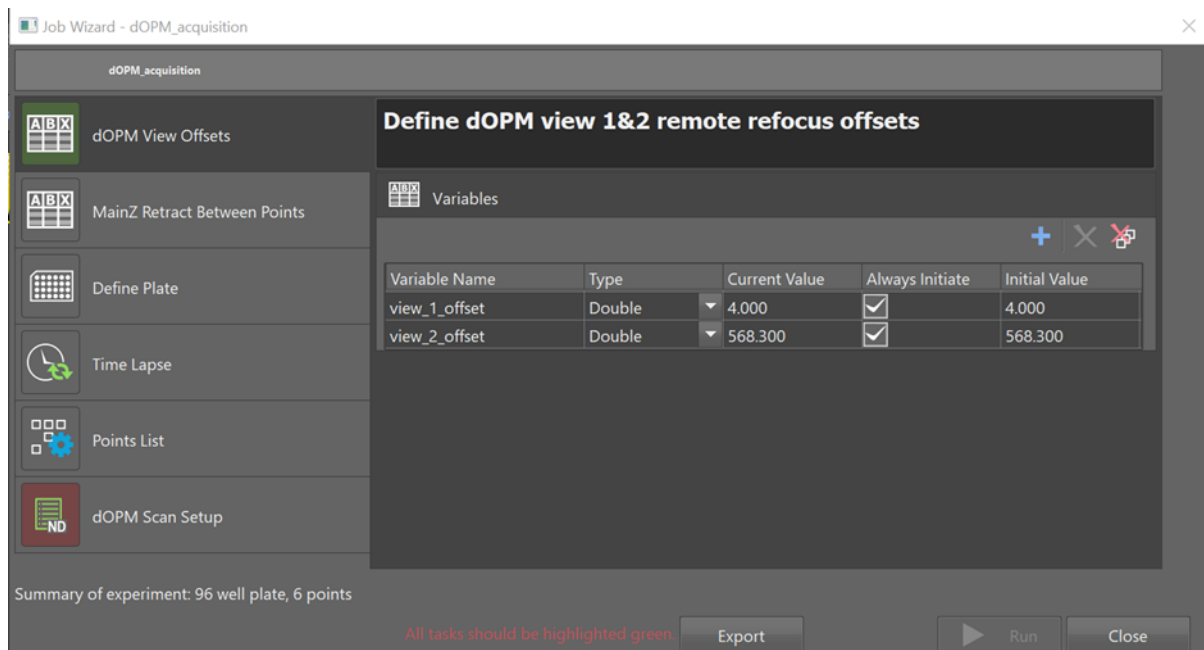
- **Any other operations involving z-positions i.e., the normal use of the microscope, with the right-hand port should be carried out with z-piezo set to the home position which is zero.**
- For example, if we want to record a position list of interesting locations in the sample, we first make sure the z-piezo is set to zero otherwise NIS-elements sets the target position as main z-drive value + z-piezo drive value i.e. $Z_{Total} = Z_1 + Z_2$. This is not physically meaningful since the z-piezo drive (Z_2) is not attached to the microscope objective. For any position list we always work with $Z_{Total} = Z_1$

Run acquisition JOBS script



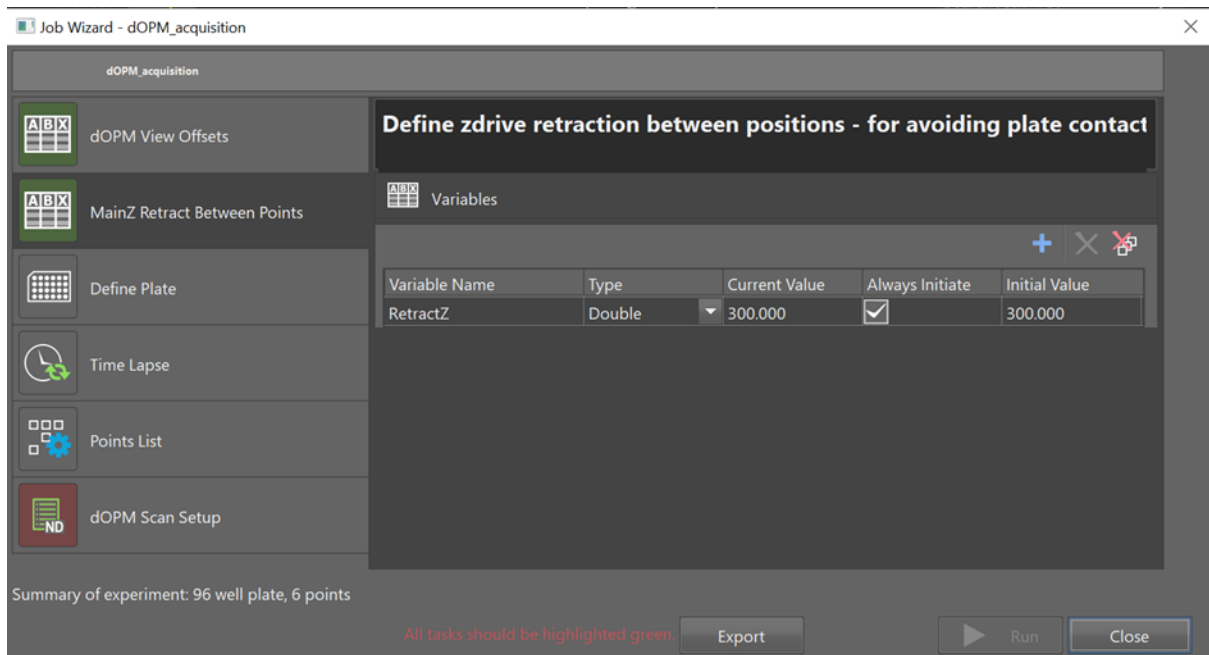
- Run the 'dOPM_acquisition' JOBS script
- Use the NIS-Elements help tool to work out how and why you would want to:
 - Use JOBS explorer

defined dOPM view 1&2 refocus offsets



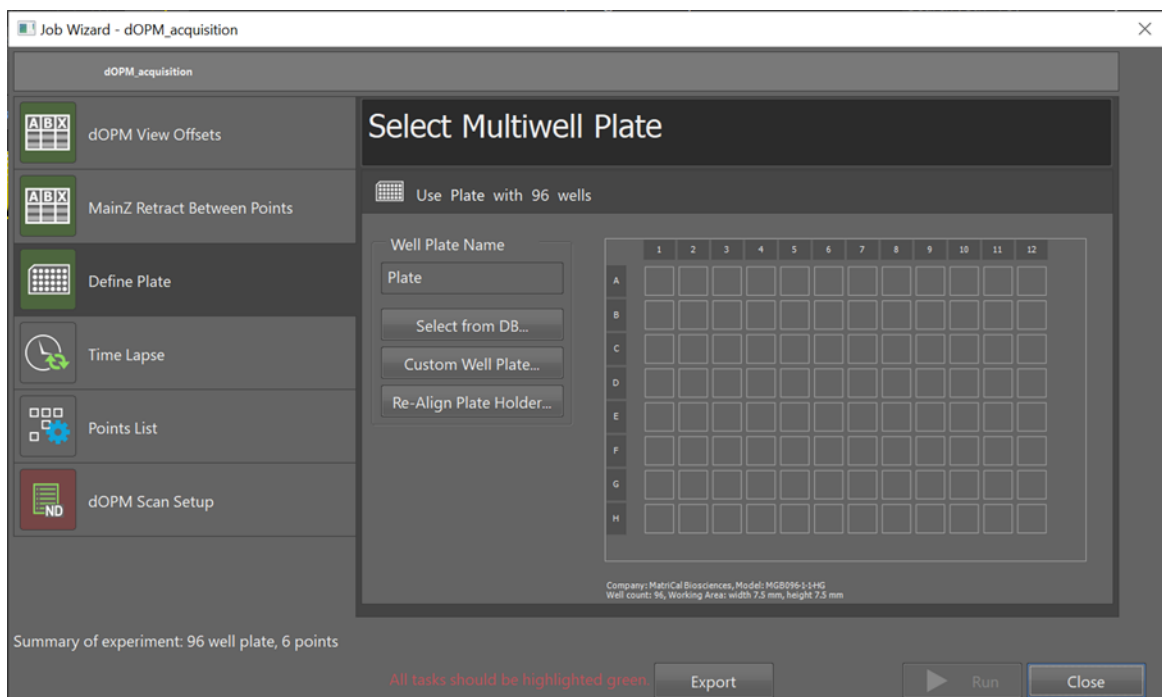
- Enter the zero remote refocus offset values for dOPM

Set z_retract parameter



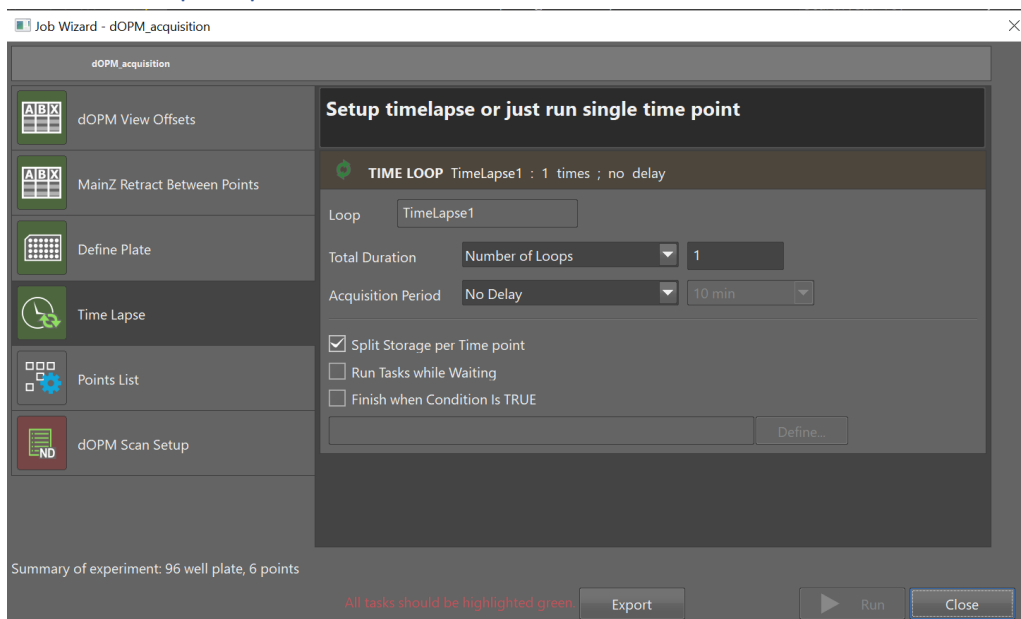
- Set the z_retract variable to a number that avoids the chance of the objective colliding with the plate during stage movements.
- Here a conservative 300µm is used but depends on the plate being used – see JOBS script 'GetPlateZProfile' for a way to measure plate flatness.

Select multi-well plate



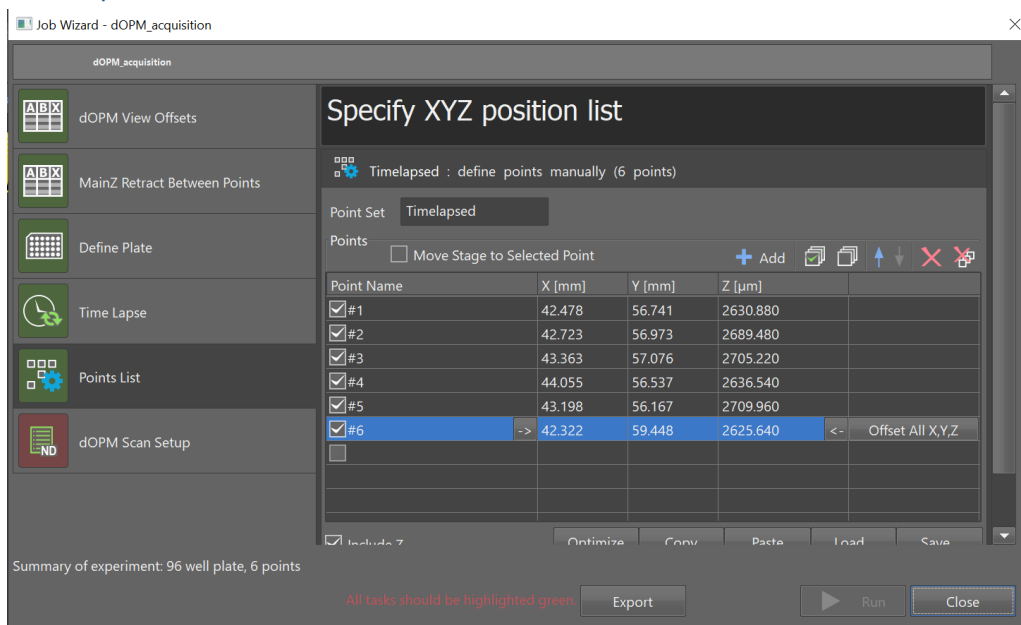
- choose correct multi-well plate definition

Set timelapse parameters



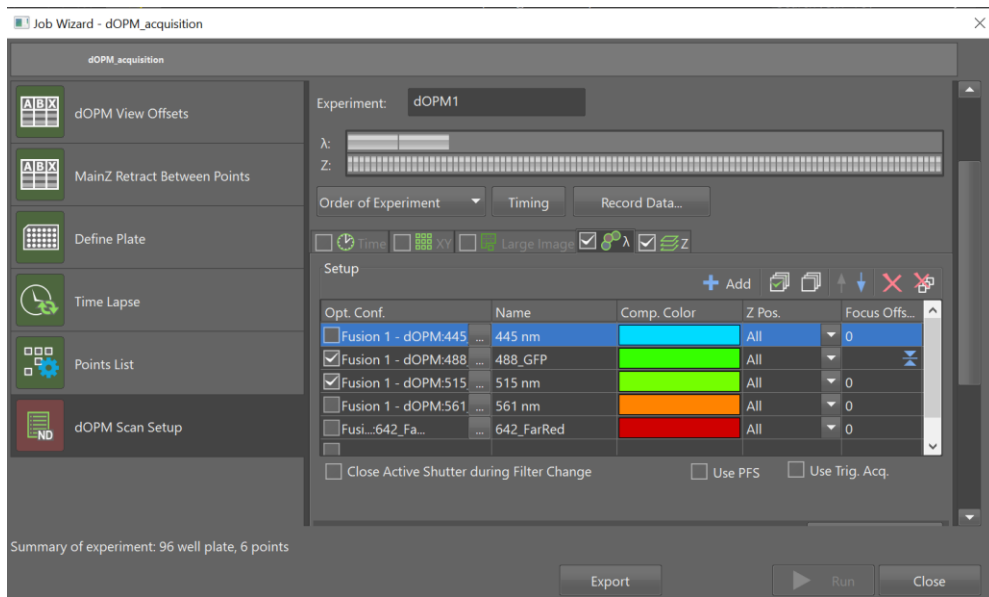
- choose whether time-lapse or not and the frequency of time points
- need to choose frequency that is longer than acquisition time for position list
- Use the NIS-Elements help tool to understand timing requirements in timelapse imaging

Load position list



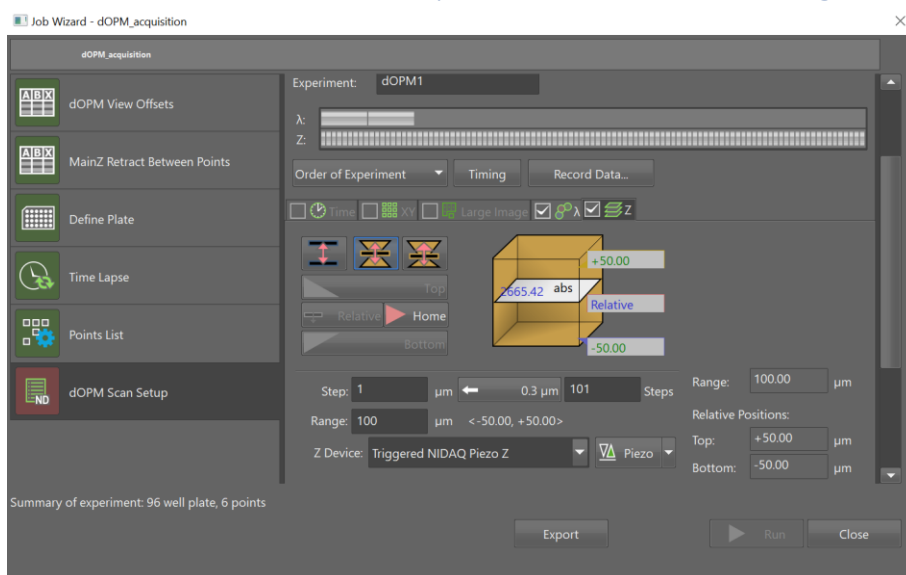
- load or define position list xml or csv file

Define dOPM volume acquisition – spectral channels



- Setup the dOPM ND acquisition parameters - lambda tab
 1. suitable dOPM optical configurations are selected
 2. advanced tab at bottom that following command boxes are ticked and filled in with
 3. execute command before capture `StgMovePiezoZ(dOPM_offset,0)`
 4. execute command after capture `StgMovePiezoZ(dOPM_offset,0)`

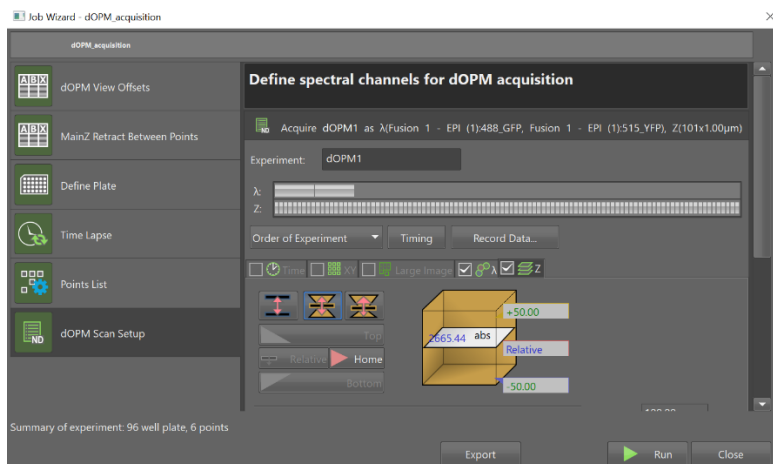
Define dOPM volume acquisition - z-scan settings



- setup the dOPM ND acquisition parameters - z tab
 - a. **NOTE:** there are two tabs to use and the others should be empty and unchecked
 - b. for the z tab, set z scanning device to either
 - i. NIDAQ piezo
 1. **NIDAQ piezo is software timed and and there will be no motion blur but relatively slow.**

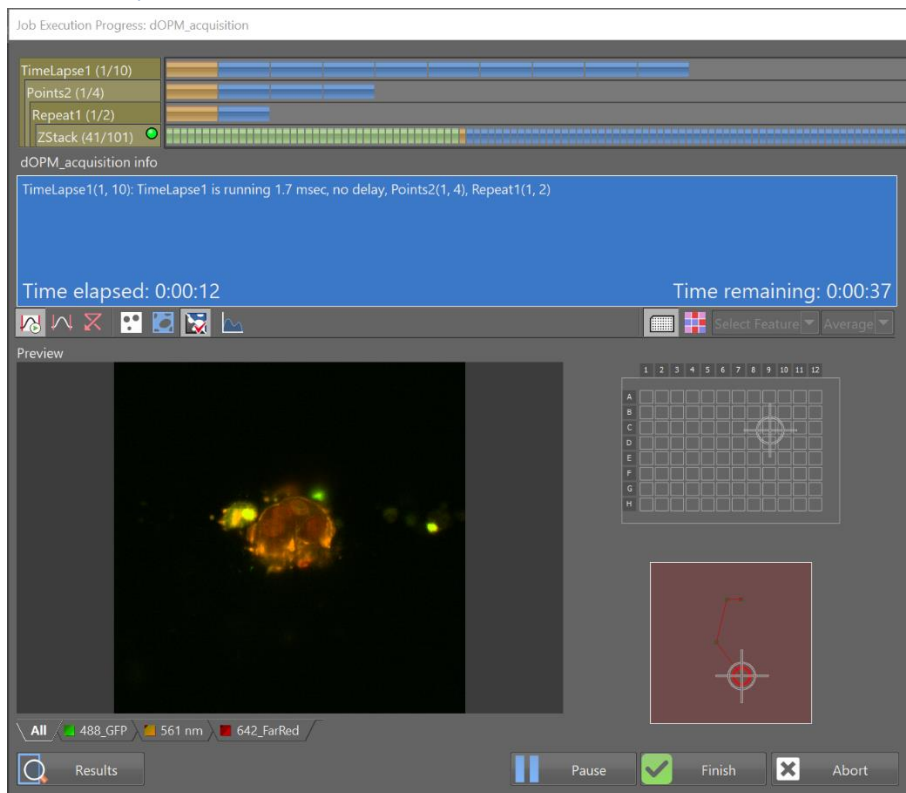
- ii. Triggered NIDAQ piezo
 1. **Triggered NIDAQ piezo is hardware timed and there will be motion blur but relatively fast.**
- c. **NOTE:** stick with option 1 above if unsure. Choice depends on height of image in pixels, exposure time, z step size

Wizard completed



- once all steps in the JOB script setup have been addressed the green button is enabled and the acquisition can be started

Run acquisition



- Acquisition is initiated by pressing green button. Progress bars at the top indicate acquisition timing details e.g. above
 1. Timelapse – x10 time points
 2. Points2 – x8 xyz positions
 3. Repeat1 – View 1&2 dOPM
 4. Zstack – View1&2 dOPM zstack planes

Assumptions

- **Acquiring data with both dOPM views**
- **Well locations used are compatible with 60x objective and any water immersion cap on top – recall that 60x objective cannot typically reach the edges of a plate. Do not proceed with any 60x based experiment if this is not clear to user and ask for help.**