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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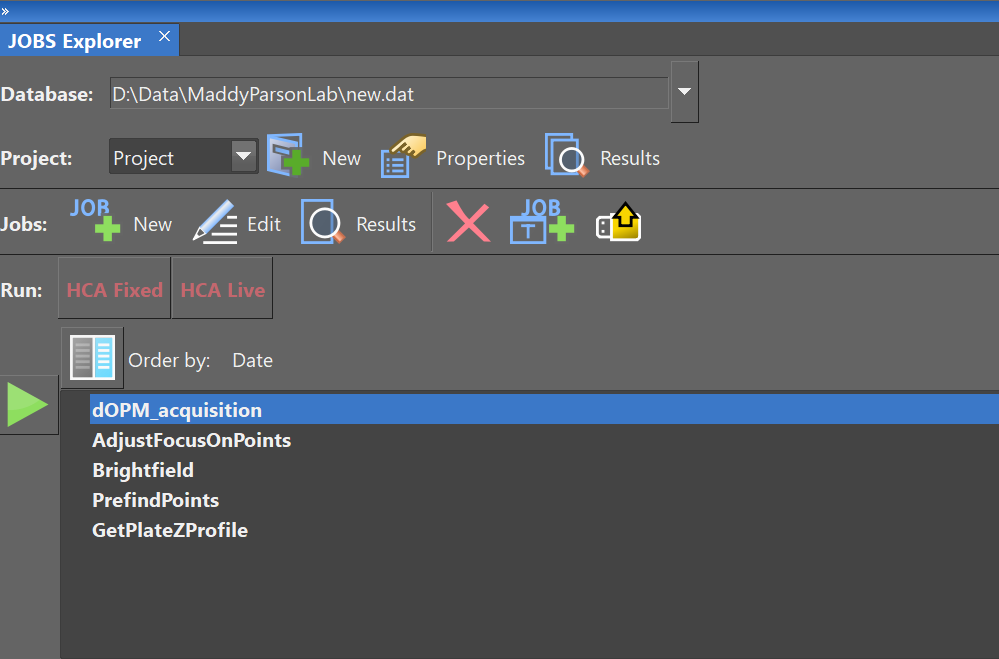
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Introduction

**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. **ZTotal = Z1 + Z2**. This is not physically meaningful since the z-piezo drive (**Z2**) is not attached to the microscope objective. For any position list we always work with **ZTotal = Z1**

Run acquisition JOBS script



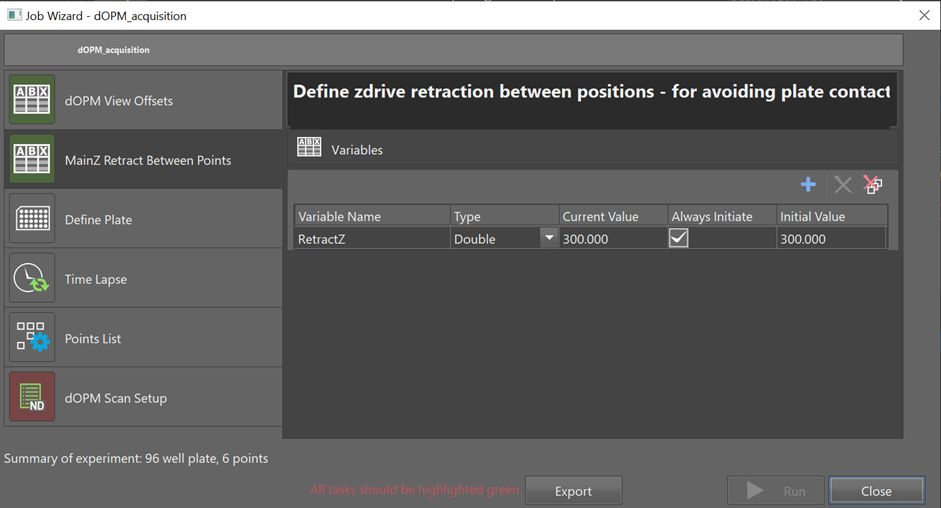
* Run the ‘dOPM\_acquisition’ JOBS script
* Use the NIS-Elements help tool to work out how and why you would want to:
  + Use JOBS explorer

defined dOPM view 1&2 refocus offsetsA screenshot of a computer

Description automatically generated

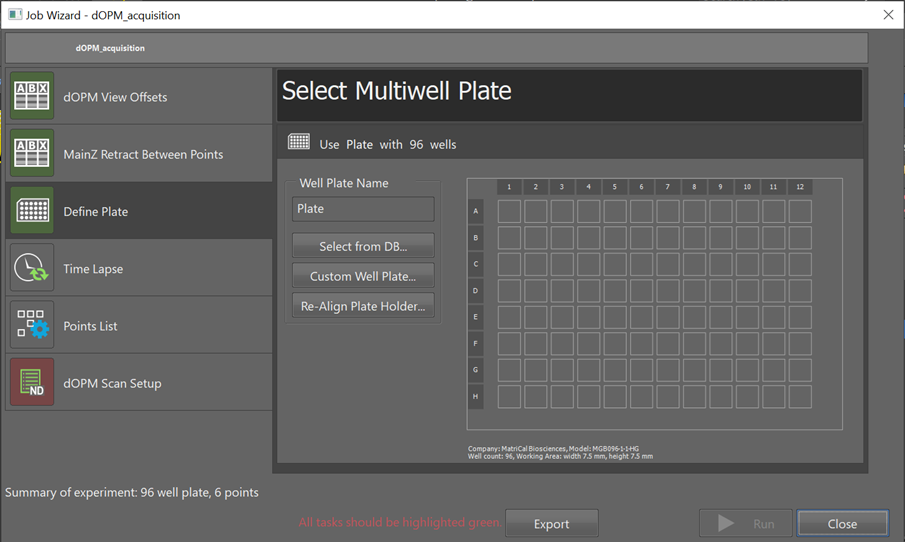
* Enter the zero remote refocus offset values for dOPM

# Set z\_retract parameter

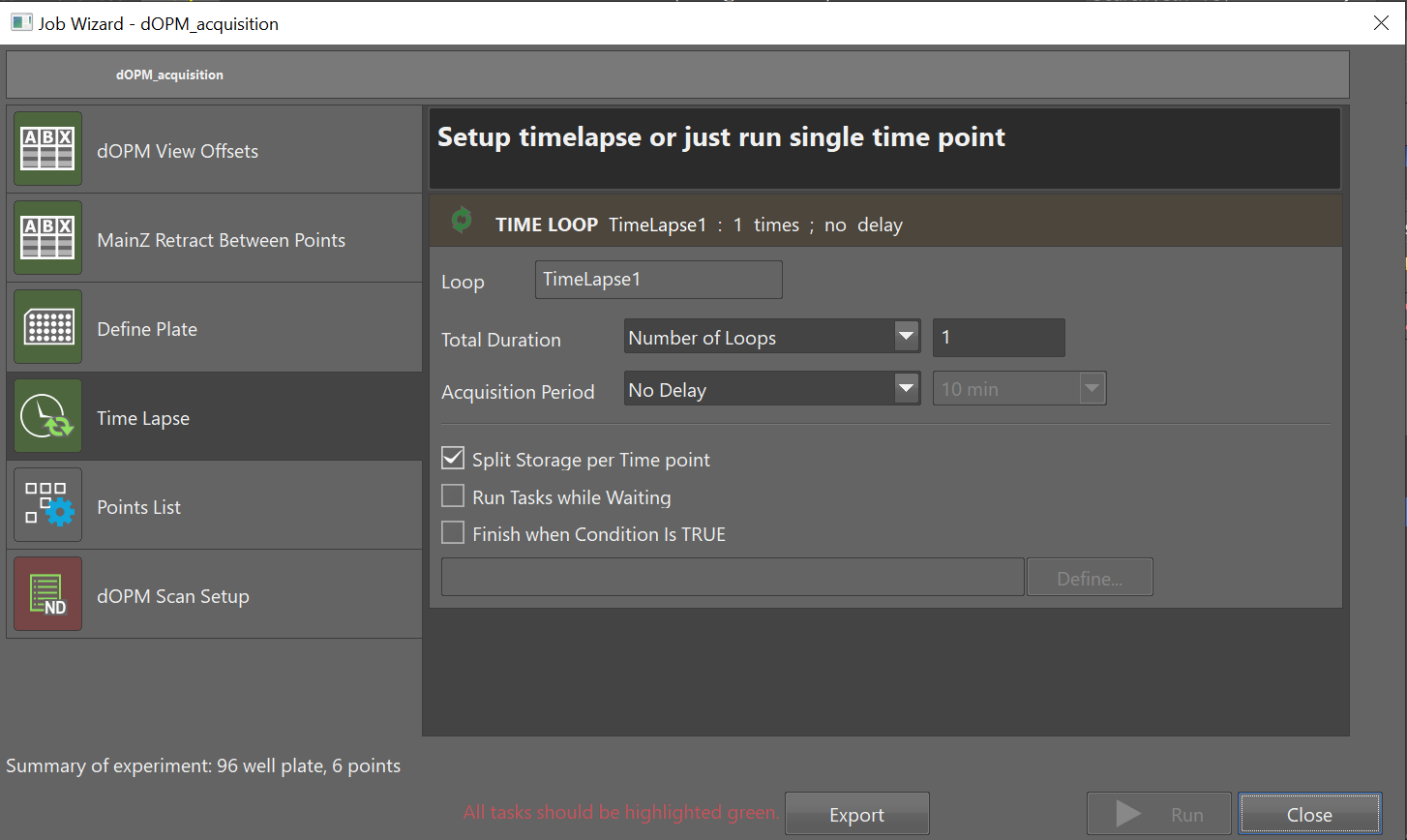


* Set the z\_retract variable to a number that avoids the chance of the objective colliding with the plate during stage movements.
* Here a conservative 300µm is used but depends on the plate being used – see JOBS script ‘GetPlateZProfile’ for a way to measure plate flatness.

# Select multi-well plate



* choose correct multi-well plate definition

Set timelapse parameters

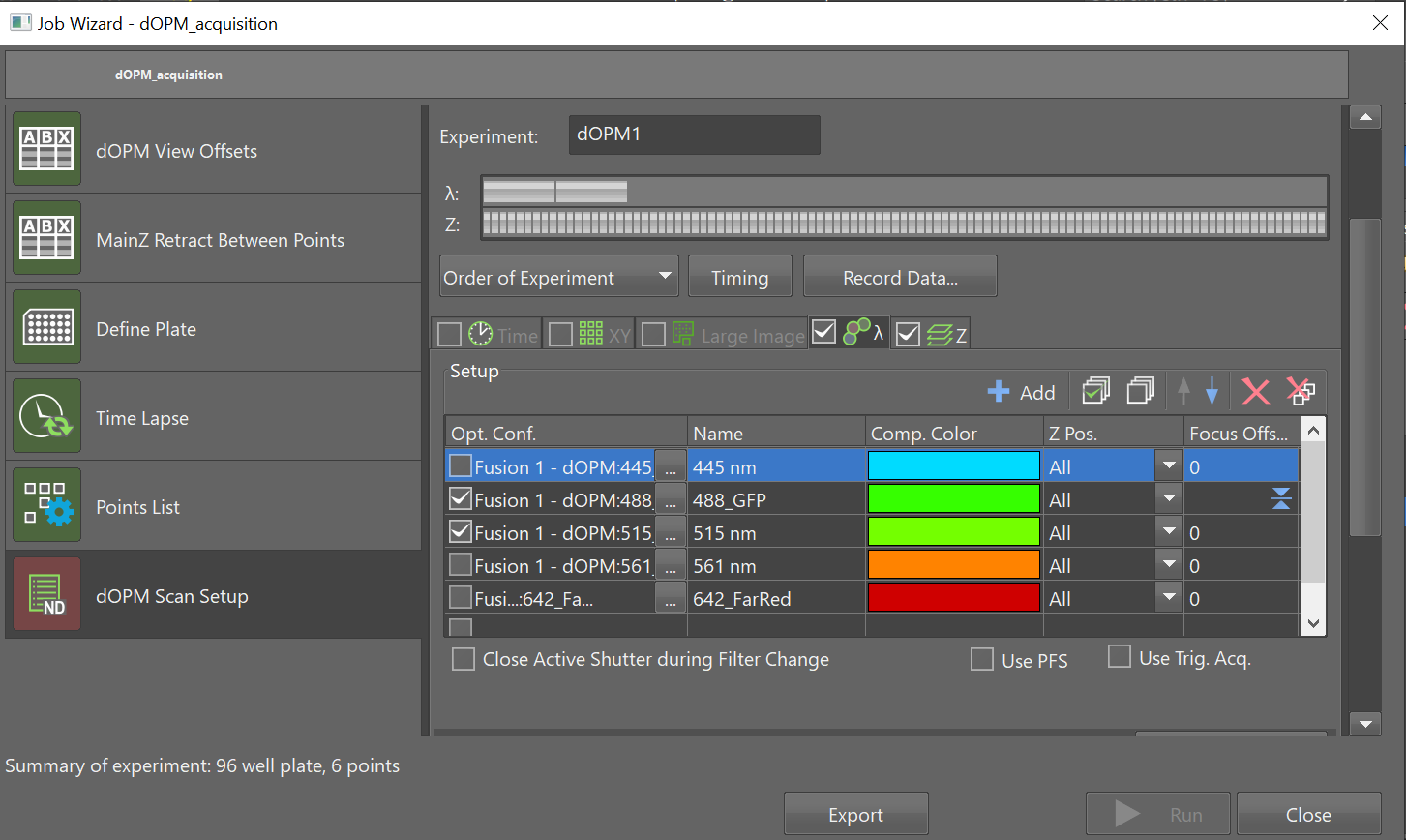
* choose whether time-lapse or not and the frequency of time points
* need to choose frequency that is longer than acquisition time for position list
* Use the NIS-Elements help tool to understand timing requirements in timelapse imaging

# Load position list

A screenshot of a computer

Description automatically generated

* 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)

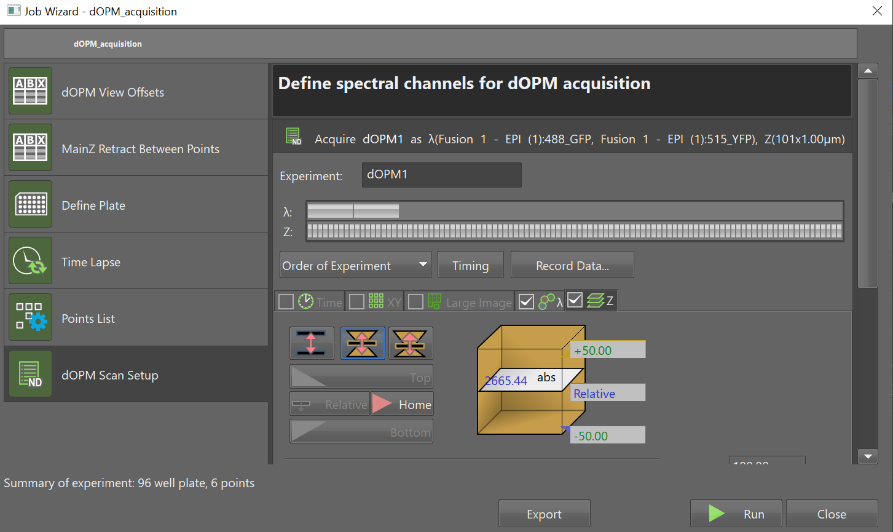
# Define dOPM volume acquisition - z-scan settings

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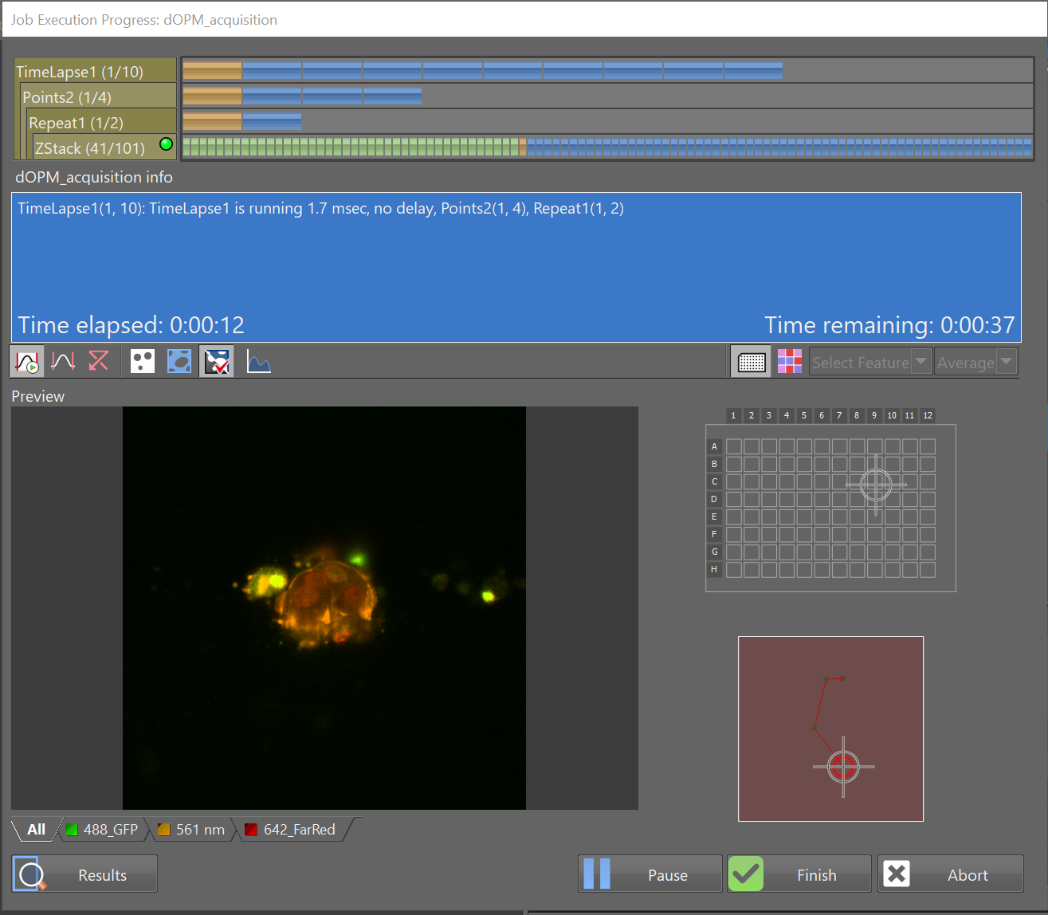
* setup the dOPM ND acquisition parameters - z tab
  1. **NOTE**: there are two tabs to use and the others should be empty and unchecked
  2. for the z tab, set z scanning device to either
     1. NIDAQ piezo
        1. **NIDAQ piezo is software timed and and there will be no motion blur but relatively slow.**
     2. Triggered NIDAQ piezo
        1. **Triggered NIDAQ piezo is hardware timed and there will be motion blur but relatively fast.**
  3. **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.**