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Organoid well plate scanning

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Protocol status: Working
We use this protocol and it's working however customization of the channels, thresholding settings, and optical configurations will be specific for each individual use case.

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Keywords: organoid, well plate, automated, Nikon, NIS elements, Z-stack, Z stack, spheroid , stack positions for each organoid, plate at 4x magnification, imaging routine, xy position of organoid, imaging, stack at 20x magnification, 4x magnification, 20x magnification, nis elements software, jobs module within nikon, nikon, xy position, stack position, plate

Disclaimer

Abstract

This is a conditional or 'smart' imaging routine written using the JOBs module within Nikon's NIS elements software (version 6.02.03). The JOB is designed to serially scan each well within a well-plate at 4x magnification and identify the XY position of organoids in each well. The program will then loop over those XY positions, identifying the top and bottom Z-stack positions for each organoid and then perform a Z-stack at 20x magnification.

Materials

Z_abs_bounds_20x.ga3 269.1KB | Z_abs_bounds_4x.ga3 272.7KB | Organoid_wellplate_3.bin 70.1KB

Required:
4x air objective
20x air objective
Nikon Ti2E microscope with motorized nosepiece and stage
96 well-plate

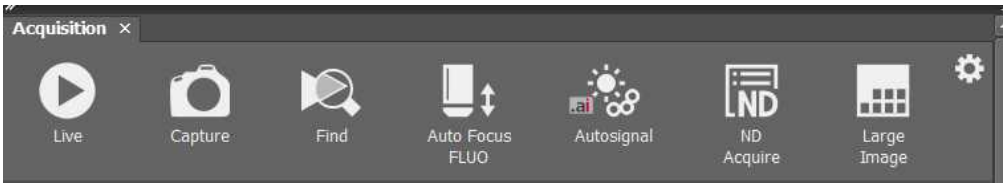
Attached is a NIS elements JOB designed to:
1) Scan over a 96 well-plate using the 4x objective and identify the XY coordinates of organoids in each well
2) Return to the XY coordinates of identified organoids and switch to the 20x air objective
3) Perform a coarse Z stack with a large step size at 20x magnification
4)Threshold the coarse Z stack to automatically segment and identify the top and bottom of the organoid
5) Perform a high resolution Z stack at 20x using the Z dimensions from the segmentation

Manual focusing on the first well

1 Create optical configurations (OCs) for the fluorophores you wish to image. For this JOB you will need an OC linked to a 4x objective and and OC linked to a 20x air objective.

2 Using the joystick move the stage so that the objective is beneath the first well to be imaged (usually top left corner of the well plate).

3 'Go live' and focus on the organoid using the 4x objective. Focus on the brightest point of the organoid.



Importing and running the JOB

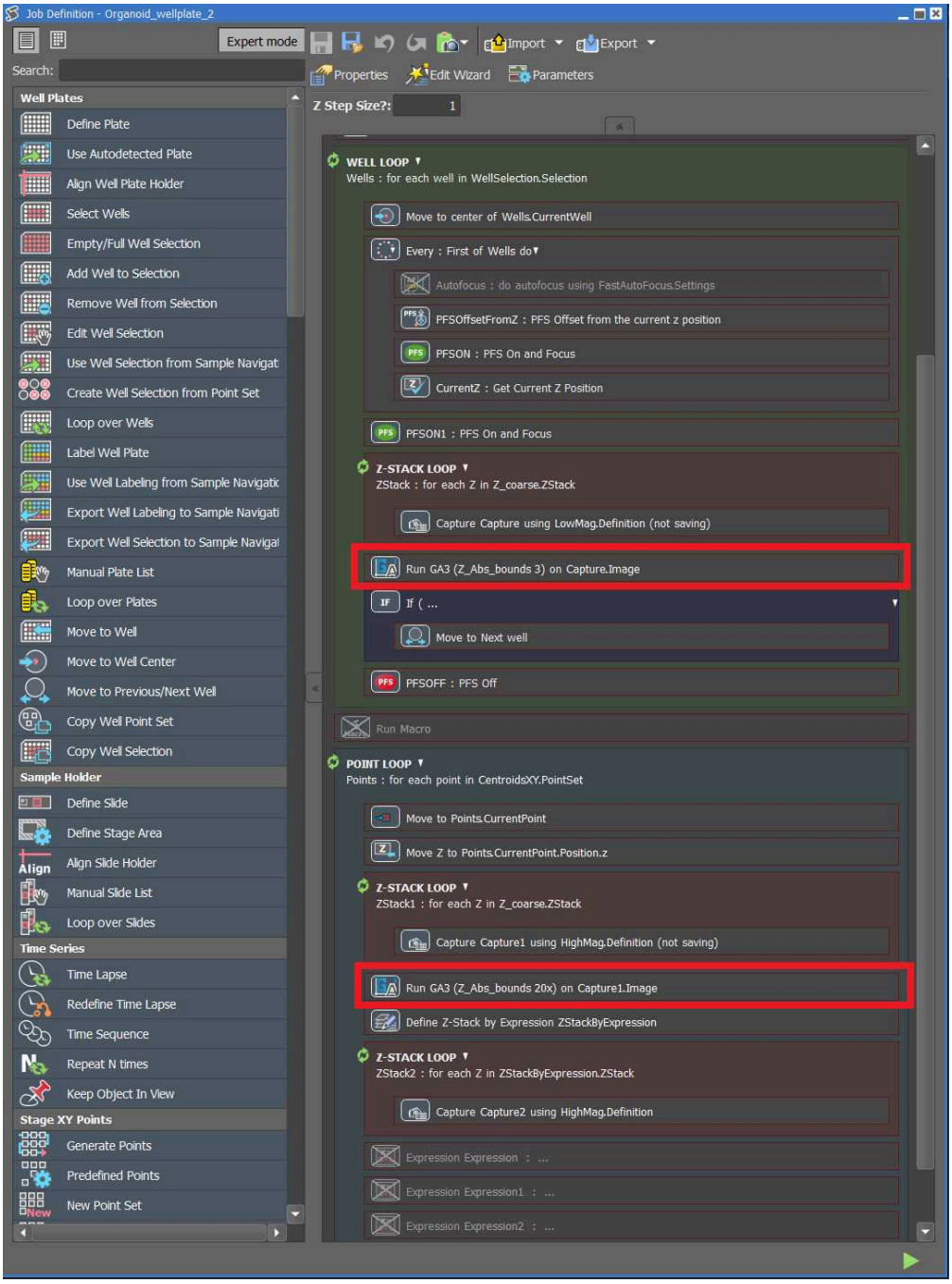
4 Search 'JOBs explorer' in the search bar in the top right corner of NIS elements.

5 Import the JOB into the JOBs explorer

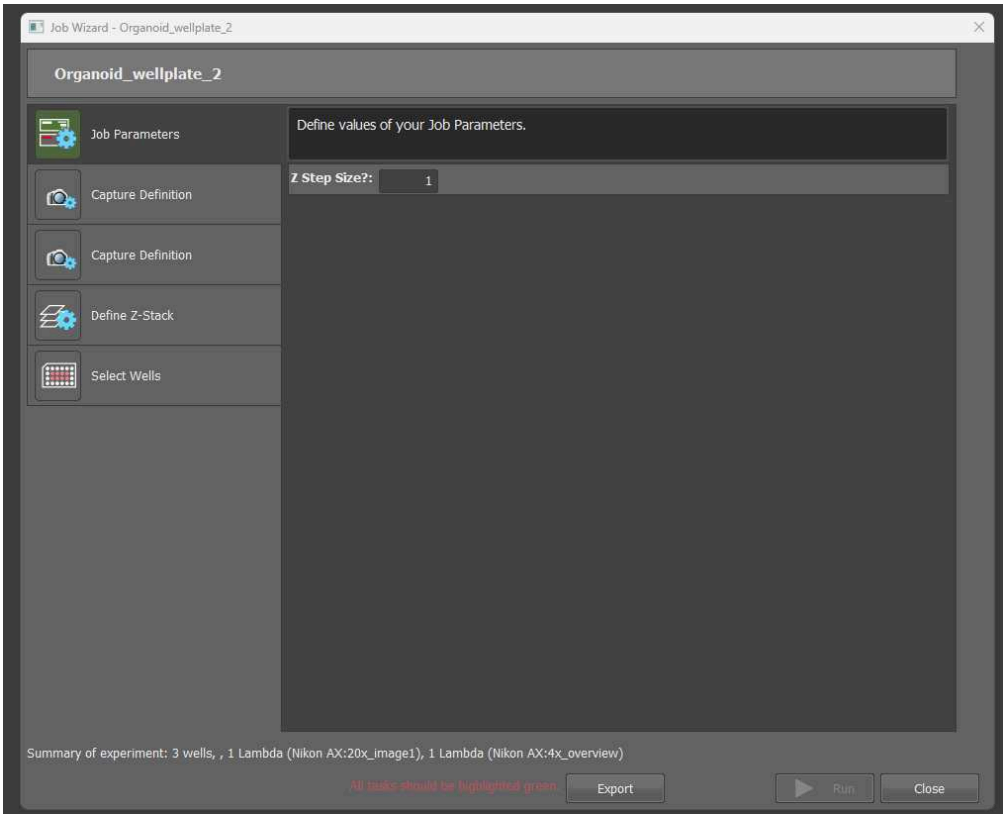
Select the JOB named 'Organoid_wellplate_3'

6 Run the JOB by clicking the button that looks like the 'play' symbol. This will open the JOB setup wizard where you can select imaging parameters.

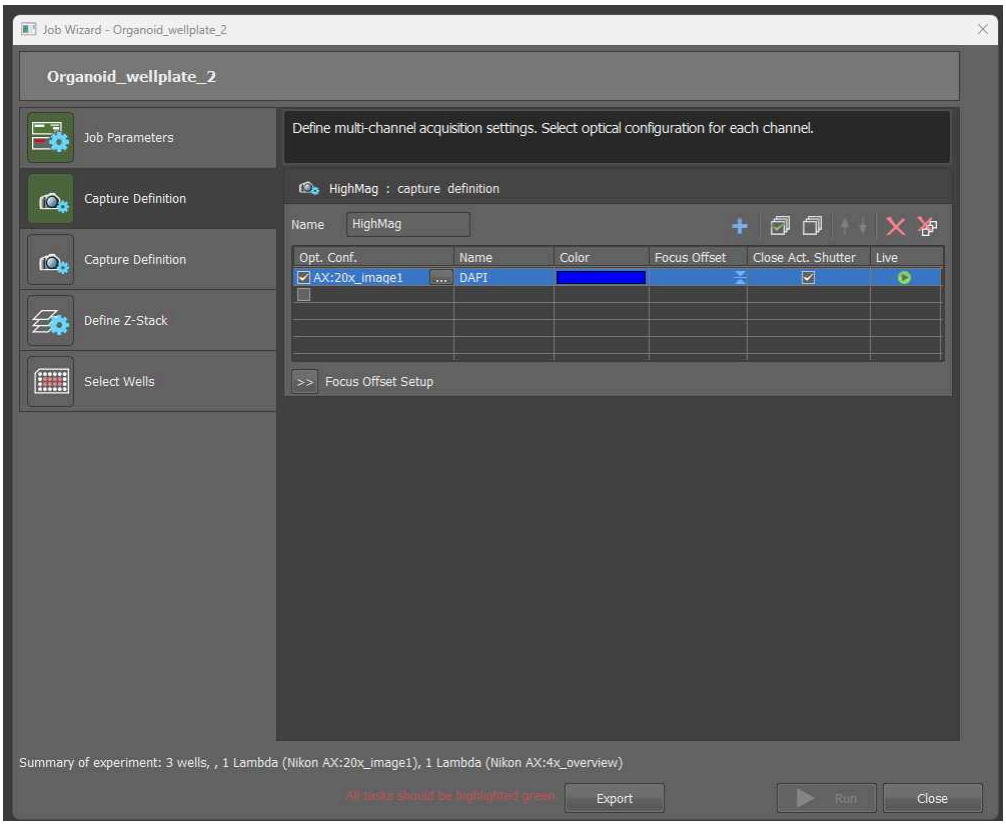
7 Import the required general analysis 3 (GA3) recipes that work within the JOB to segment and save the XYZ locations of the organoids. You can do this by double clicking on the GA3 command in the JOB highlighted in red in the image below and then importing the GA3 recipes. The upper GA3 command should have the 'Z_abs_bounds_4x' GA3 recipe imported, and the lower GA3 command should have the 'Z_abs_bounds_20x' GA3 recipe imported.



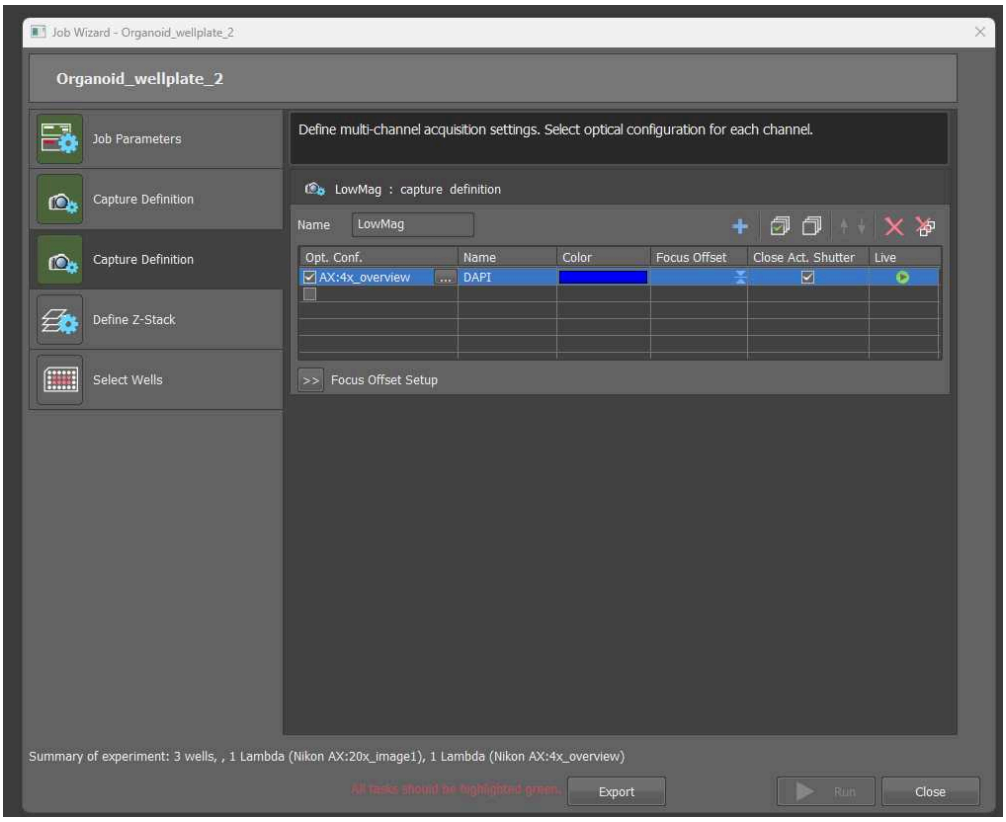
8 Select the step size you would like to use for the high resolution Z-stacks. This is at the top of the setup wizard in the field named: 'Z Step Size?' and takes an integer.



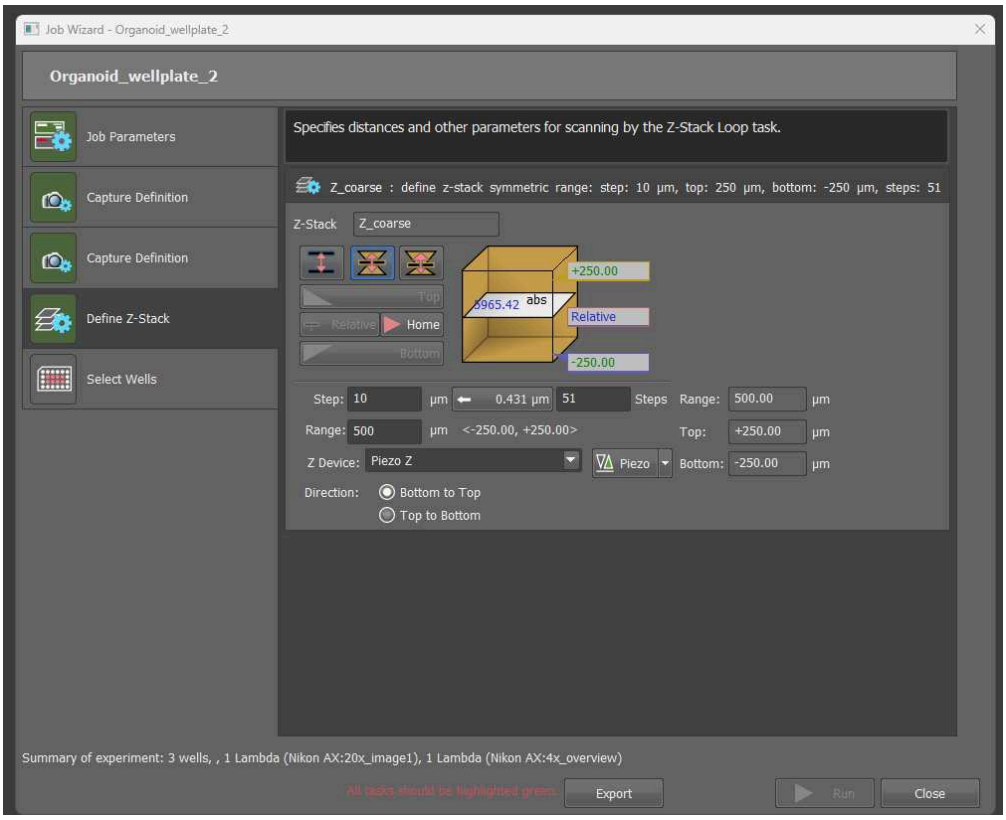
9 Select the high magnification capture definition. This is where you'll input the 20x magnification optical configurations (OC) you made earlier.



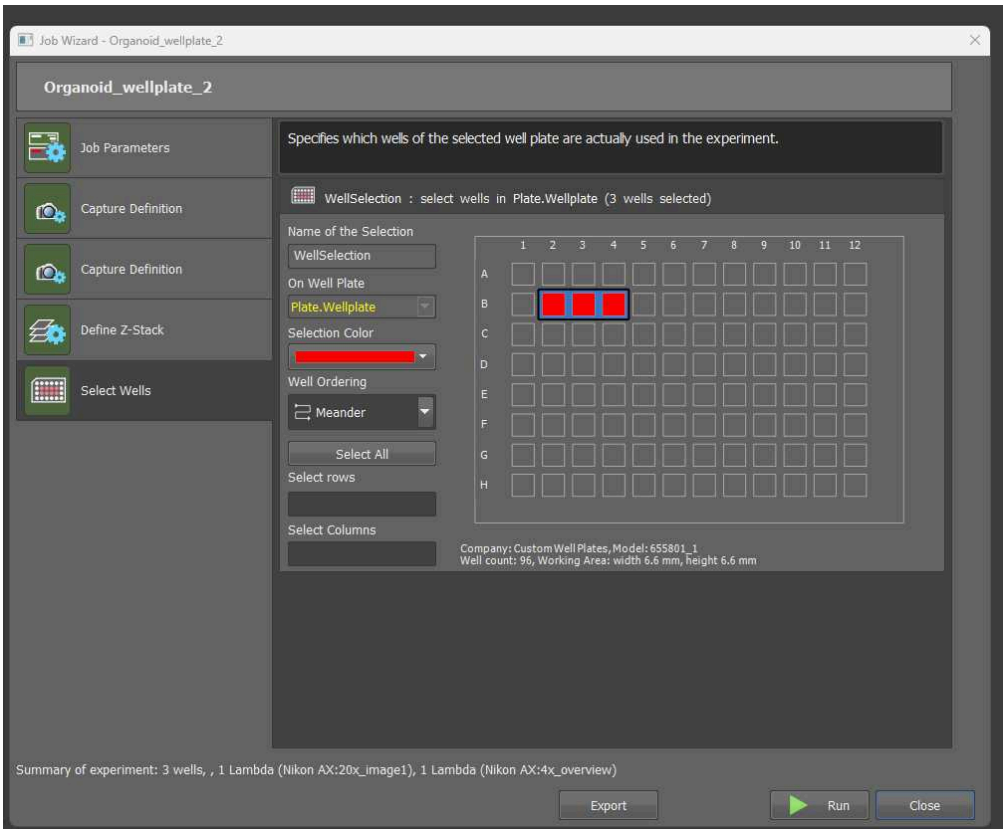
10 Select the low magnification capture definition. This is where you'll input the 4x magnification optical configurations (OC) you made earlier.



11 Define the depth (in Z) and step size (in micrometers) you would like the microscope to search over when looking for organoids in each well. This is by default 500um range with 10um steps.



12 Select which wells you would like to image by clicking and dragging across the well area


Job Wizard - Organoid_wellplate_2
Organoid_wellplate_2
Job Parameters
Capture Definition
Capture Definition
Define Z-Stack
Select Wells
Specifies which wells of the selected well plate are actually used in the experiment.
WellSelection : select wells in Plate.Wellplate (3 wells selected)
Name of the Selection
WellSelection
On Well Plate
Plate.Wellplate
Selection Color
Well Ordering
Meander
Select All
Select rows
Select Columns
1 2 3 4 5 6 7 8 9 10 11 12
A
B
C
D
E
F
G
H
Company: Custom Well Plates, Model: 655801_1
Well count: 96, Working Area: width 6.6 mm, height 6.6 mm
Summary of experiment: 3 wells, , 1 Lambda (Nikon AX:20x_image1), 1 Lambda (Nikon AX:4x_overview)
Export
Run
Close

13 Click 'Run' !