Jan 03, 2025

Automated sample detection (slide) and tiled Z-stacks

DOI
https://dx.doi.org/10.17504/protocols.io.261ger2ool47/v1

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Protocol Citation: Joe Airey 2025. Automated sample detection (slide) and tiled Z-stacks . **protocols.io** https://dx.doi.org/10.17504/protocols.io.261ger2ool47/v1

Protocol status: Working
We use this protocol and it's working but will need to be tweaked to individual users samples.

Created: January 02, 2025

Last Modified: January 03, 2025

Protocol Integer ID: 117554

Keywords: tissue section, slide, slide scanning, Z-stack, Z stack, Nikon, jobs, GA3, general analysis 3, microscopy, NIS Elements, Elements, microscopy, in-vivo imaging, confocal, fluorescence, conditional imaging routine, automated sample detection, 3d data, magnification at the start, magnification, nis elements software, jobs module within nikon, detected sample, cover slip at low magnification, sample, desired color channel, such as tissue section, 3d, detection

Abstract

This is a conditional imaging routine written using the JOBs module within Nikon's NIS elements software (version 6.10.01) on a Ti2-E widefield microscope. The JOB is designed to scan over a cover slip at low magnification, automatically find the sample (such as tissue sections) and then collect 3D data (Z-stacks) which tile across the detected sample. The user needs to input their desired color channels and magnification at the start of the JOB. This script can run on confocal with minor modification.

Materials

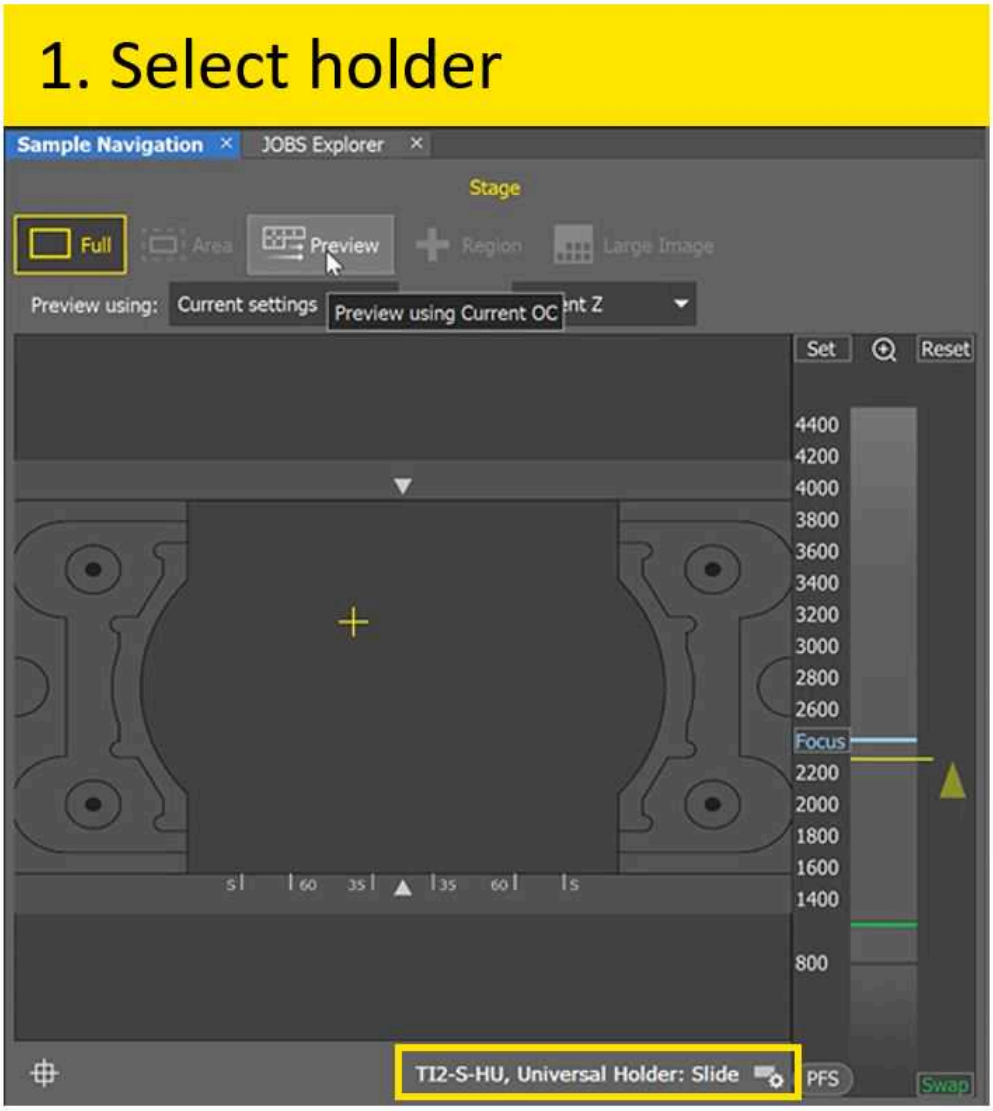
Tis1_GA3.ga3 Tis3.bin

Materials:
Ti2-E motorized microscope
NIS elements AR version 6.10 or above
4x Plan Apo Lambda D objective
10x, 20x, or 40x air immersion objective

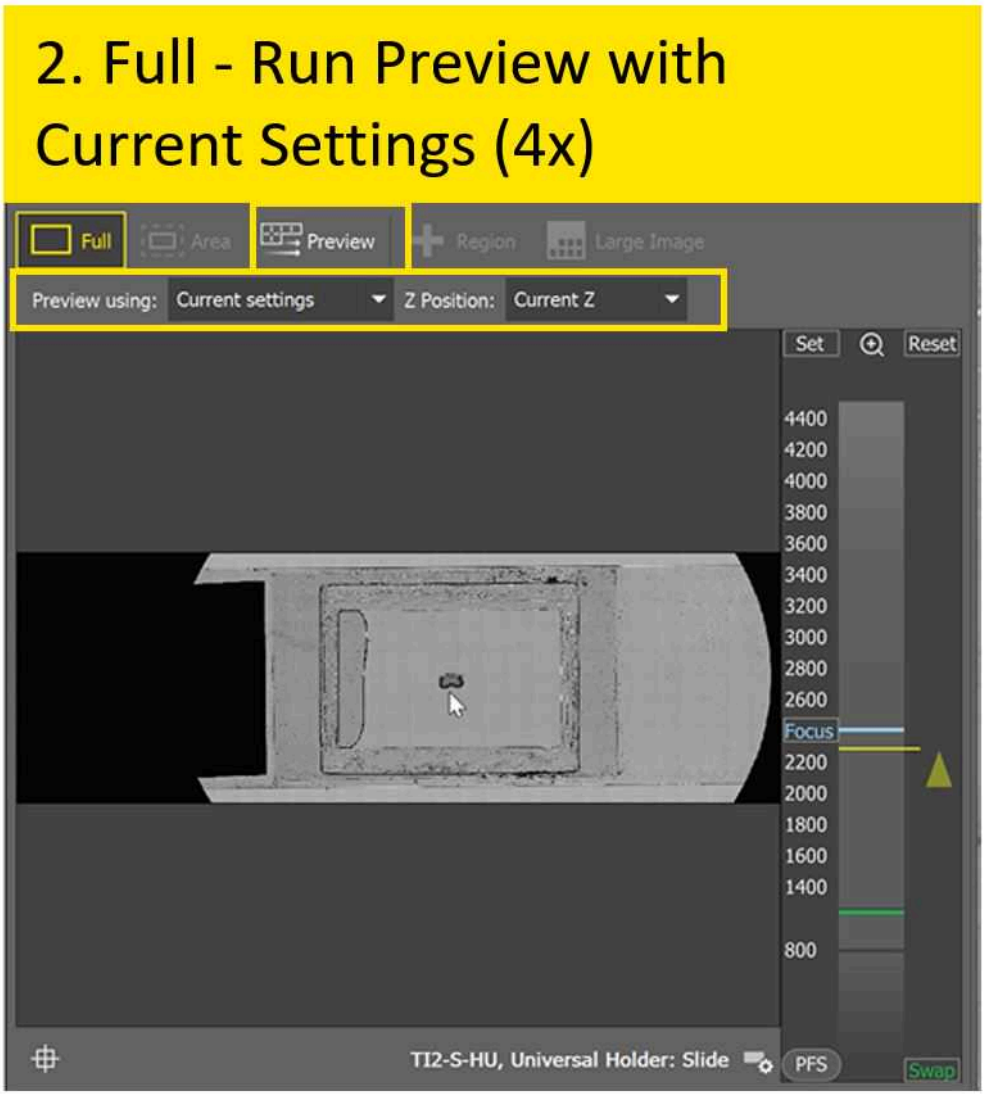
Sample Navigation / Slide Area Scan

1 Load the slide into the slide holder of the microscope and focus on the sample using a low magnification objective.

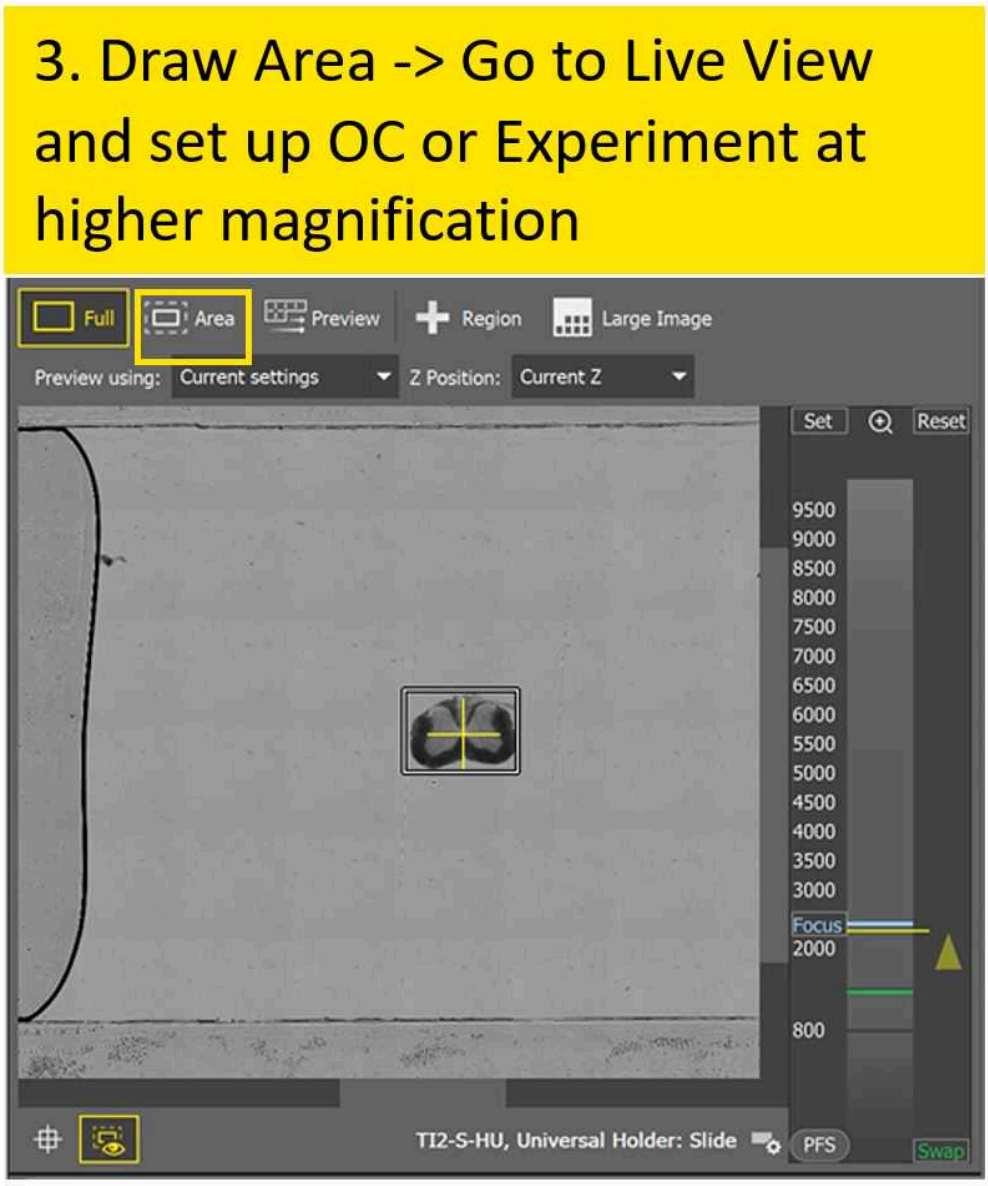
2 In the 'Sample Navigation' tab select which slide holder you are using:



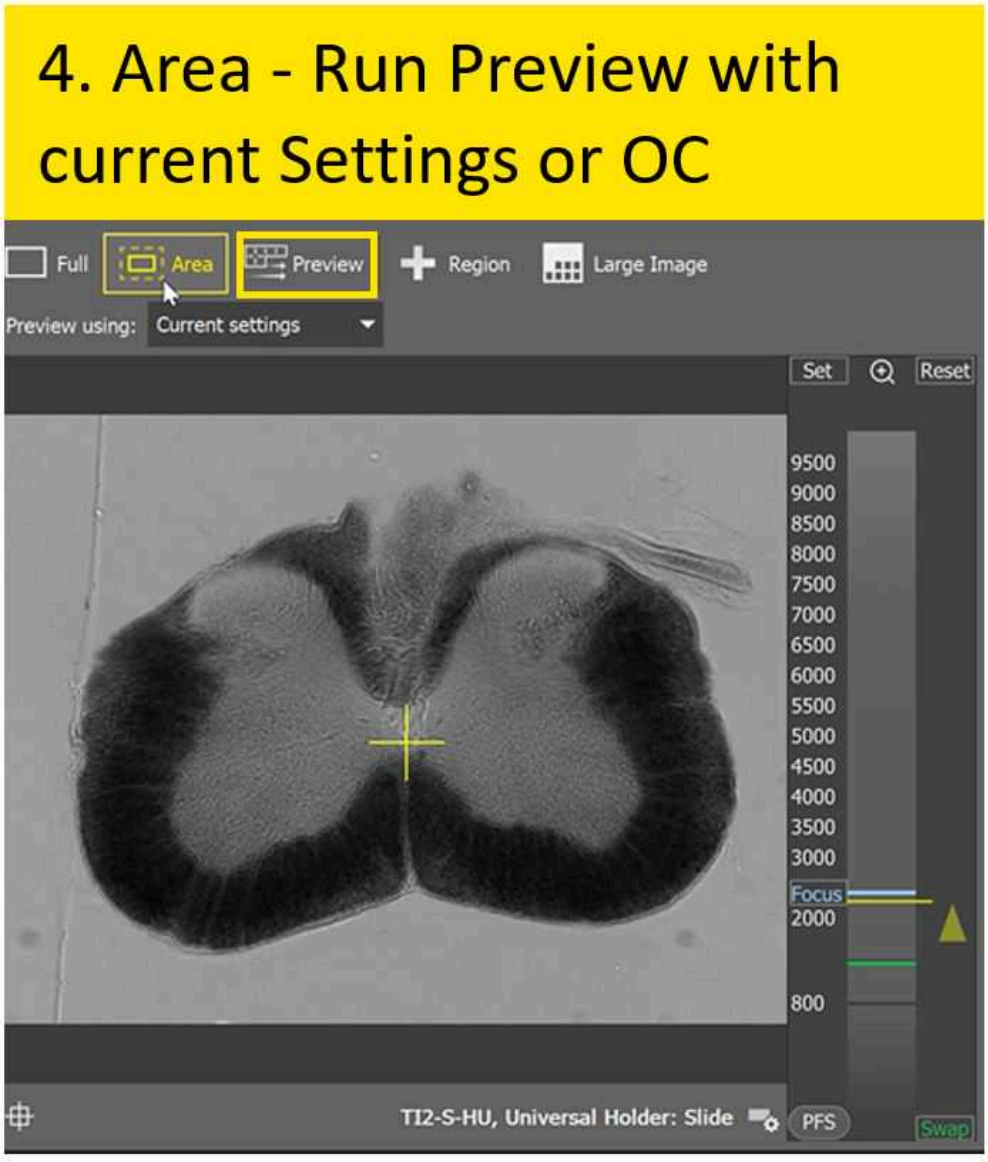
3 Capture an overview image of the current slide by clicking on the 'Full' or 'Preview' buttons. Clicking 'Full' will capture an image in 'Brightfield' while clicking 'Preview' will allow the choice of imaging in either brightfield or select an imaging channel or optical configuration.



4 Refine the area to be imaged by drawing a region of interest (ROI) by clicking the 'Area' button



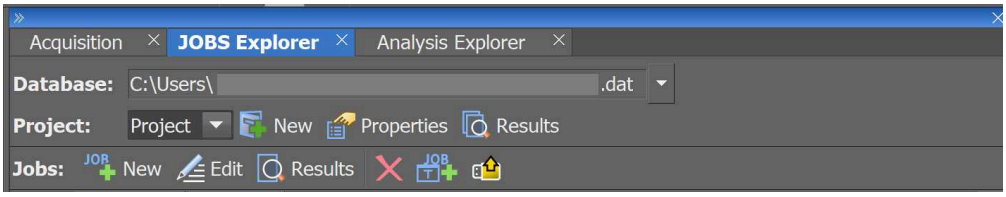
5 Clicking 'Area' will now capture an overview of the area of interest



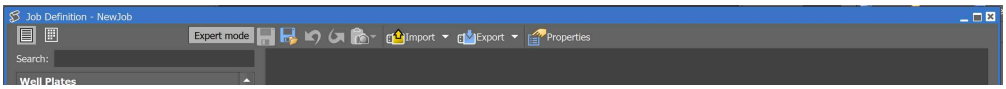
6 Elements can now use these images to navigate around the tissue section image at higher magnification and with Z-stacks.

Running the JOB to tile Z-stacks over the tissue section at higher magnification

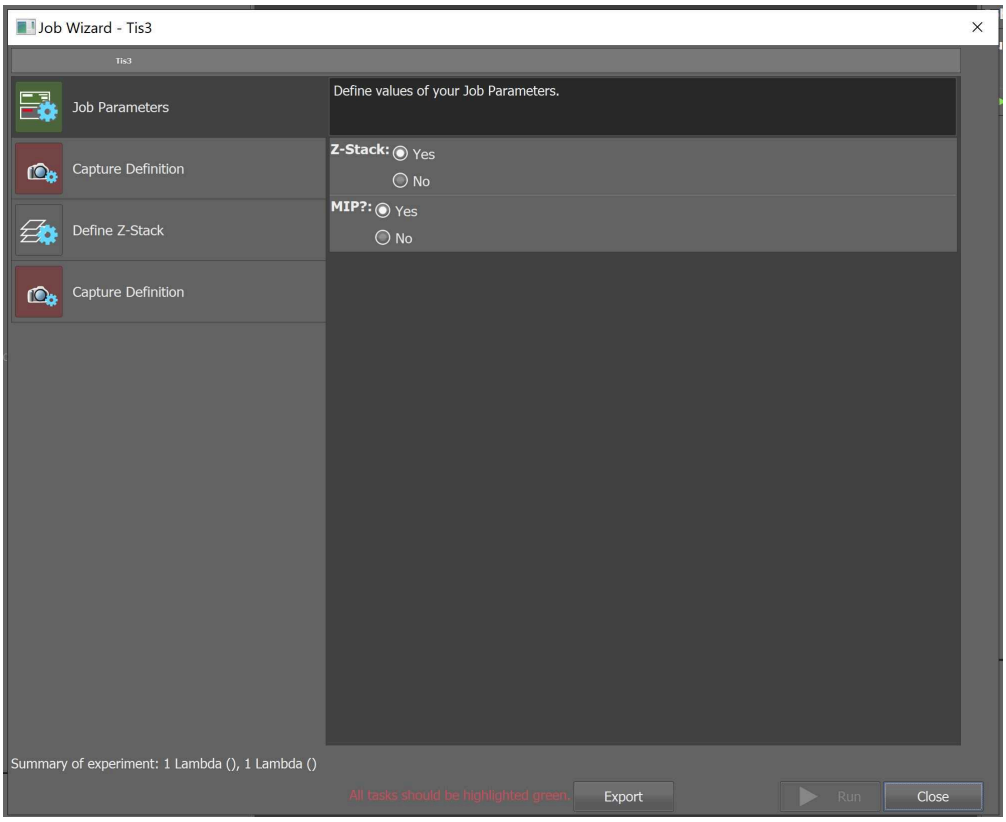
7 Navigate to the JOBs explorer tab and select 'New' to import the new JOB for the first time. Note once imported this step does not need to be repeated on subsequent runs of the JOB.



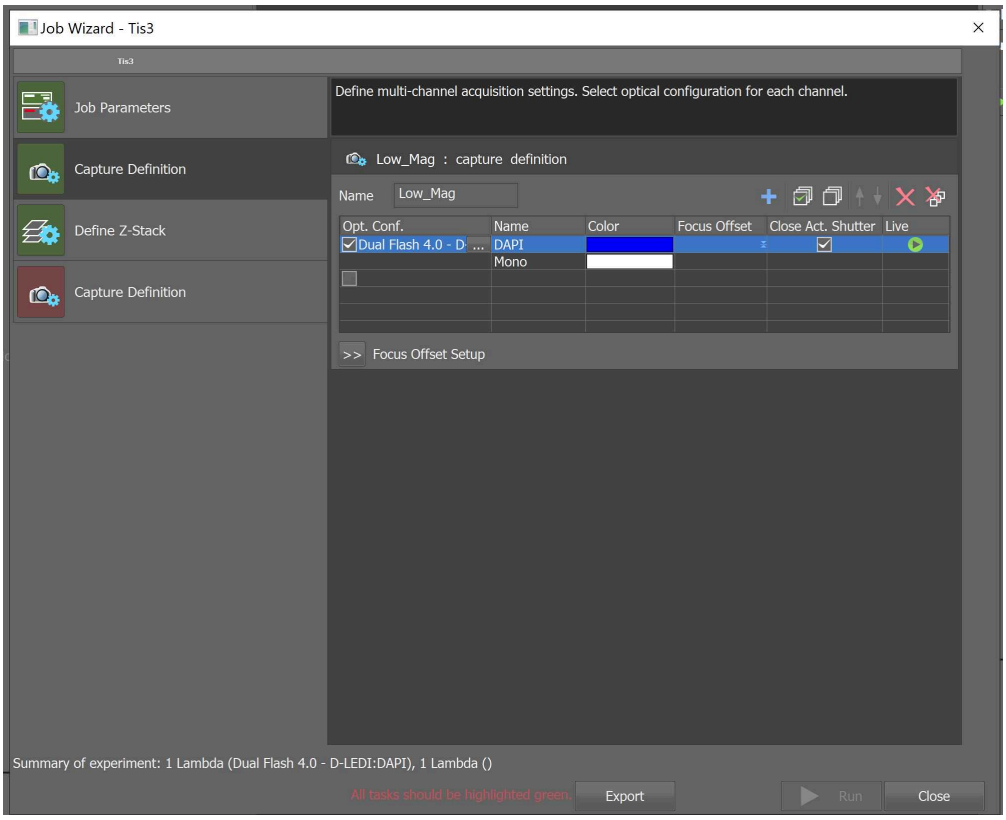
8 Import the JOB saved as .bin titled 'Ti3.bin'



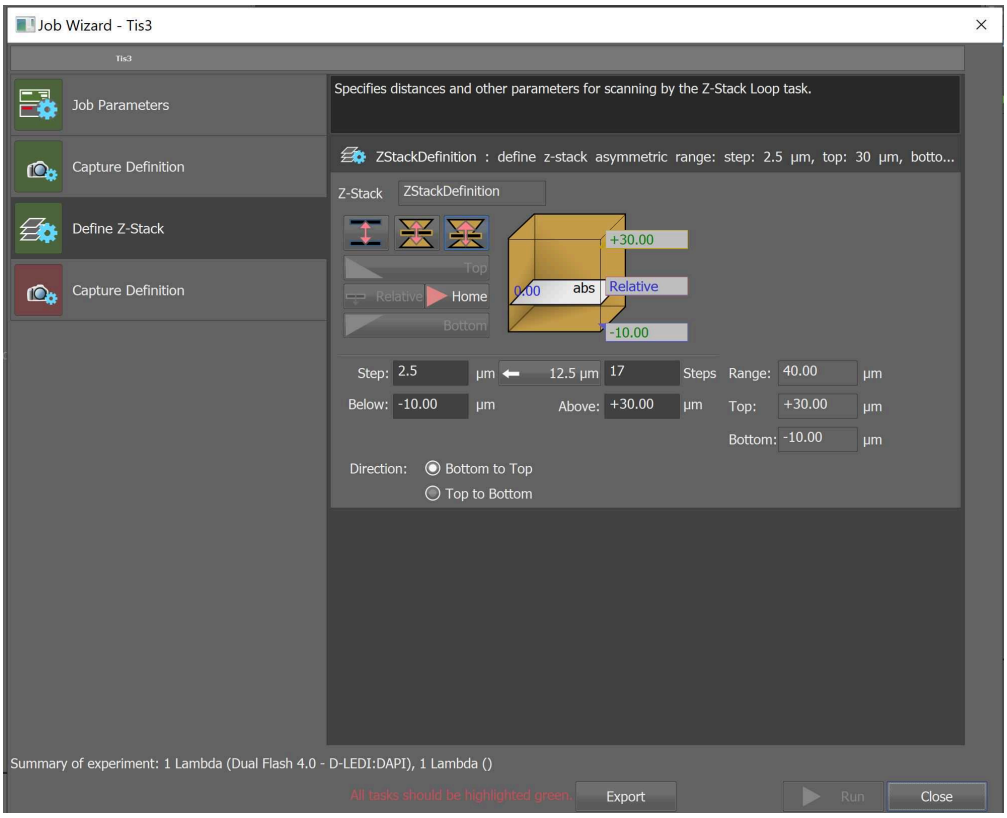
9 Run the JOB by clicking the play button at the lower right corner of the edit window. This will open the JOB wizard where you can input settings. The first is where you can enter if you would like to collect Z-stacks, and also maximum intensity projections:



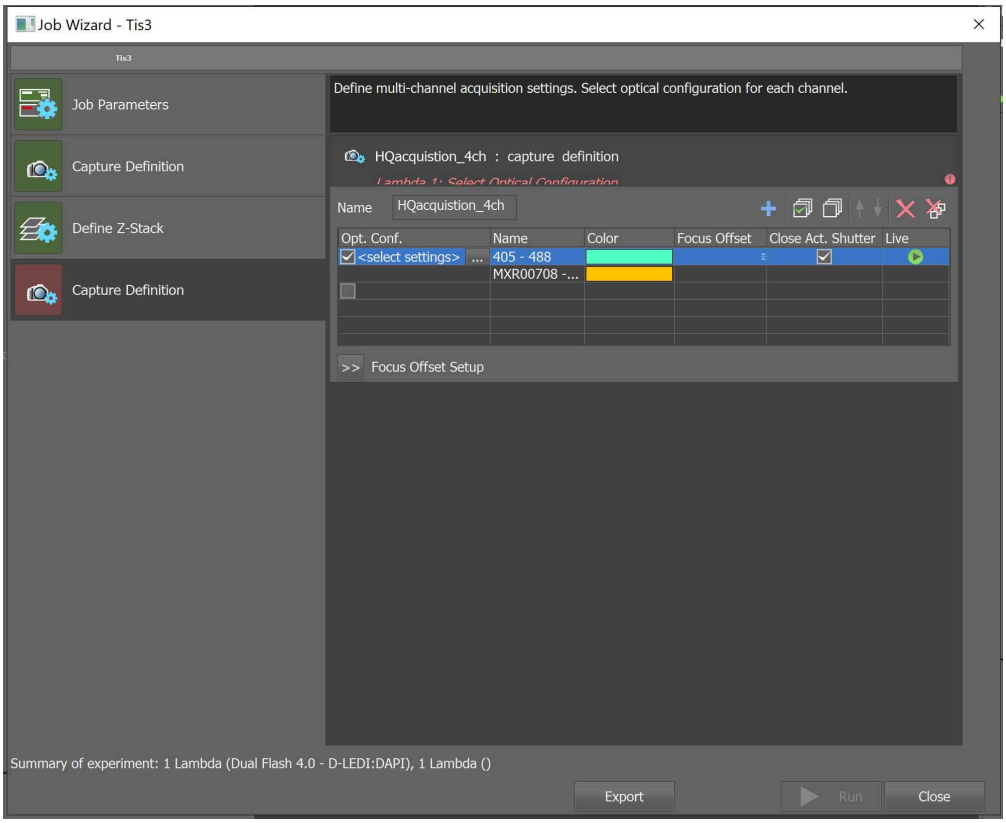
10 Select the first 'capture definition' which will be the channel (OC or experiment) used to perform the low magnification overview images. If using fluorescence, select the channel with the most homogeneous signal.



11 Define the thickness of the Z-stacks. By default the job will perform an asymmetrical Z-stack from the point of autofocus -10um below and 30um above the focus on an inverted microscope.



12 Select the second 'capture definition' which will be the channels (OCs or experiment) used to perform the high magnification images and Z-stacks.


Job Wizard - Tis3
Tis3
Job Parameters
Capture Definition
Define Z-Stack
Capture Definition
Define multi-channel acquisition settings. Select optical configuration for each channel.
HQacquistion_4ch : capture definition
Name
HQacquistion_4ch
Opt. Conf.
Name
Color
Focus Offset
Close Act. Shutter
Live
<select settings>
405 - 488
MXR00708 -...
>> Focus Offset Setup
Summary of experiment: 1 Lambda (Dual Flash 4.0 - D-LEDI:DAPI), 1 Lambda ()
Export
Run
Close

Click ' Run'