

STANDARD OPERATING PROCEDURE

Olympus VS 200 Slide Scanner

INTRODUCTION

This document describes the operational setup and procedures for Olympus VS 200 Slide Scanner (VS 200). This document offers users, particularly beginners, a brief step-by-step instruction on how to operate the VS 200, specifically scanning of single slides.

The Olympus VS 200 is an advanced auto slide scanner allowing whole slide imaging at high resolution, high speed, and high throughput. It supports bright field (BF), fluorescence, polarisation and darkfield with the possibility to scan a sample using multiple techniques at the same time. The batch scanning can run up to 216 slides that can be queued for acquisition. The TruAI technology and post-processing deep learning enable accurate segmentation of morphological features on a sample.

This system can image different types of samples, such as cells, tissue, model organisms, and organoids and the ability to therefore it's a powerful solution for research projects in many fields such as brain, cancer and stem cell research, and drug discovery etc.

SCOPE

This procedure applies to basic operations of single slide scan including starting up the system, loading the sample (loading slides to the tray and loading the tray to the tower), creating and/or editing an existing project for BF and fluorescence scanning, 2D image acquisition for BF and fluorescence, post-acquisition processing (data viewing, data saving and access, data policy), and shutting down the system.

For more detailed operational instructions and other advanced applications including batch scanning, Z-stacking, focus extended imaging (FEI), polarization imaging, and dark field imaging, users are to refer to the full manual by the vendor which is accessible from Cell imaging internet site: <https://sydneyuni.atlassian.net/wiki/spaces/WIF/pages/768016621/Manuals+Protocols>.

SAFETY

The operator must exercise caution while the instrument is engaged or while loading the sample, as to avoid placing fingers or any foreign object into the stage area for when the microscope is required to initialise or required to move.

In addition, safe operation of the instrument also requires the awareness of other risks e.g. biological hazard considerations, exposure to UV light, fatigue and gesture discomfort/injury etc. Refer to the latest Safe Work Procedure (SWP) published on the Westmead Imaging internet site "WIMR-SWP-WHS-GEN-63.03 Use of a fluorescence or laser scanning microscope" <https://sydneyuni.atlassian.net/wiki/spaces/WIF/pages/768016621/Manuals+Protocols>.

Acquisition of samples on the VS 200 system must only occur after the approval of an associated project in PPMS. Any projects to be run on the VS 200 involving hazardous chemicals and/or biohazardous samples must have the appropriate PPMS project approval. Live biohazardous samples are not allowed to be imaged on the instrument. When applicable, proper cleaning of the oil objective is required at the end of the experiment.

OGTR requirements for safe work in a PC2 laboratory apply.

TRAINING / COMPETENCIES

All personnel require training prior to independent operation of the instrument. Training is conducted by staff with competency demonstration necessary before authorisation.

Competency is assessed via demonstration of independent instrument operation, in conjunction with verbal explanation of all aspects of operation and troubleshooting common faults. After training is carried out, a training competency quiz is to be completed and passed before instrument access is authorised by imaging staff. All instrument operation is to be conducted by trained operators.

EQUIPMENT & SUPPLIES

The Olympus VS 200 is located in the Microscopy Lab 4 (previously J2.08), Level 2 WIMR.

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PROCEDURE

1. System startup

Turn on the power of the following three components in the specific order by following the numbers 1-3 marked next to the buttons/key of:

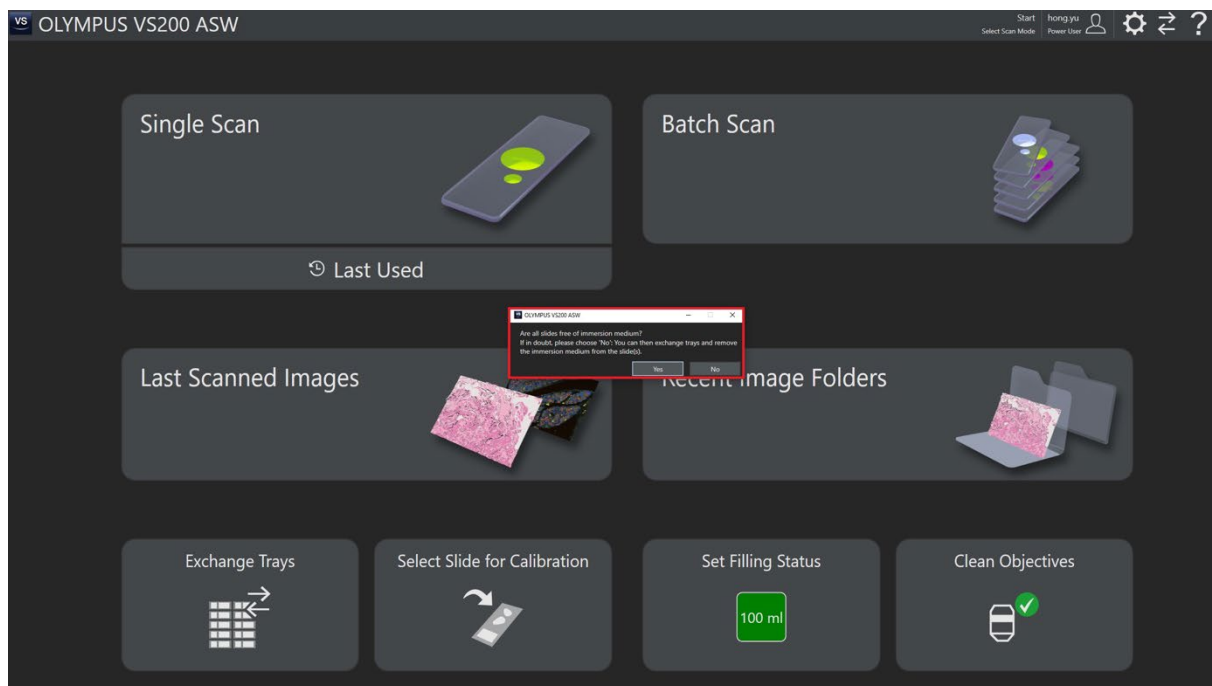
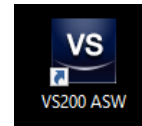
1st LED light source

2nd Microscope

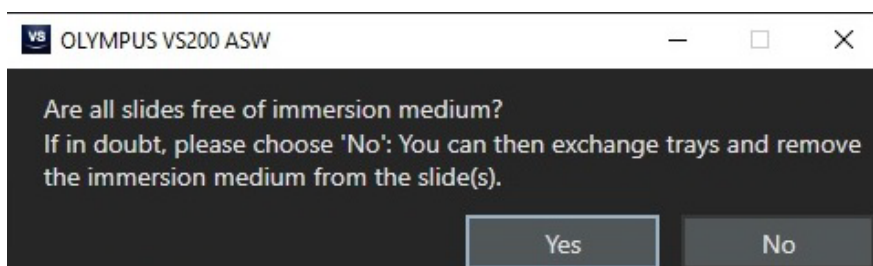
3rd Fluorescence camera (BF camera gets turned on automatically when the system is powered on)

2. Getting ready for your experiment

2.1 Open the acquisition software VS200 ASW, there is a desktop shortcut. The layout of the software looks like this:



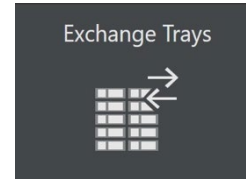
2.2 By clicking yes on the initial pop-up message, confirm all loaded slides are free of immersion oil



2.3 Follow the below steps to load the sample(s) into the rack

2.3.1 In VS200 ASW, click “Exchange Trays”

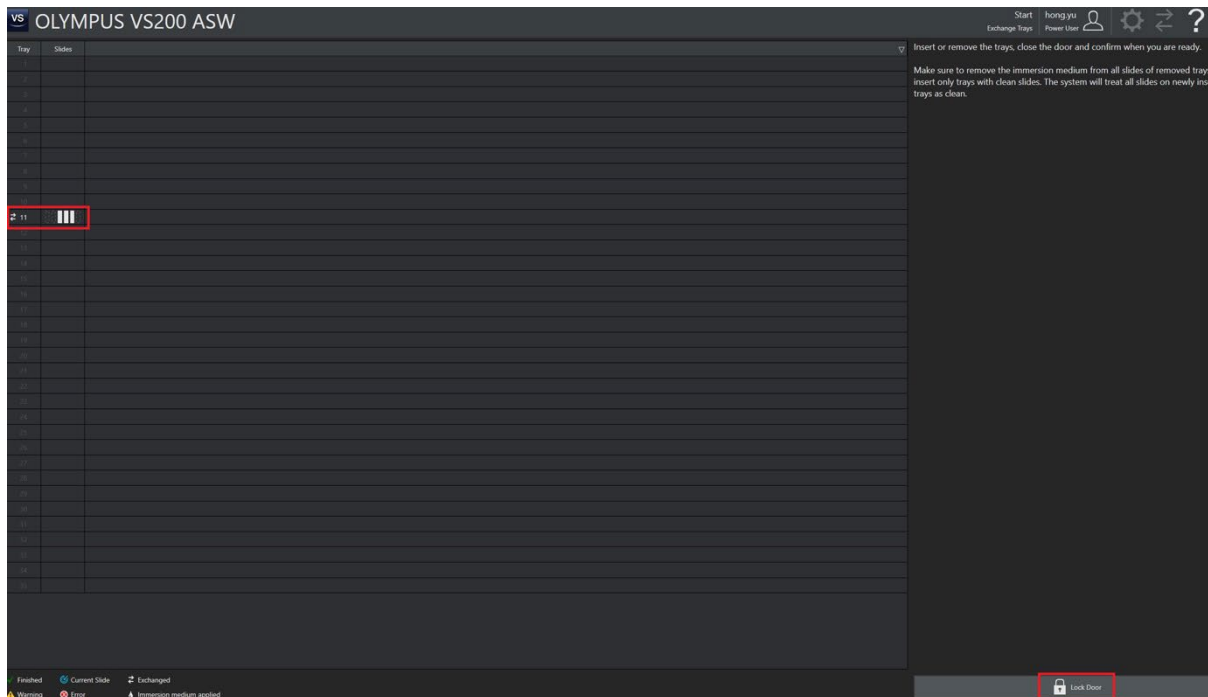
2.3.2 On the lab bench, place the slides into the tray(s)



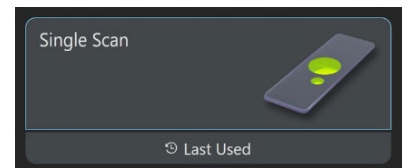
Note: ensure the “Door Locked” light is off before opening the tower!

2.3.3 Open the tower door, and insert the trays into empty slots (any available slots are fine) Close the tower door afterwards

2.3.4 Now, all trays and slides are automatically recognised and displayed in the VS200 ASW. Click “Lock Door” to finalise the sample loading step

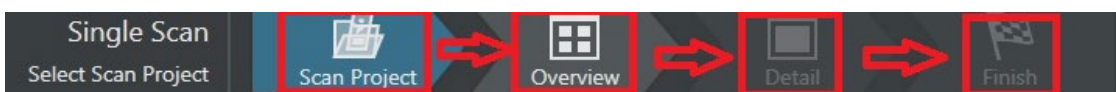


2.3.5 Click “Single Scan” in the VS200 ASW to initialise the single scan



3. Single scan (BF)

Tip: The overall workflow of BF single scan includes 4 steps in the following order:



Step 1 Set up a Scan Project for subsequent scanning of your slides. You can choose to create a brand new project or edit an existing one

Step 2 Overview the whole slide. This step scans the whole slide using the Overview settings from the chosen Project. You'll need this overview image to select a ROI for next step of detail scan

Step 3 Detail scan the ROI(s)

Step 4 Finish the scan

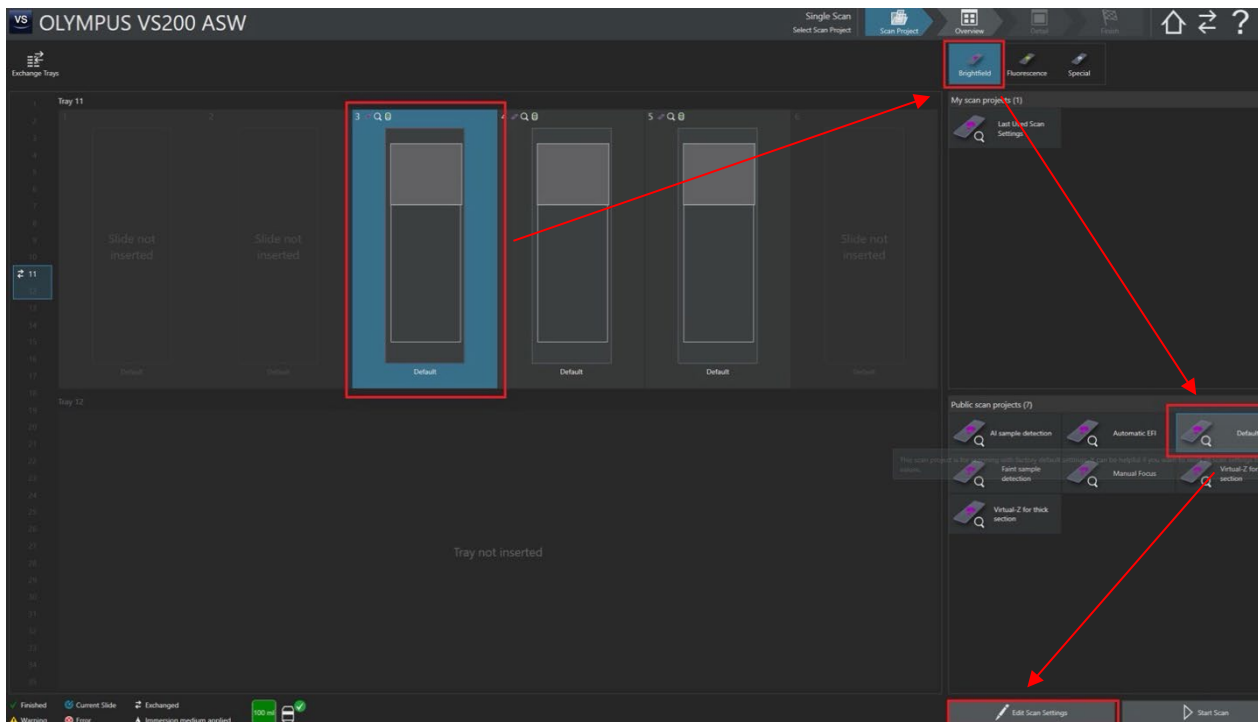
Detailed procedures are as follows:

3.1 Set up a Scan Project

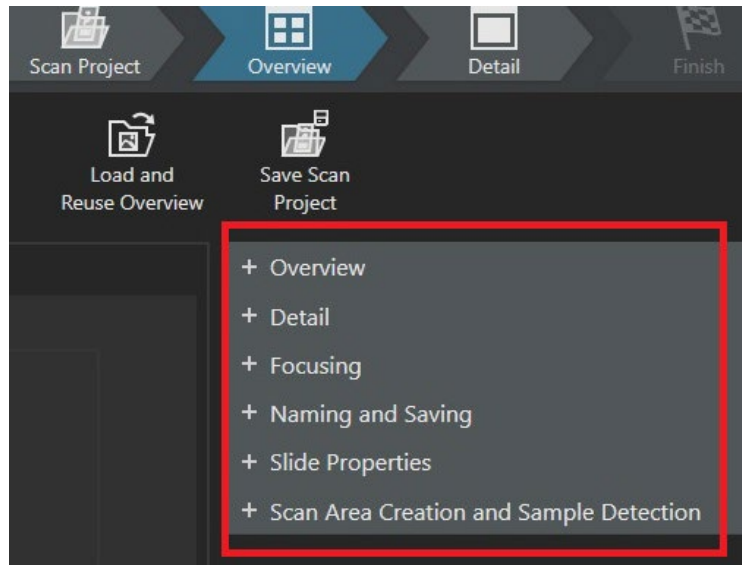
3.1.1 Create a new project or select an existing project to start with

3.1.1.1 Create a new project by editing the built-in "Default template". Highlight the to-be-scanned slide in the tray/slide view window. Click: "Brightfield"---"Default"---"Edit Scan Settings"

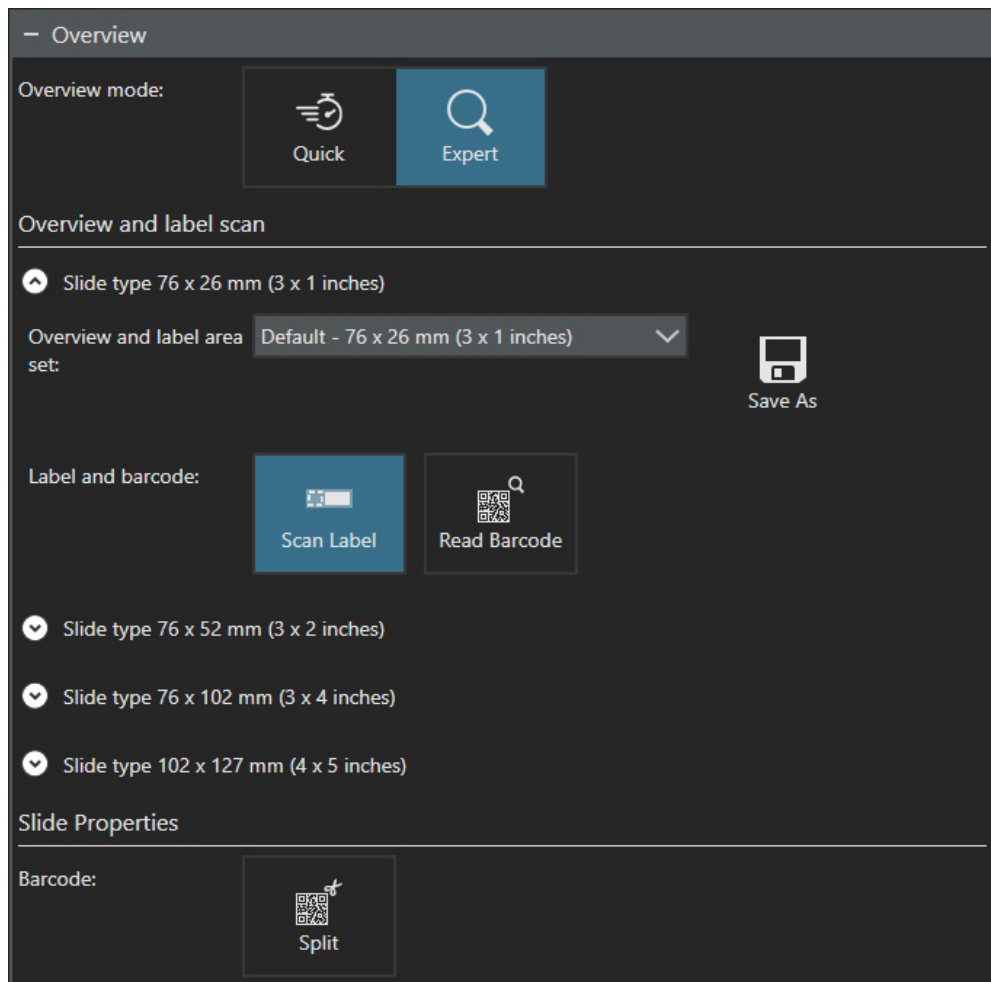
3.1.1.2 Alternatively, select a project from "My scan projects" and "Edit Scan Settings" as needed



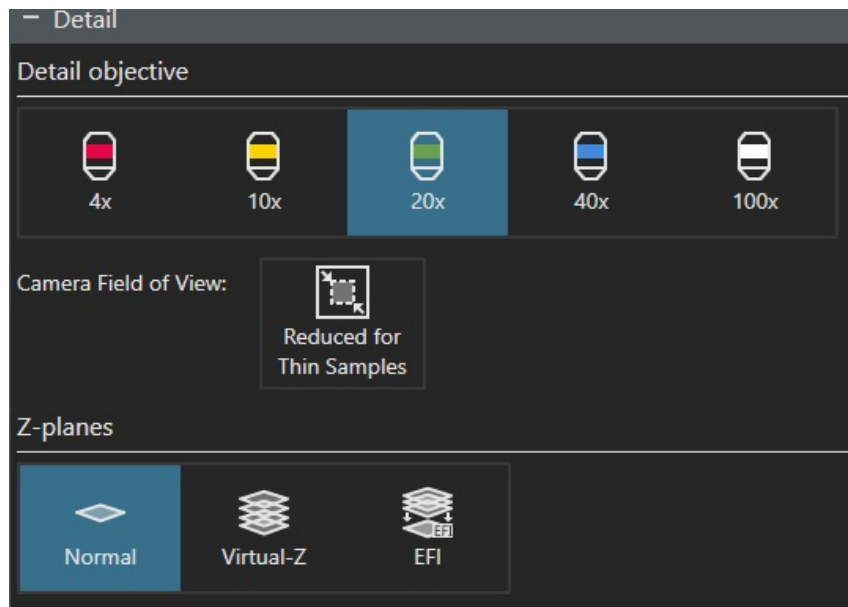
Note: To create your new project, you'll need to go through these settings including Overview, detail, focusing, naming and saving, slide properties, scan area creation and sample detection as shown in the below screenshot



3.1.2 Review/edit “Overview and label scan” settings

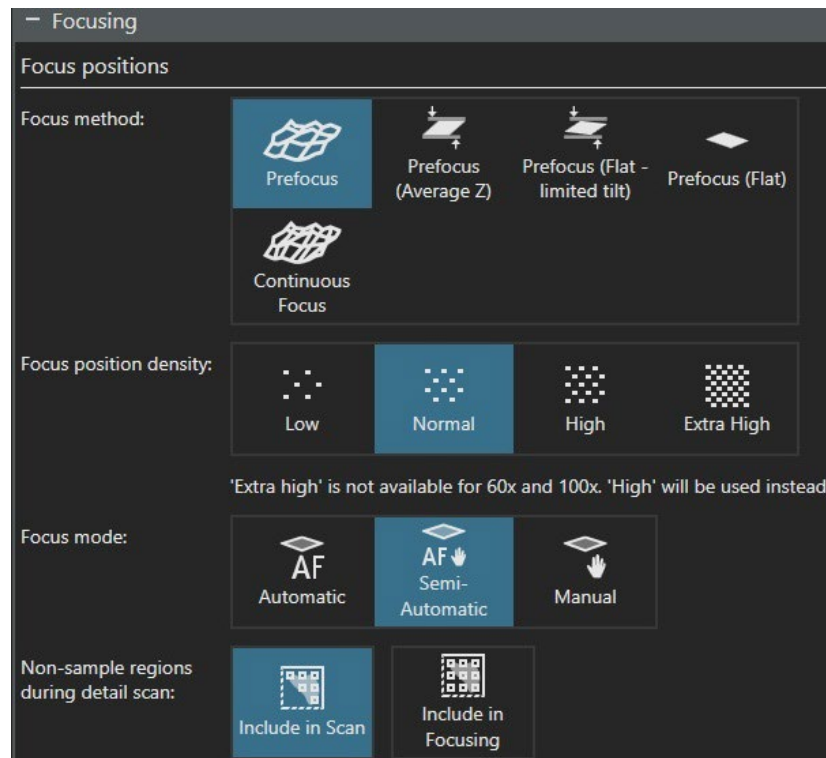


3.1.3 Review/edit “Detail” settings



3.1.4 Review/edit “Focusing” settings

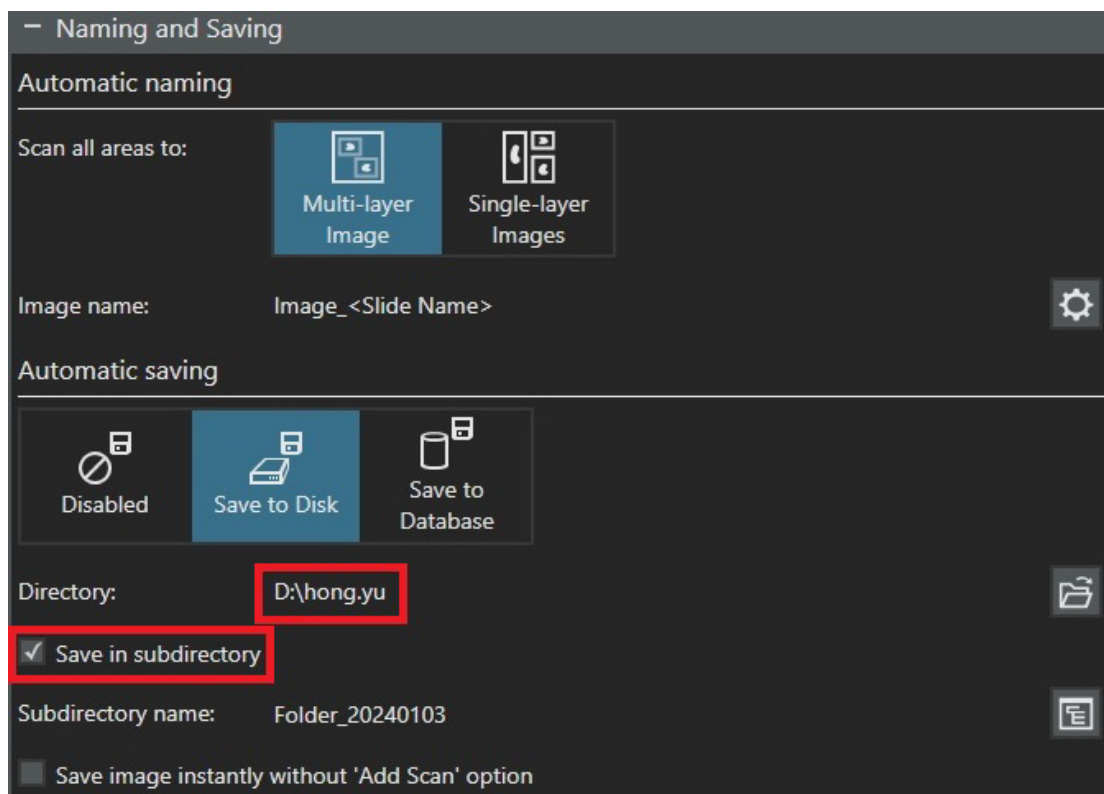
Tip: it is recommended to choose either “Semi-auto” or “Manual” for your Focus Mode. This way, you’ll be able to take advantage of the option to review all focus points and further to adjust focus either manually and/or automatically (auto focus) in case default auto focusing does not work well.



3.1.5 Review/edit “Naming and Saving” settings

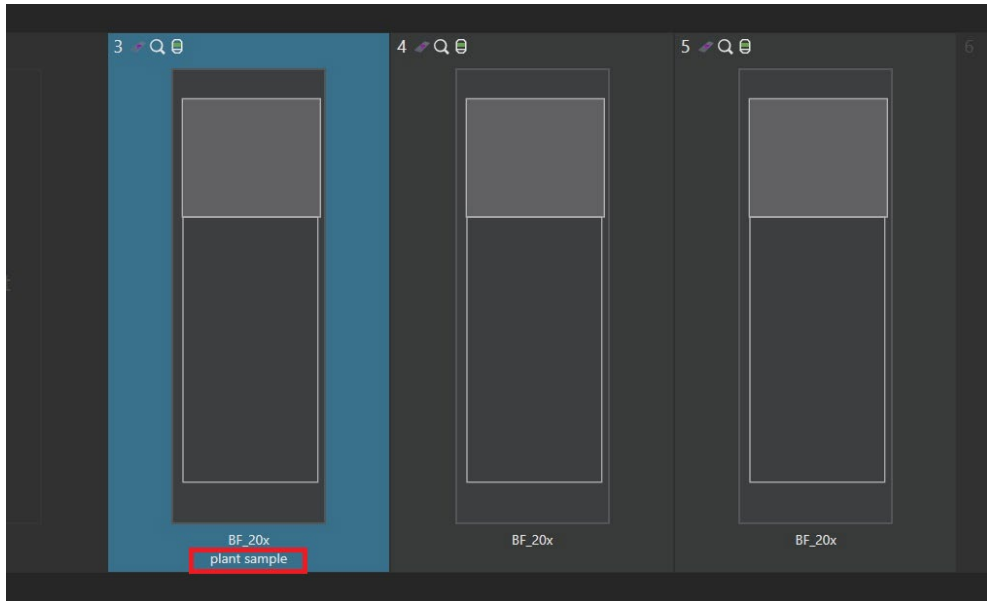
Naming: “Slide name” as programmed in the below screenshot can be typed in the next step

Saving: make sure your data path is correct: D:/your folder



3.1.6 Type in the slide name in the Slide properties

The slide name will display in the live tray view window and will be used after scan is completed to name your image as set up in the above step (refer to 3.1.5 for Naming and Saving)



3.1.7 Review/edit “Scan Area Creation and Sample Detection” settings

Note: This step is to set up the settings for auto-detecting the sample area to be used for overview scanning. There is little need to worry too much about perfection here while defining the settings as you’ll always have a chance to manually adjust the sample ROI in a later scan step.



– Scan Area Creation and Sample Detection

Scan area creation

Scan area creation method:

Automatic Predefined

Automatic scan area creation:

One for All Subsamples One for Each Subsample

Scan area type:

Rectangle Circle Polygon

Scan area sorting:

From Top to Bottom From Left to Right

Non-sample regions during detail scan:

Include in Scan Include in Focusing

Scan area during detail scan:

Enlarge

Sample detection

Sample detection method:

Generic Detection AI (Neural Network)

Sample detection sensitivity:

0

Non-colored sample detection weight:

0 %

Only keep objects of size between:

0.203 mm² 13000 mm²

Fill holes smaller than:

0.203 mm²

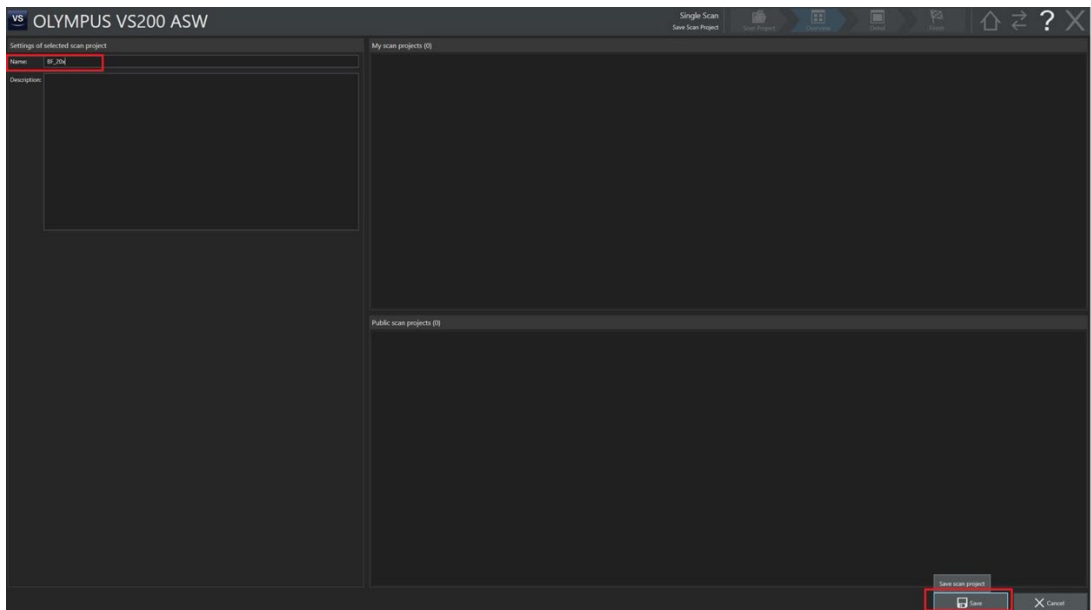
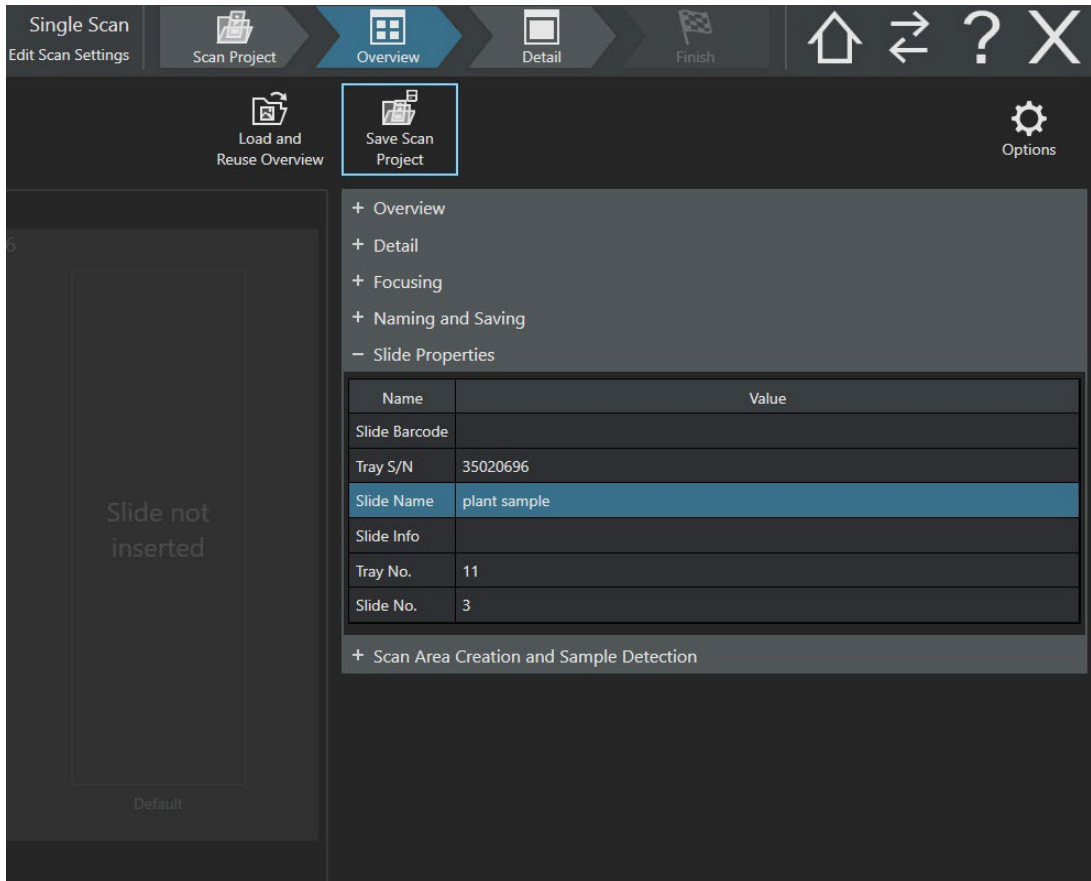
Coverslip borders:

Remove

Advanced sample detection

Marker detection

3.1.8 Save your project at the end of editing i.e. “BF 20x” in this example. Otherwise, your default template will be overwritten with your edits

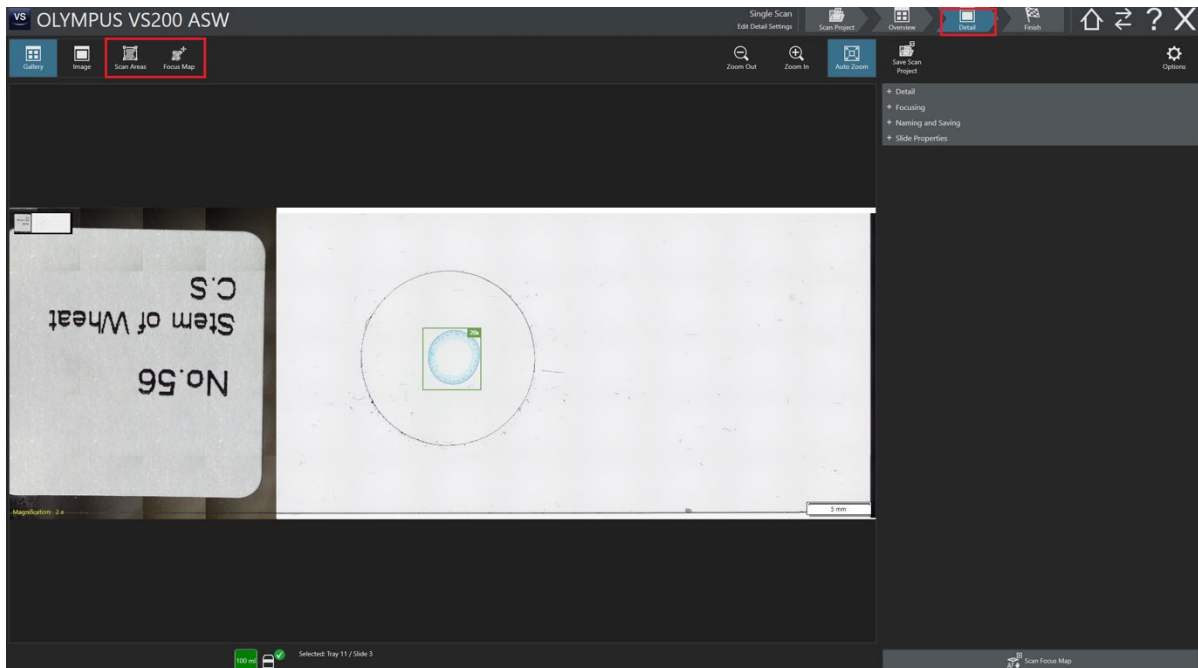


3.2 Overview scan

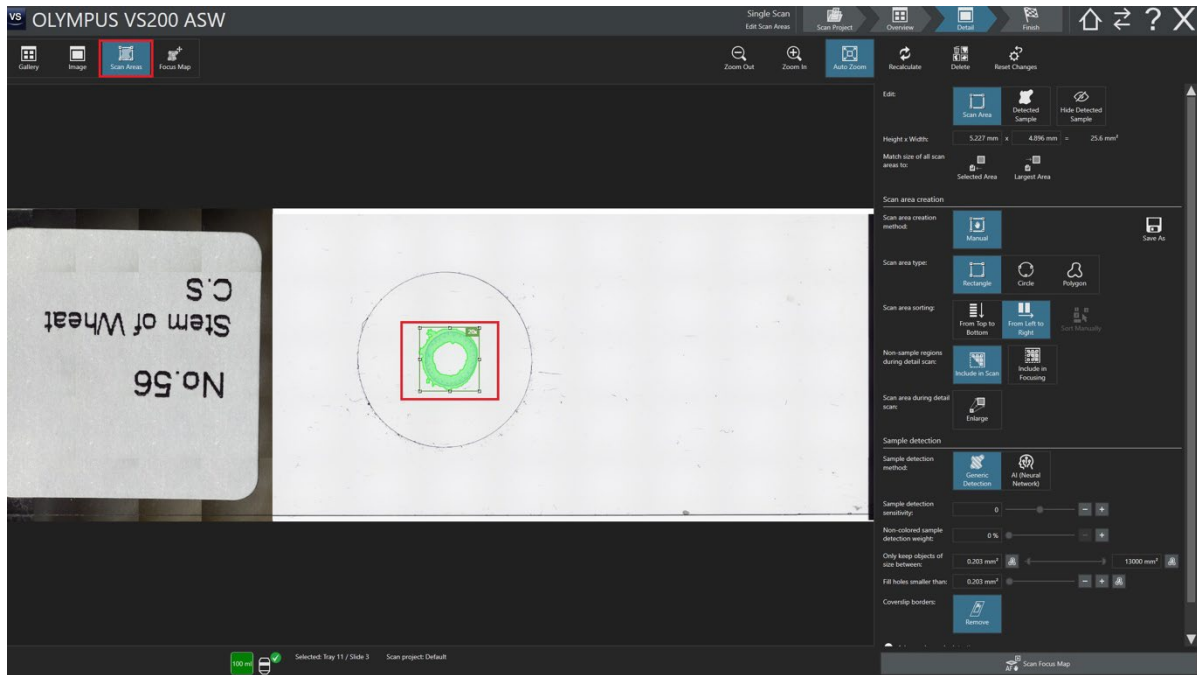
Once your project is created, click “Start Scan” to proceed overview scan using the settings set up in this new project

3.3 Detail scan

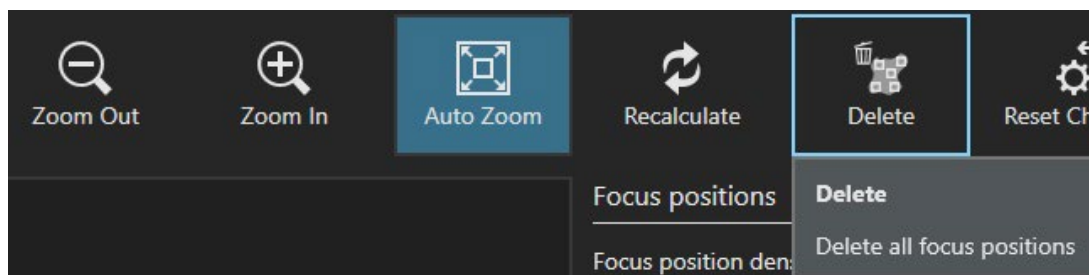
You’ll need to go through sub-steps to further set up your detail scan including selecting “Scan Area” and putting focus points to create a “Focus Map” in the selected Scan Area.



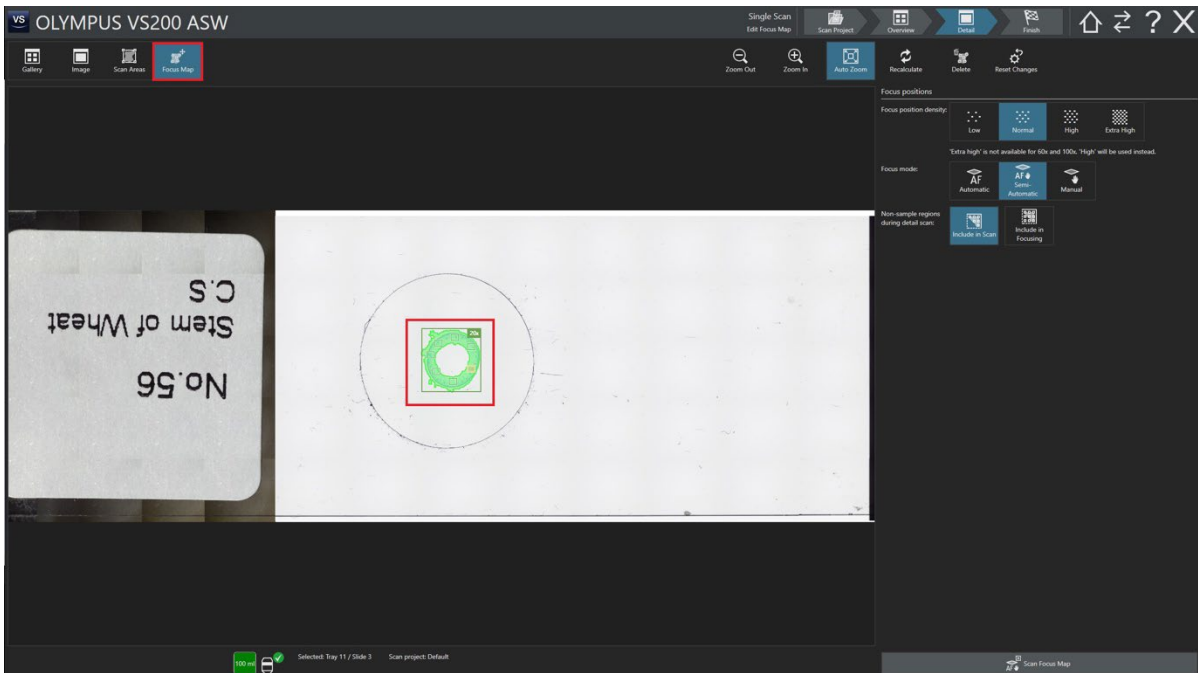
3.3.1 Scan area. Click “Scan Area” and manually select a ROI



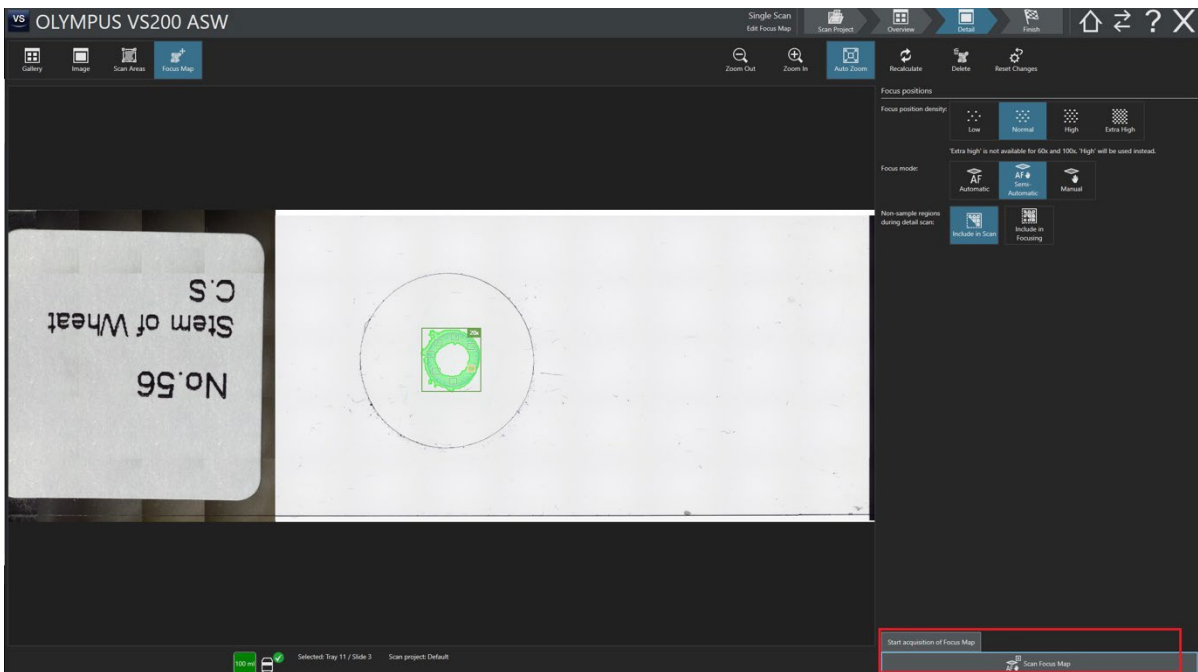
Note: if you are not happy with the auto-detected area, just click “Delete” to clear them and then manually draw a ROI



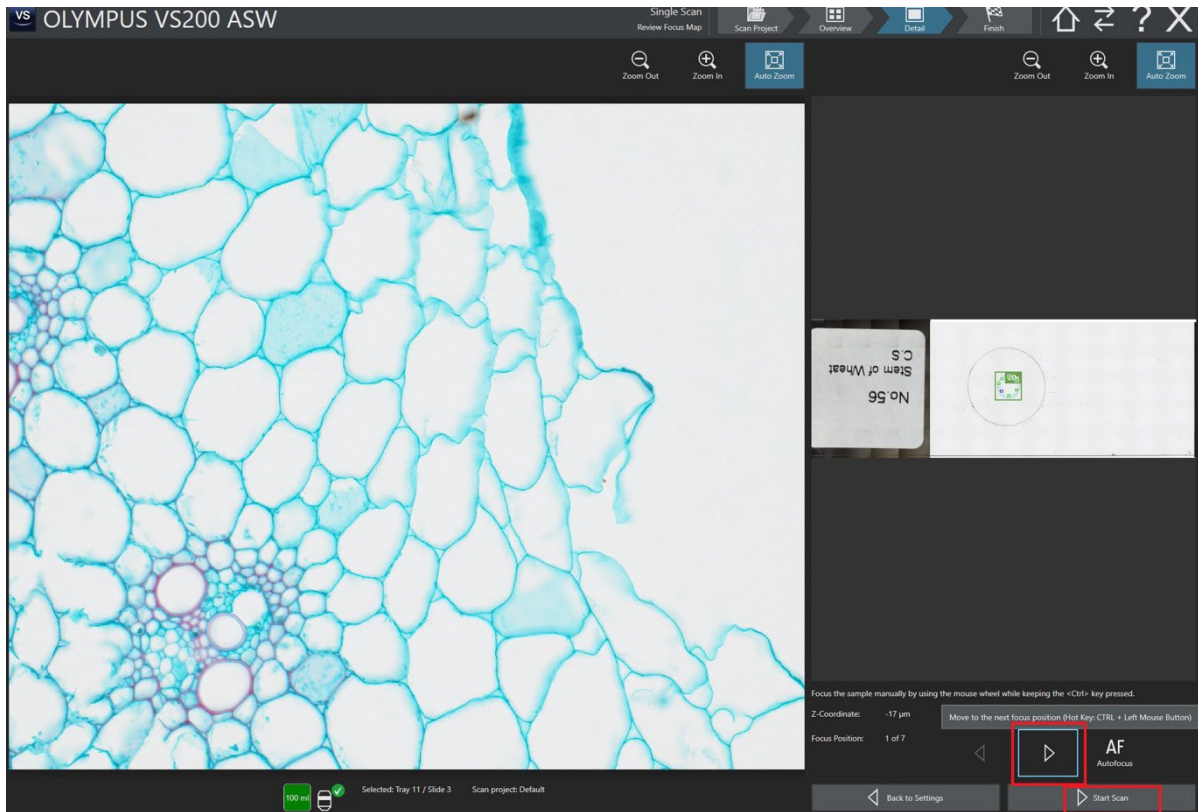
3.3.2 Focus Map. Now add focus points in the selected ROI



3.3.3 Click “Scan Focus Map” to visit all the focus points you just added to the ROI



3.3.4 Review the default auto-focusing result. If needed, you can manually adjust focusing of the focus points (ctrl key + mouse scrolling wheel for fine focusing or ctrl + shift + mouse scrolling wheel for coarse focusing)

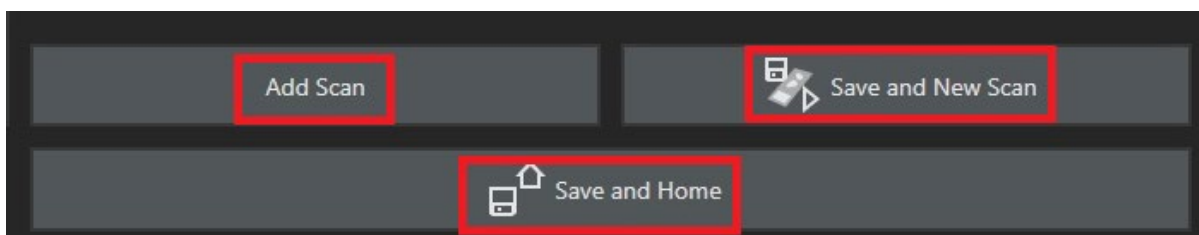


3.3.5 Proceed detail scan. Click “Start Scan” to proceed Detail Scan

3.4 Finish scan

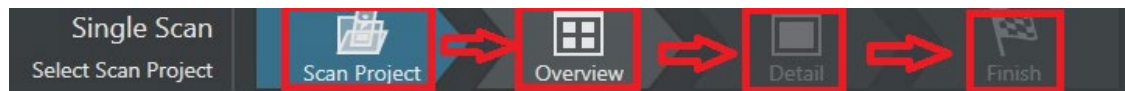
Finish the scan. Click “Save and New Slide” to scan another BF slide or “Save and Home” to return to Home where you’ll have an option to run either single or batch scan

Tip: before you finish this scan, you can choose to “Add Scan” to the current slide if you need to add additional scan i.e. adding a new ROI or change to a different objective etc



4. Single scan (Fluorescence)

Tip: Same as BF single scan, the overall workflow of fluorescence single scan includes 4 steps in the following order:



Step 1 Set up a Scan Project for subsequent scanning of your slides. You can choose to create a brand new project or edit an existing one

Step 2 Overview the whole slide. This step scans the whole slide using the Overview settings from the chosen Project. You'll need this overview image to select a ROI for next step of detail scan

Step 3 Detail scan the ROI(s)

Step 4 Finish the scan

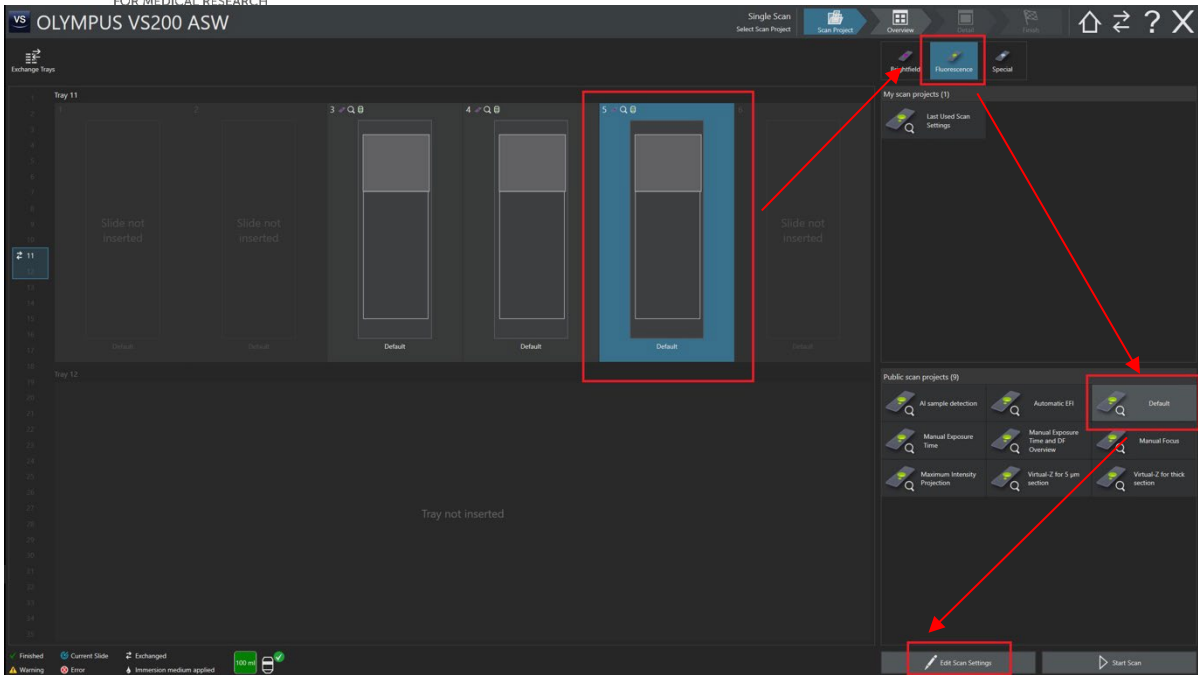
Detailed procedures are as follows:

4.1 Set up a Scan Project

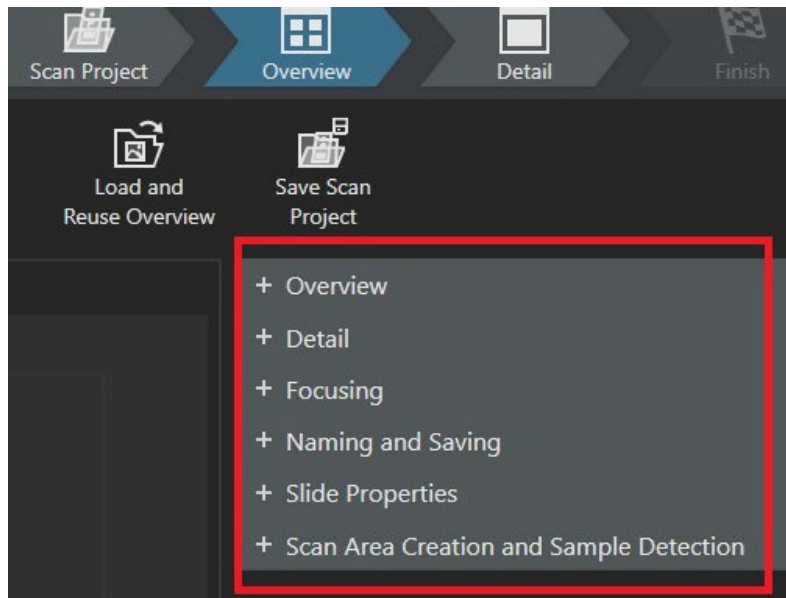
4.1.1 Create a new project or select an existing project to start with

4.1.1.1 Create a new project by editing the built-in "Default template". Highlight the to-be-scanned slide in the tray/slide view window. Click: "Fluorescence"--- Default"---"Edit Scan Settings"

4.1.1.2 Alternatively, select a project from "My scan projects" and "Edit Scan Settings" as needed





Note: To create a new project, you'll need to go through these settings including Overview, detail, focusing, naming and saving, slide properties, scan area creation and sample detection as shown in the below screenshot



4.1.2 Review/edit "Overview and label scan" settings






— Overview

Overview mode:  Quick  Expert

Overview and label scan

Slide type 76 x 26 mm (3 x 1 inches)

Overview and label area Default - 76 x 26 mm (3 x 1 inches) set: 





Label and barcode:  Scan Label  Read Barcode

Slide type 76 x 52 mm (3 x 2 inches)

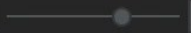


Slide type 76 x 102 mm (3 x 4 inches)




Slide type 102 x 127 mm (4 x 5 inches)

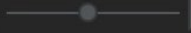


Overview focusing


Focus mode:  No Focusing  Manual Z  Quick Focus  Use Focus Map


Overview exposure

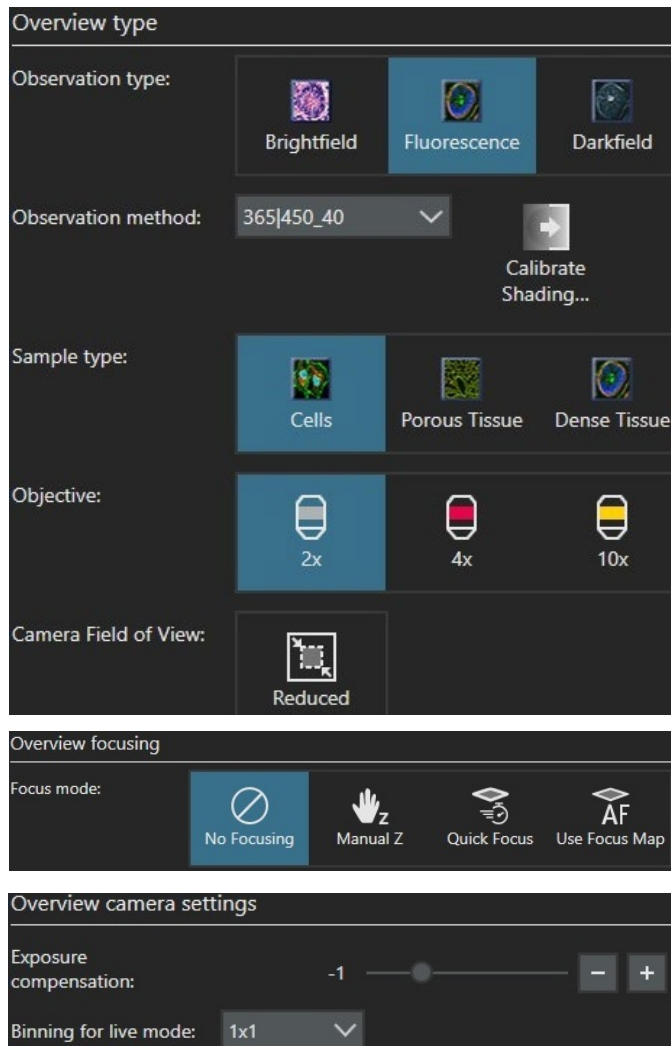
Light intensity: 70 %   

Exposure mode:  Auto Exposure  Manual Exposure  Start Live

Exposure time: 100.00 ms   

Deblur:  Online Deblur

Display limits:  Automatic



4.1.3 Review/edit "Detail" settings



- Detail

Detail objective

4x 10x 20x 40x 100x

Camera Field of View: Reduced for Thin Samples

Z-planes

Normal Virtual-Z EFI MIP

Channels

Add FL Channel Add TMO Channel Remove Channel First Channel: Focusing Only

#	Channel Name	Intensity	Exposure	Deblur	Display Limits
1	365 450_40	70%	Auto	-	Auto
2	645 700_50	70%	Auto	-	Auto
3	725 800_40	70%	Auto	-	Auto
4	490 526_36	70%	Auto	-	Auto
5	555 590_20	70%	Auto	-	Auto

Use Z-Offset Calibrate Shading...

Channel Settings

Light intensity: 70 %

Exposure mode: AE Auto Exposure Manual Exposure Start Live


Maximum exposure time (all channels): 1.00 s


Deblur: Online Deblur


Display limits:


Tips: how to set up your fluorescence channels - Add or remove channel(s), you can also change the Channel Name (right click and type) or re-order the fluorescence channels (click the up/down arrows next to the FL channel(s))

Channels


 Add FL Channel

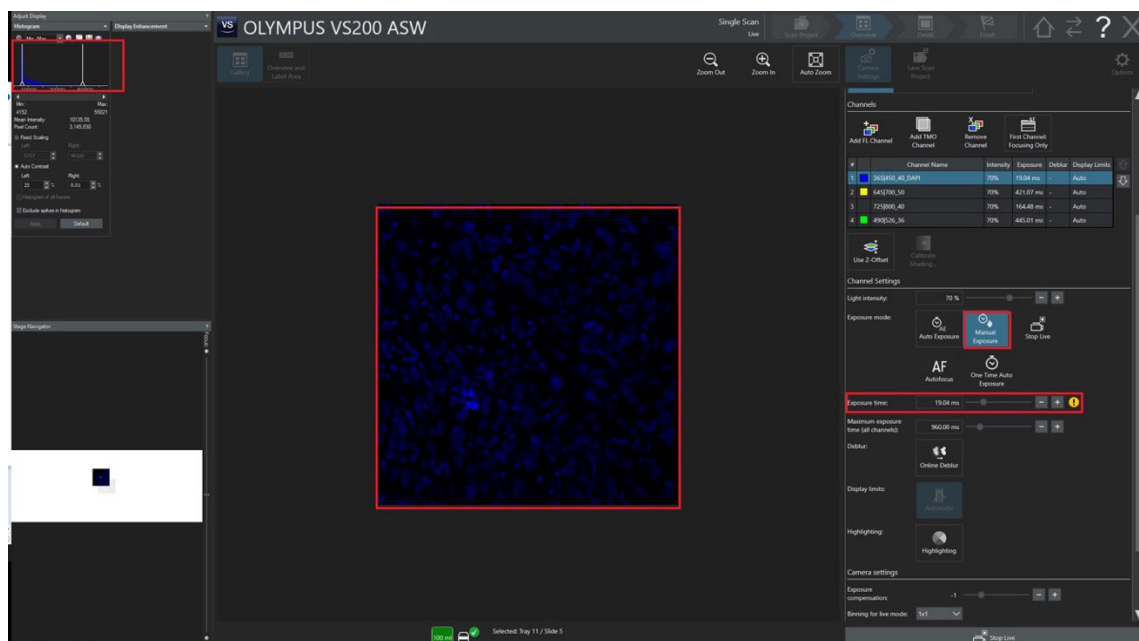

 Add TMO Channel


 Remove Channel


 First Channel: Focusing Only

#	Channel Name	Intensity	Exposure	Deblur	Display Limits
1	365 450_40_DAPI	70%	Auto	-	Auto
2	645 700_50	70%	Auto	-	Auto
3	725 800_40	70%	Auto	-	Auto
4	490 526_36	70%	Auto	-	Auto
5	555 590_20	70%	Auto	-	Auto

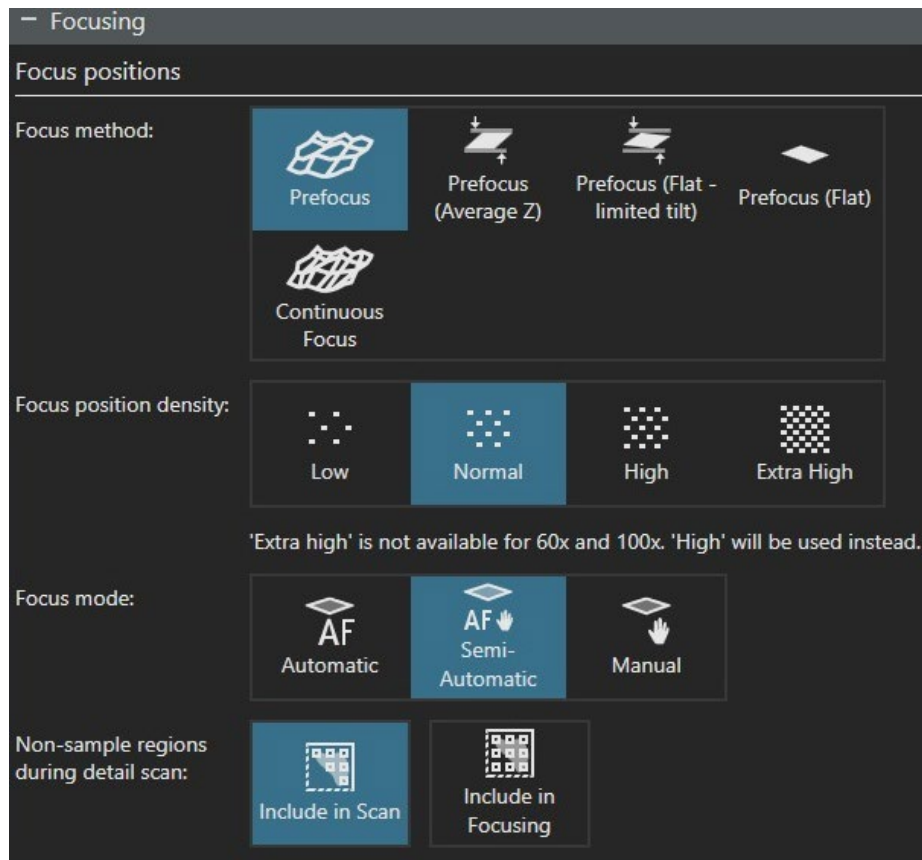
Tips: how to set up your fluorescence channels - manually set up exposure time



The screenshot shows the Olympus VS200 ASW software interface. The main window displays a fluorescence image of a cell sample. On the right side, the 'Channels' panel is visible, showing a list of channels with their respective intensity, exposure, and display limits. The 'Channel Settings' panel is also visible, showing the 'Manual Exposure' mode selected, with an exposure time of 19.04 ms. The 'Detail' settings are visible on the right side of the interface.

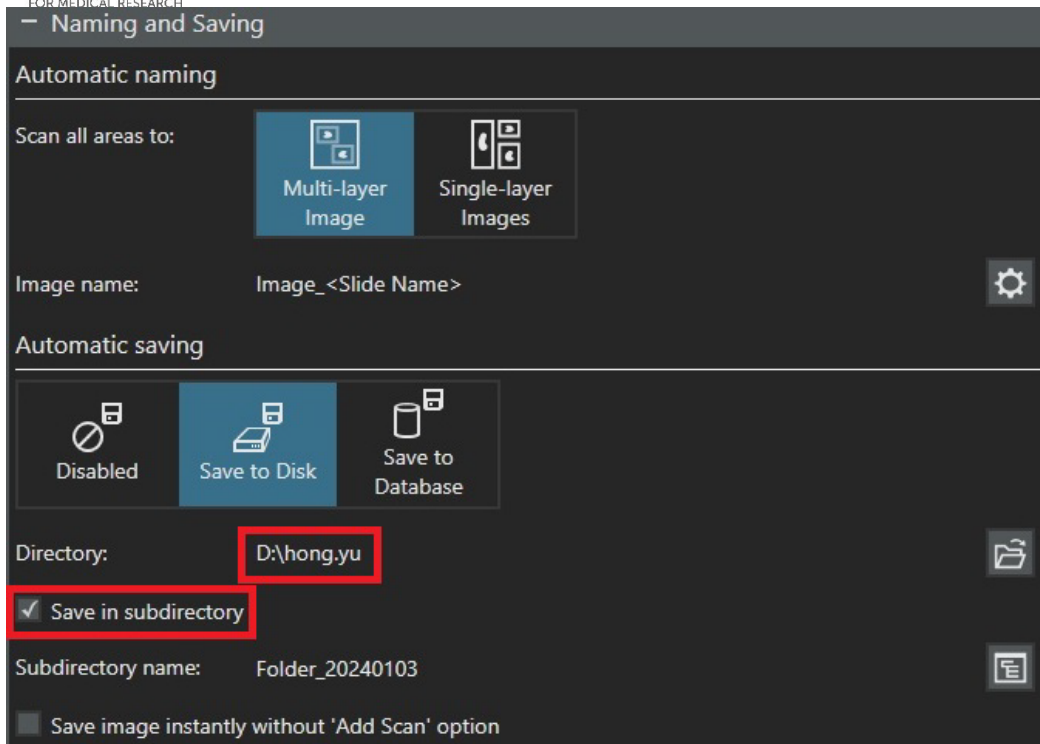
Note: complete the rest of “Detail” settings

4.1.4 Review/edit “Focusing” settings



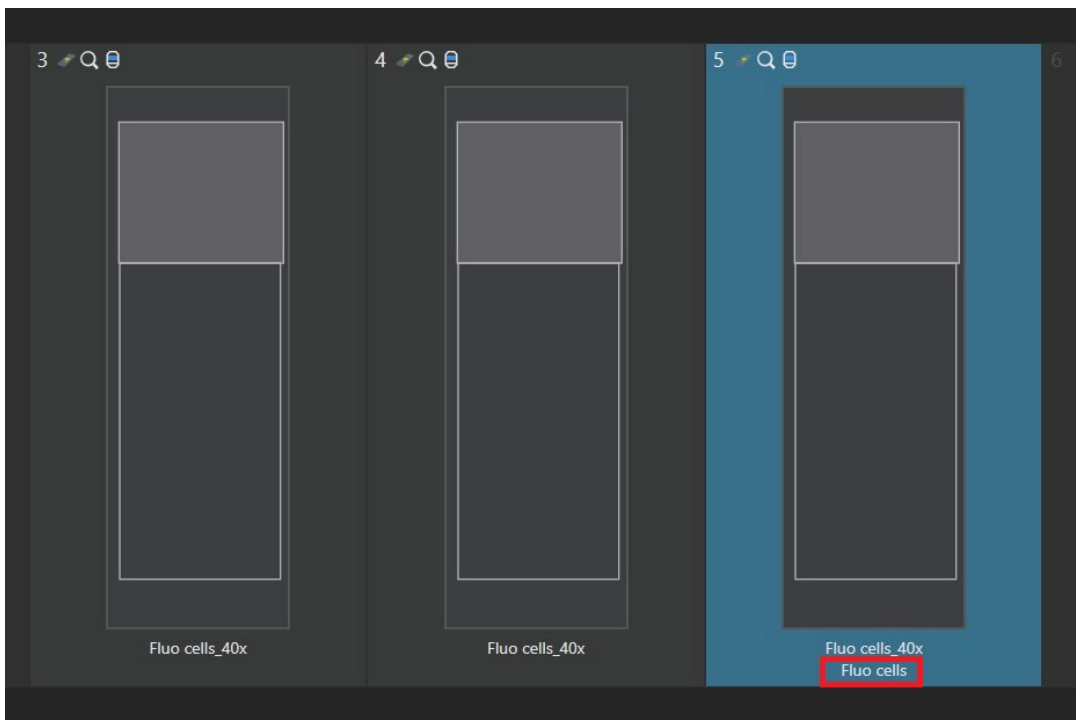
Tip: it is recommended to choose either “Semi-auto” or “Manual” for your Focus Mode. This way, you’ll be able to take advantage of the option to review all focus points and further to adjust focus either manually and/or automatically (auto focus) in case default auto focusing does not work well.

4.1.5 Review/edit “Naming and Saving” settings

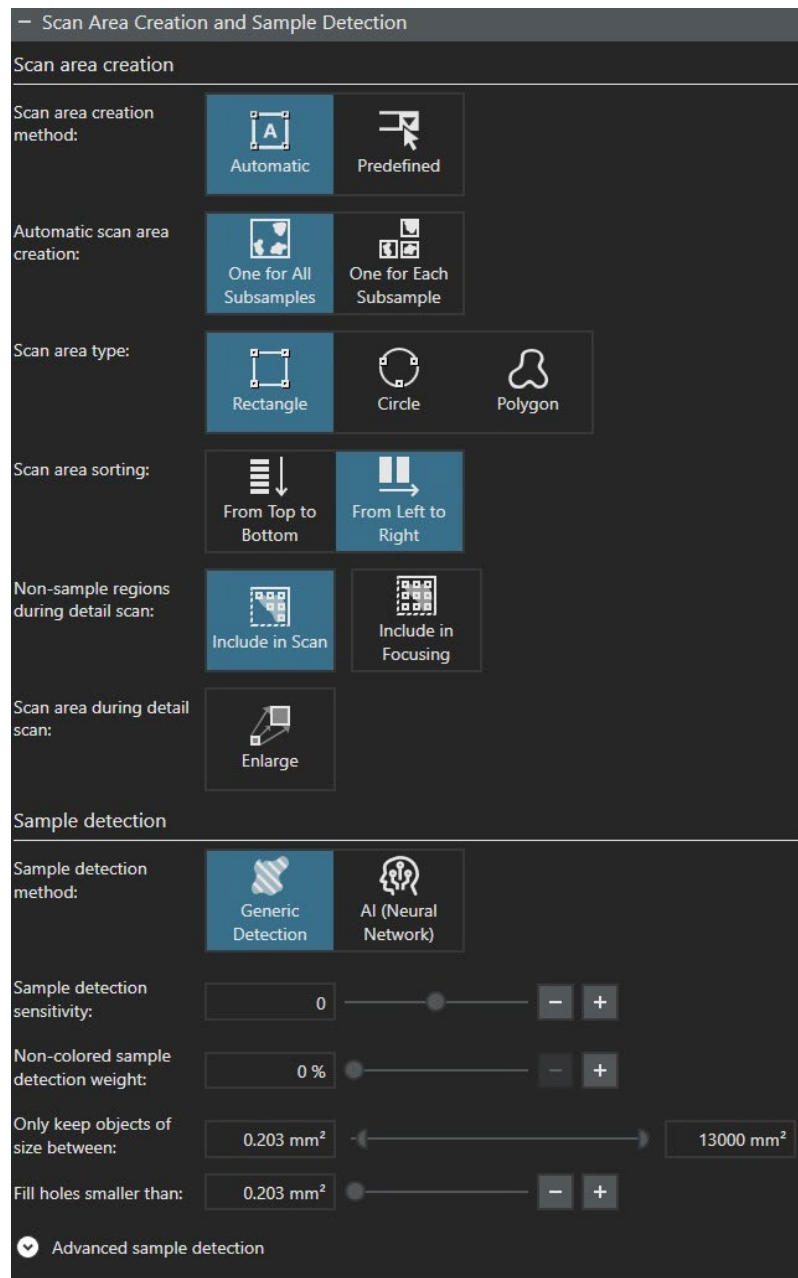


4.1.6 Type in the slide name in the “Slide properties”

The slide name will be displayed in the tray view window and be used after scan is completed to name your image as set up in the above step (refer to 4.1.5 for Naming and Saving)



4.1.7 Set up the settings for Scan Area Creation and Sample Detection



Note: This step is to set up the settings for auto-detect the sample area to be used for overview scanning. No need to worry too much about finely defining the settings as you'll always have a chance to manually adjust the sample ROI in the next detail scan step

4.1.8 Save your project at the end of editing i.e. “Fluo Cells 40x” in this example. Otherwise, your default template will be overwritten with your edits

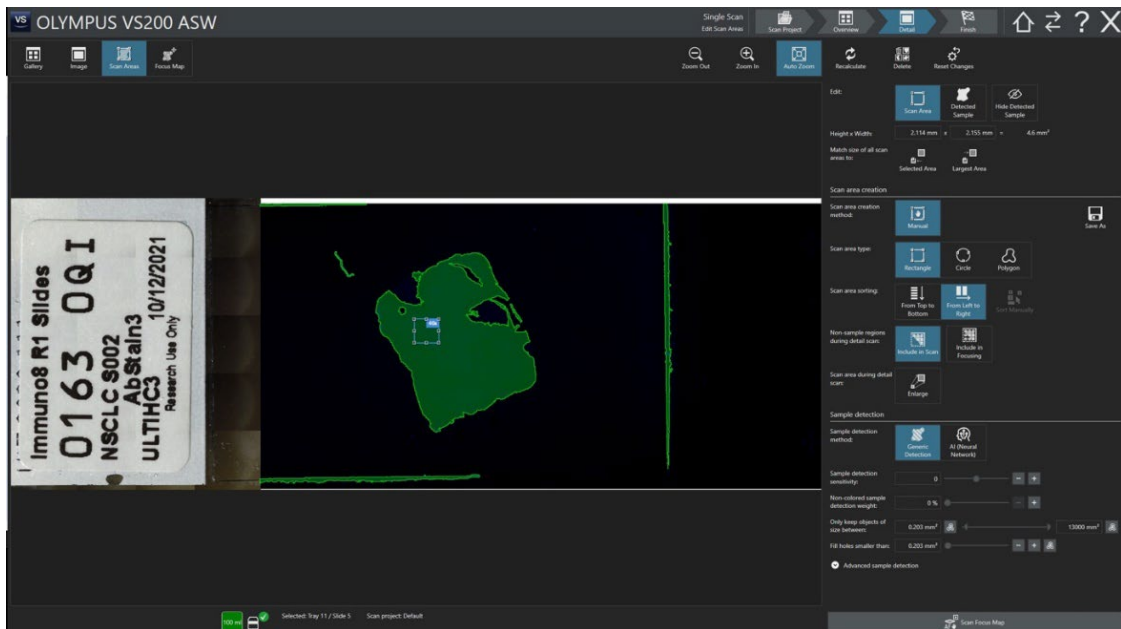
4.2 Overview scan

Once your project is created, click “Start Scan” to proceed overview scan using the settings set up in this new project

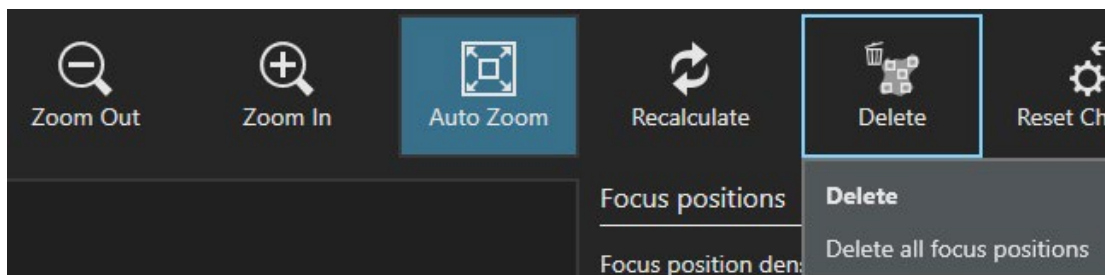
4.3 Detail scan

You’ll need to go through two sub-steps here to further set up your detail scan including selecting “Scan Area” and putting focus points to create a “Focus Map” in the selected Scan Area

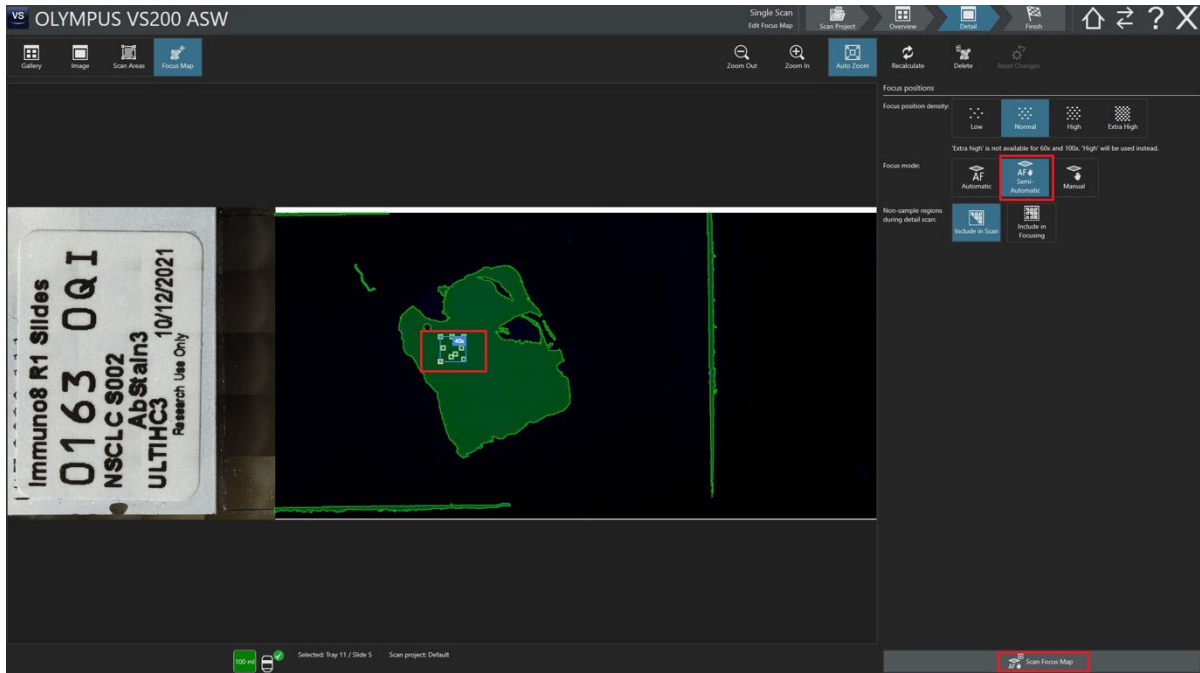
4.3.1 Scan area. Click “Scan Area” and manually select a ROI



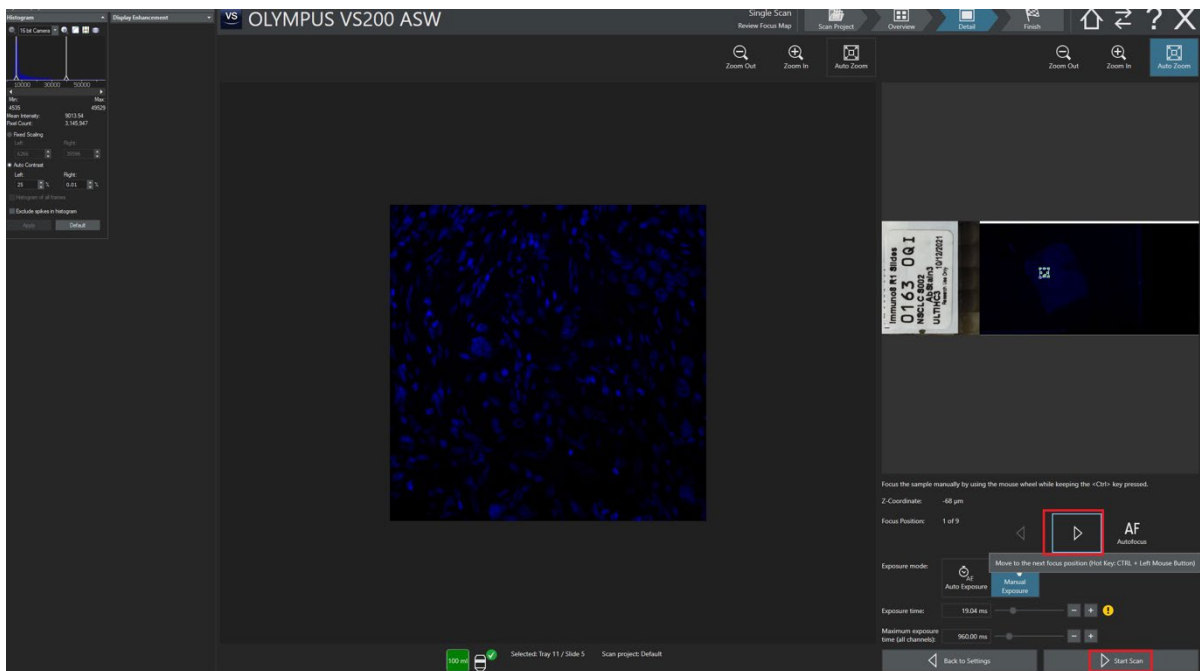
Note: if you are not happy with the auto-detected area, just click “Delete” to clear them and then manually draw a ROI.



4.3.2 Focus Map. Now add focus points in the selected ROI



4.3.3 Click “Scan Focus Map” to visit all the focus points just added to the ROI. Review the default auto-focusing result. If needed, you can manually adjust focusing of the focus points (ctrl key + mouse scrolling wheel for fine focusing or ctrl + shift + mouse scrolling wheel for coarse focusing)

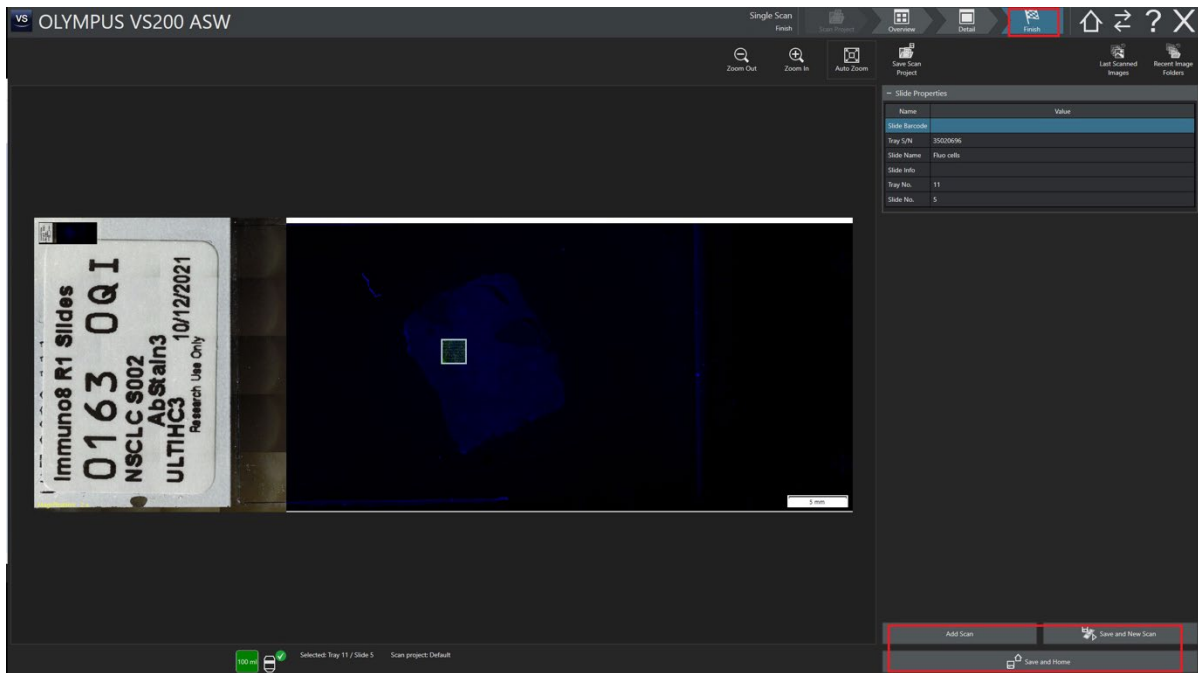


4.3.4 Proceed Detail scan. Click “Start Scan” to proceed Detail Scan

4.4 Finish the scan

Click “Save and New Slide” to scan another BF slide or “Save and Home” to return to the home screen offering an option to run single or batch scan

Note: before you finish this scan, you can choose to “Add Scan” to the current slide

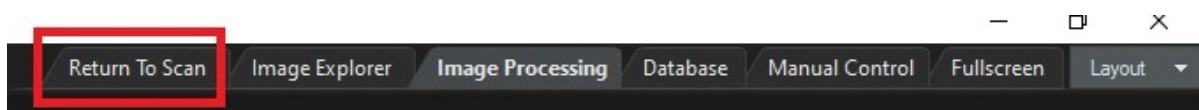


5. Image viewing in VS 200 ASW

View images in VS 200 ASW. To view your images with more options for processing, switch to VS 200 ASW’s viewing interface. To access click “double-way arrows” icon on the top right corner of the screen



Note: if you want to return to acquisition in VS 200 ASW, click “Return To Scan”



6. System shutdown

6.1 Clean the 100X oil objective if applicable.

- 6.1.1 Click the Home icon on the top right corner of the screen to go to the home page of VS 200 ASW

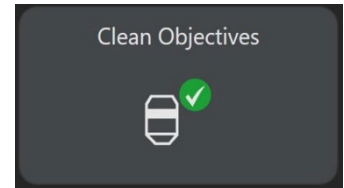


- 6.1.2 Click “Clean Objectives” to release the 100X objective on the microscope for better cleaning access.

- 6.1.3 Open the front door of the microscope

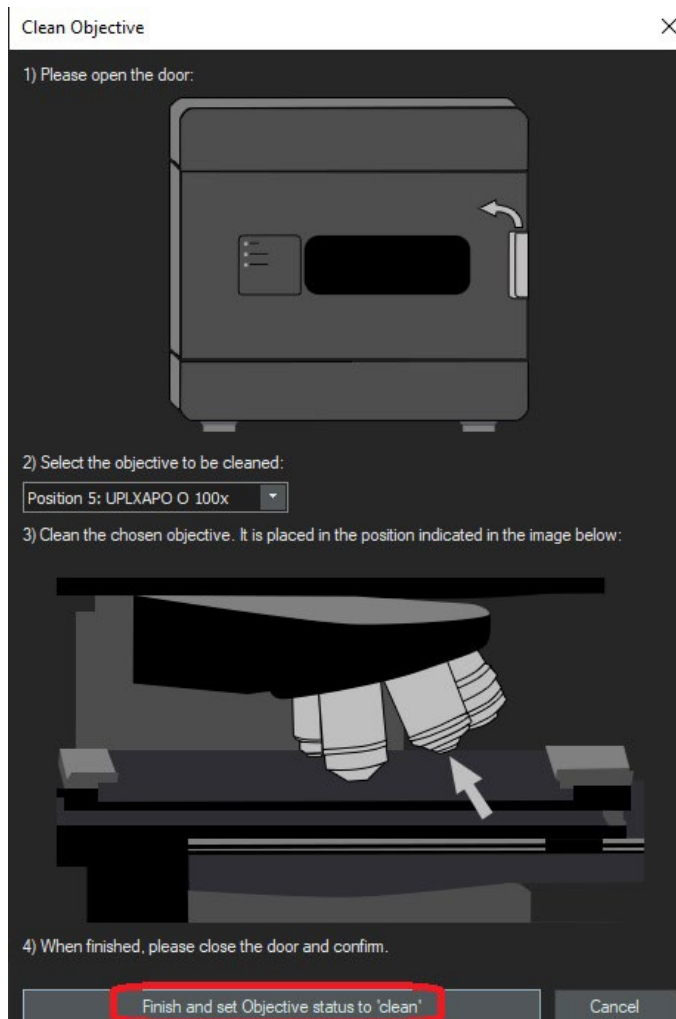
- 6.1.4 Clean the 100x objective using provided single use disposable lens tissue wipes

- 6.1.5 Once finished, close the microscope door



Warning: Do not take off the oil objective as the microscope is aligned based on the current objective position

- 6.1.6 Click “Finish and set Objective status to clean” to complete the objective cleaning step



6.2 Close the software VS 200 ASW



6.3 Switch off the three components in 3-2-1 order (camera—microscope—LED light)

6.4 Take out the trays from the tower and close the door

DATA & RECORDS MANAGEMENT

- Data should be saved in the data drive in your named folder on the acquisition computer (e.g., D:\firstname.lastname')
- Your data will synchronise upon logout to
 - o Scientific platforms, WIMR
 - o Onedrive, Westmead Imaging
 - o splouddata, Cloud
- Note the data drives may change from time to time to reflect updated workflows, this will be communicated via email**
- (optional) Your data can also be adhoc pushed to the remote drives via a desktop shortcut on demand
- (optional) Upon completion of your training, you will have received a link to your shared onedrive folder for non-WIMR data access if required
- It is recommended you process your data using the new cloud ultra virtual data analysis machine (UltraVM)
- The UltraVM
 - o can be booked in PPMS
 - o has all the required Olympus software preinstalled, allowing for fast conversion as well as deep learning workflows (please note .vsi conversion is unfortunately a single CPU process)
 - o accesses data on the onedrive or splouddata drive (to be updated upon cost analysis)
 - o is blazingly quick... 24xEPYC CPUs, 80GB A100 GPU, 220GB RAM, 50GB SCRATCH (may change without notice).
 - o is charged at \$20 per hour in 2024 (software/storage/compute included)
 - o can be connected to by any Windows "Remote Desktop Connection" and your WIMR credentials while on the WIMR network.
- Your data can be written to either your own mapped drive, or the scratch space (this is wiped when UltraVM is shutdown).
- UltraVM access details will be provided once you are trained

REFERENCES

For more information about single scan and other advanced applications i.e. batch scan, Z stacking etc, please refer to the complete manual provided by Olympus (available on the acquisition computer desktop once you login and also on the Westmead Imaging


Website:

<https://sydneyuni.atlassian.net/wiki/spaces/WIF/pages/768016621/Manuals+Protocols>.

This SOP is also published on the Westmead Imaging Website:

<https://sydneyuni.atlassian.net/wiki/spaces/WIF/pages/768016621/Manuals+Protocols>.

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