

STANDARD OPERATING PROCEDURE

Olympus VS 120 Slide Scanner

PURPOSE

This document describes the operational setup and procedures for the Olympus VS 120 Slide Scanner (VS 120). This document offers users, particularly beginners, a brief step-by-step instruction on how to operate the Olympus slide scanner.

The VS 120 is a high throughput slide scanner offering high resolution and high throughput for both bright field and fluorescence imaging. The batch scanning/imaging allows 100 slides to be queued for acquisition.

SCOPE

This procedure applies to bright field (BF) and fluorescence imaging operations including starting up the system, preparing the system, running single and/or batch scanning mode and shutting down the system. It covers both single slide and batch modes. This procedure does not cover polarisation scanning.

Users should refer to the full manual provided by the vendor for detailed instructions. A copy of the manual is available in the instrument lab and via the Westmead Imaging internet site.

SAFETY

The operator must exercise caution during loading or removing slides from the stage, never put fingers into the sample loading area while the stage is moving or initialising.

In addition, safe operation of the instrument also requires the awareness of other risks, e.g. glass cuts, exposure to mercury lamp explosion, fatigue and gesture discomfort/injury etc.

Refer to Safe Work Procedure WIMR-SWP-WHS-GEN-16.01, "Using a fluorescence or laser scanning microscope": <https://sydneyuni.atlassian.net/wiki/spaces/WIF/pages/768016621/Manuals+Protocols>

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 120 Slide Scanner is located in J.2.08, Level 2 WIMR.

PROCEDURE

Starting up

- Turn on the computer. Log in (AD login).
- Turn on the system according to the component order (numbered in handwriting on each component/part of the system):
 1. Controller
 2. Microscope control box
 3. Mercury burner for fluorescence scanning if applicable
 4. Power switch of the slide loader
 5. Fluorescence camera (push the switch on the top of the camera from right to left to turn power on).
The BF camera is always on.
- After the system has started up, double click the [VS-ASW-L100] icon on the desktop to start up the software.

Loading slides to the cassettes

- Insert the slides into the slots with the coverslip facing upwards and the label area pointing outwards.
DO NOT set more than 1 slide into one slot
DO NOT set the slide to span two slots
- Up to 50 sides can be loaded into one cassette (total 100 slides for 2 cassettes can be scanned in one go).



Important notes on slide preparation for batch scanning!

- **NEVER** load slides that are wet or have mounting medium not completely set.
- If a coverslip is sticking out, or if there is a lump of mounting medium on any edges of a slide, use the provided razor blade and/or paper to make the edge smooth.
- Make sure sticky labels are not sticking out beyond slides. Slice any edges that stick out with the razor blade.
- Clean the slides with 100% ethanol before loading if needed.

Scanning modes

Got to instructions for the scanning mode you wish to use:

- Single BF (Quick mode) – p.3
- Single BF (Expert mode) – p.4
- Single Fluorescence (Expert mode) – p.5
- Batch BF (Expert mode) – p.6
- Batch Fluorescence (Expert mode) – p.7

Placing a single slide onto the stage (*for single mode*)

- Release the stage from the software (recommended - need to enter the virtual slide scanning wizard first or by manually controlling the joystick).
- Pull the silver metal clip to release the grabbing claws, in the meantime place the slide onto the stage with the coverslip upwards and the labelled side to the left.

Single BF (Quick mode)

The wizard will take you through by following the steps below to set up scanning



- Scan mode: click “Quick” under brightfield category.
- Slide input: click “manual” to release the stage, then load the slide onto the stage as described on Page 2. Then click “ok” in the pop-up window that displays “please insert a new slide”. Click “next” to continue.
- Slide properties: enter slide info here. Click “option” at the bottom, click “overview scan” tab, under “overview scan parameter”, tick “scan label” and under “exposure settings”, tick “manual exposure”.
- Auto naming: click “...” to set up your naming rule, e.g. “slide name_1...”. Click the “save to disk” icon.
- Auto saving: **all data must be saved here: F:\Virtual Slide Images\“your folder” (kept for 7 days before auto deleting). Your data will be transferred to the server instantly: Scientific platforms G:\Cell Imaging\VS120\“your folder” (kept for 14 days before auto deleting)**
- Tick “substructure” and then click “next”.
- Click “overview” to process overviewing.
- Magnification: choose an objective for final scanning and a Z-scan mode for final scanning, i.e. single layer, then “next”.
- Choose an objective for final scanning then “next”.
- Choose a Z-scan mode for final scanning, i.e. single layer.
- Click “Scan Now”.
- Finish: Click either “save and scan new slide” or “save and finish” to exit the current protocol (this will also save the scanned images to defined folder as set up earlier in the autosaving step). If you like to further modify the scanning conditions of the current slide, click “add scan” to go through the steps as you like to modify.

Single BF (Expert mode)

The wizard will take you through by following the steps below to set up scanning

Scan Mode → Slide Input → Slide Properties → Magnification → Scan Area → Focus Settings → Review Focus Map → Finish

- Scan mode: click “Expert” under brightfield category.
- Slide input: click “manual” to release the stage then load the slide onto the stage as described on Page 2. Then click “ok” in the popping up window that displays “please insert a new slide”. Click “next” to continue.
- Slide properties: type in the slide info here. Click “option” at the bottom, click “overview scan” tab, under “overview scan parameter”, tick “scan label”; under “exposure settings”, tick “manual exposure”.
- Auto naming: click “...” to set up your naming rule i.e. “slide name_1...”, click the “save to disk” icon
- Auto saving: **all data must be saved here: F:\Virtual Slide Images\“your folder” (kept for 7 days before auto deleting). Your data will be transferred to the server instantly: Scientific platforms G:\Cell Imaging\VS120\“your folder” (kept for 14 days before auto deleting)**
- Tick “substructure” and then click “next”.
- Click “overview” to process overviewing.
- Magnification: choose an objective for final scanning and a Z-scan mode for final scanning, i.e. single layer, then “next”.
- Scan area: click “Edit Scan Area”. You will be directed to an Edit Scan Area window... “Clear All” first, then choose a “Scan and Focus logic” and a “Scan area type”. In the live window, draw a ROI to select your scanning area.
- After selecting a ROI, down in the same tab, click “edit focus map”, you’ll be directed to an Edit Focus Map window (if you miss this step here, don’t worry, this can be done in the next step). You can either “Clear All” auto picked focus points first and add focus points as you like, or later edit the auto generated focus points (based on chosen “Grid Density”) in the selected ROI, i.e. moving or deleting these points. Choose a “Scan and focus logic” (middle option is recommended). Finally click “Confirm input”.
- Focus settings: click “Edit focus map” to set up focus points (skip this step if already done in the previous step)
- Focus logic: the middle option is recommended (half auto focus half manual focus)
- Click “scan focus map”.
- Review focus map: you are now offered to review all the selected focus points. If you are not happy with the focus effect, you can either manually adjust the focus handle or use “AF” to improve. After reviewing all points, click “Scan Now”.
- Finish: Click either “save and scan new slide” or “save and finish” to exit the current protocol (this will also save the scanned images to defined folder as set up earlier in the autosaving step). If you like to further modify the scanning conditions of the current slide, click “add scan” to go through the steps as you like to modify.

Single Fluorescence (Expert mode)

The wizard will take you through by following the steps below to set up scanning

Virtual Slide Acquisition Wizard - Fluorescence Expert Mode

Scan Mode → Slide Input → Slide Properties → Overview → Magnification → Scan Area → Illumination Settings → Focus Settings → Review Focus Map → Finish

- Scan mode: click “Expert” under Fluorescence category.
- Slide input: click “manual” to release the stage, then load the slide onto the stage as described on Page 2. Then click “ok” in the popping up window that displays “please insert a new slide”. Click “next” to continue.
- Slide properties: type in the slide info here. Click “option” at the bottom, click “overview scan” tab, under “overview scan parameter”, tick “scan label”; under “exposure settings”, tick “manual exposure”.
Auto naming: click “...” to set up your naming rule i.e. “slide name_1...”, click the “save to disk” icon.
Auto saving: **all data must be saved here: F:\Virtual Slide Images\“your folder” (kept for 7 days before auto deleting). Your data will be transferred to the server instantly: Scientific platforms G:\Cell Imaging\VS120\“your folder” (kept for 14 days before auto deleting)**
Tick “substructure” and then click “next”.
- Overview: depending on your sample, can choose either BF (BF mono) or fluorescence imaging to overview the slide. Overview lens: 2X or 10X.
- Magnification: choose an objective for final scanning and a Z-scan mode for final scanning, i.e. single layer, then “next”.
- Scan area: click “Edit Scan Area”. You’ll be directed to an Edit Scan Area window. “Clear All” first, then choose a “Scan and Focus logic” and a “Scan area type”. In the live window, draw a ROI to select your scanning area. After selecting a ROI, down in the same tab, click “edit focus map”, you’ll be directed to an Edit Focus Map window (if you miss this step here, don’t worry, this can be done in the next step). You can either “Clear All” auto picked focus points first and add focus points as you like or later edit the auto generated focus points (based on chosen “Grid Density”) in the selected ROI, i.e. moving or deleting these points. Choose a “Scan and focus logic” (middle option is recommended). Finally click “Confirm input”.
- Illumination settings:
Add/select channels: if you can, always use the filters from the same position i.e. “DAPI-tri”+“FITC_Tri”+ “TexRed_Tri”. A bad combination example: “DAPI_Tri”+“FITC”+“TRITC”. **Note: the 1st channel in the filter list will be the representative one used for focus purpose across the sample.**
Click “start Live” to bring up live window so you can manually adjust the exposure time for individual channels. Click “next”.
- Focus settings: click “Edit focus map” to set up focus points (skip this step if already done in the previous step).
Focus logic: the middle option is recommended (half auto focus half manual focus)
Click “scan focus map”.
- Review focus map: you are now offered to review all the selected focus points. If you are not happy with the focus effect, you can either manually adjust the focus handle or use “AF” to improve. After reviewing all points, click “Scan Now”.
- Finish: Click either “save and scan new slide” or “save and finish” to exit the current protocol (this will also save the scanned images to defined folder as set up earlier in the autosaving step). If you like to further modify the scanning conditions of the current slide, click “add scan” to go through the steps as you like to modify.

Batch BF (Expert mode)

The wizard will take you through by following the steps below to set up scanning

Scan Mode → Slide Properties → Scan Settings → Batch Scan Overview → Review Batch Scan Settings → Batch Scan → Finish

- Load slides to the cassette(s) and place the cassette(s) to the slide loader as described in Page 2.
- Scan mode: click “Expert batch” under brightfield category. Slides will be automatically delivered to the stage one by one.
- Slide properties: type in the slide info here. Click “option” at the bottom, click “overview scan” tab, under “overview scan parameter”, tick “scan label”, then click “next”.
- Scan settings:
Magnification and image document: choose an objective for final scanning and a Z-scan mode for final scanning, i.e. single layer.

Auto naming: click “...” to set up your naming rule i.e. “slide name_1...”, click the “save to disk” icon.

Auto saving: all data must be saved here: F:\Virtual Slide Images\“your folder” (kept for 7 days before auto deleting). Your data will be transferred to the server instantly: Scientific platforms (G:\Cell Imaging\VS120\“your folder”) (kept for 14 days before auto deleting).

Tick “substructure” and then click “next”.

Focus settings: no need to do much about focusing here but just apply the current “Scan Settings” to the slide(s) (one or All).

- Batch scan overview: click “overview” to process overviewing. This Batch Scan Overview may take a while depending on how many slides and brightness of the overview signal.
- Review batch scan settings.
Scan area: click “Edit Scan Area”. You will be directed to an Edit Scan Area window. “Clear All” first, then choose a “Scan and Focus logic” and a “Scan area type”. In the live window, draw a ROI to select your scanning area.

After selecting a ROI, down in the same tab, click “edit focus map”, you’ll be directed to an Edit Focus Map window (if you miss this step here, don’t worry, this can be done in the next step). You can either “Clear All” auto picked focus points first and add focus points as you like, or later edit the auto generated focus points (based on chosen “Grid Density”) in the selected ROI, i.e. moving or deleting these points. Choose a “Scan and focus logic” (middle option is recommended).

Finally click “Confirm input”.

- Click “scan now”.
- Finish.

Batch Fluorescence (Expert mode)

The wizard will take you through by following the steps below to set up scanning

Virtual Slide Acquisition Wizard - Fluorescence Expert Batch Mode

Scan Mode → Slide Properties → Scan Settings → Batch Scan Overview → Review Batch Scan Settings → Batch Scan → Finish

- Load slides to the cassette(s) and place the cassette(s) to the slide loader as described in Page 2.
- Scan mode: click “Expert batch” under Fluorescence category. Slides will be automatically delivered to the stage one by one.
- Slide properties: type in the slide info here. Click “option” at the bottom, click “overview scan” tab, under “overview scan parameter”, tick “scan label”; under “exposure settings”, tick “manual exposure” then click “next”.
- Scan settings:
Magnification and image document: choose an objective for final scanning and a Z-scan mode for final scanning, i.e. single layer.

Auto naming: click “...” to set up your naming rule i.e. “slide name_1...”, click the “save to disk” icon

Auto saving: **all data must be saved here: F:\Virtual Slide Images\“your folder” (kept for 7 days before auto deleting). Your data will be transferred to the server instantly: Scientific platforms G:\Cell Imaging\VS120\“your folder” (kept for 14 days before auto deleting)**

Tick “substructure” and then click “next”.

Overview settings: depending on your sample, can choose either BF (BF mono) or fluorescence imaging to overview the slide. Overview lens: 2X or 10X. **Note: the “start Live” here is not available here in Batch mode. This means you need to get the exposure time right beforehand**

Illumination settings:

Add/select channels: if you can, always use the filters from the same position i.e. “DAPI_Tri”+“FITC_Tri”+“TexRed_Tri”. A bad combination example: “DAPI_Tri”+“FITC”+“TRITC”. **Note: the 1st channel in the filter list will be the representative one used for focus purpose across the sample.**

Exposure time: type in pre-acquired exposure time here (one channel at one time).

Focus settings: no need to do much about focusing here but just apply the current “Scan Settings” to the slide(s) (one or All).

- Batch scan overview: click “overview” to process overviewing. This Batch Scan Overview may take a while depending on how many slides and brightness of the overview signal.
- Review batch scan settings
Scan area: click “Edit Scan Area”. You will be directed to an Edit Scan Area window... “Clear All” first, then choose a “Scan and Focus logic” and a “Scan area type”. In the live window, draw a ROI to select your scanning area.

After selecting a ROI, down in the same tab, click “edit focus map”, you’ll be directed to an Edit Focus Map window (if you miss this step here, don’t worry, this can be done in the next step). You can either “Clear All” auto picked focus points first and add focus points as you like or later edit the auto generated focus points (based on chosen “Grid Density”) in the selected ROI, i.e moving or deleting these points. Choose a “Scan and focus logic” (middle option is recommended).

Finally click “Confirm input”.

- Click “scan now”.
- Finish.

Shut down

- Remove your slide from the stage (single mode) or the loader (batch mode) and return racks to the overhead storage shelf.
- Exit from the software (the computer can be left on).
- Turn off the fluorescence lamp (holding on the button for 3''), and all other components (order is not important).
- Clean the bench and leave the room tidy.

DATA & RECORDS MANAGEMENT

All data must be saved here: F:\Virtual Slide Images\“your folder” (kept for 7 days before auto deleting).

Your data will be transferred to the server instantly in Scientific Platforms: G:\Cell Imaging\VS120\“your folder” (kept for 14 days before auto deleting).

REFERENCES

A hardcopy of brief step-by step operation instruction is provided in Lab J208. A full version e-manual is available on both the local acquisition computer and the Imaging Website:

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

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