MS57 Training guide: 2D+ Microscope slides Oasis glide + Surveyor (RBINS & RMCA)
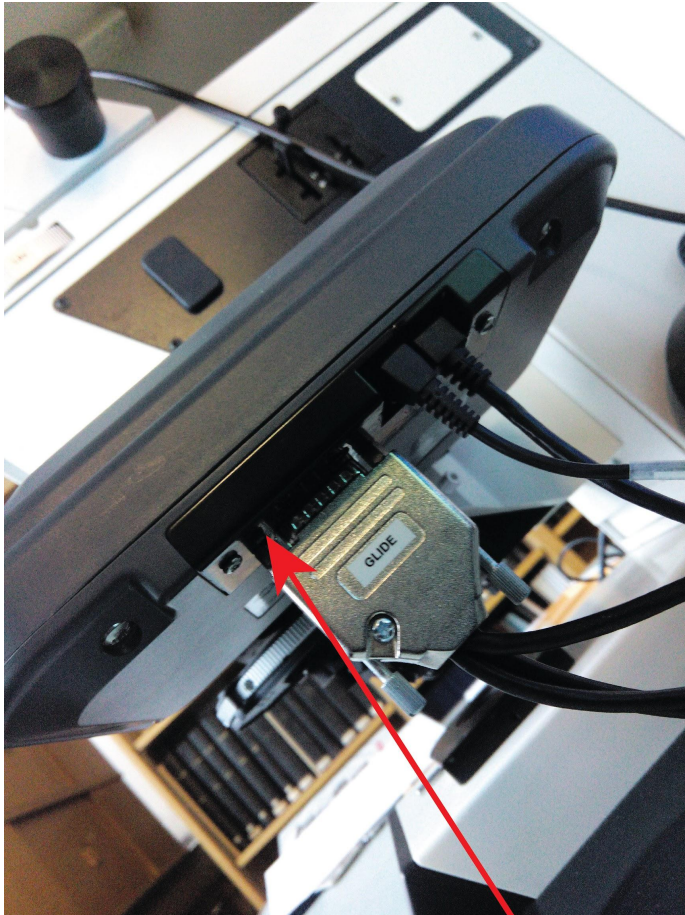
1. Starting the system and producing a gigapan image

Camera switch

LED light switch

Glide
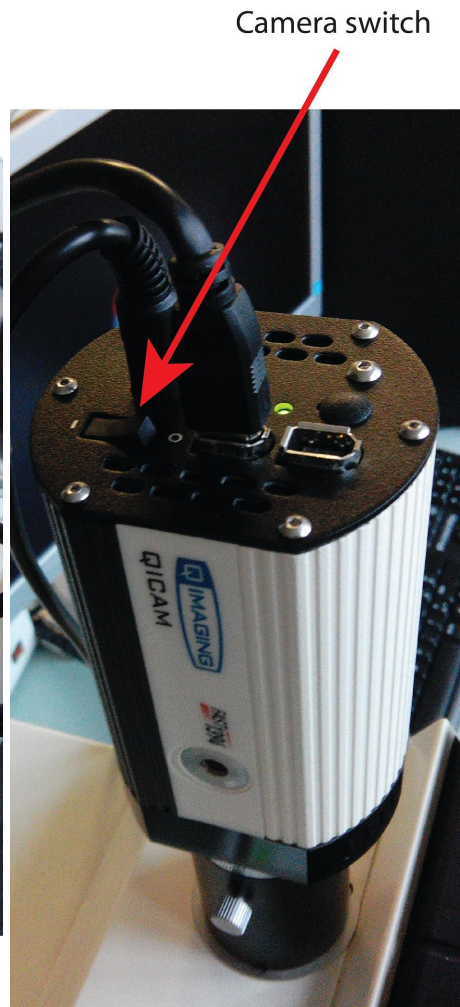
Camera switch

Glide switch

1) Turn on the computer and login.

2) Remove the protection of the microscope and:
- Switch on the camera (switch on top of the camera)
- Switch on the glide (switch under the glide)
- Switch on the light (LED module on the left of the microscope)

3) Start Surveyor (Surveyor will initialize the stage)

Figure labels: ZStack module | Image toolbar | Scan window toolbar | Image annotations tools | Live image | Scan window (Map view) | Joystick
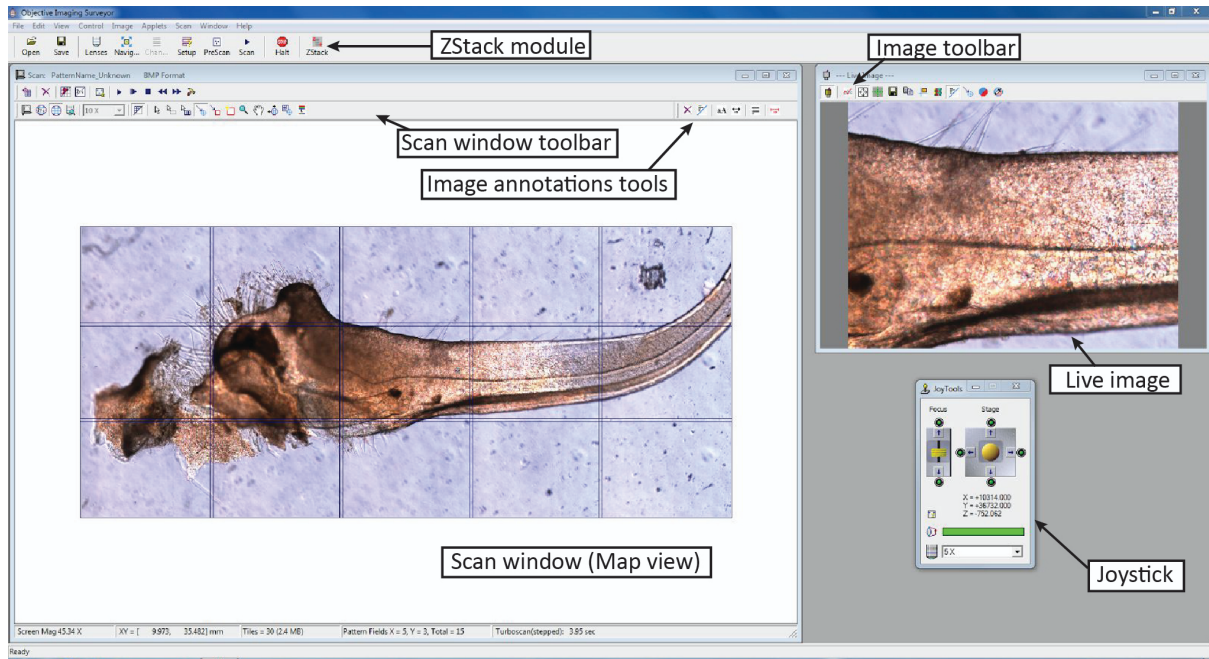
4) Place specimen slide into stage holder.

5) Select your LOWEST powered objective both on the microscope and by selecting magnification on joystick dialog box (x5).

6) By looking into the microscope, find a clear and clean area to adjust exposure and white balance. In the –Live Image- Dialogue Box select the button with the 3 colour courbes. On the **exposure** tab bring exposure to appropriate level using slide bar (or try the auto expose button).

7) Select the **color balance** tab and apply white balance.

8) Select **shading** tab. For shading correction choose a place over the hole in between of the slide and the slide holder. Select bright field shading, clear the previous shading correction and click on **setup shading correction**. And apply change and click on ok to leave the dialogue box. Tip: make sure the image is not over exposed! Eventually lower the exposition down and adjust it for the specimen after.

9) Using the soft joystick in Surveyor, position the XY stage to a position where the specimen is beneath the objective and thus visible in the live image window. Ensure it is reasonably in focus using either soft joystick or coarse focus on microscope. On the Stage View you will see that the small crosshair graphic moves as you move the stage in X and Y, always indicating the current position.

10) Select the scan define tool   and use it to draw a small area around the current position.

To position the scan area more accurately, you can use the Position Scan Tool   .

11) Now switch to the Map View by clicking on the   button.

12) Press the "Start Scan" button :

13) You will now see a dialog box asking whether you wish to use Turboscan Cruise, Turboscan Stepped, Zstack Scan, Standard Scan, or Track Focus Scan. It is best to select **Turboscan Stepped**. Let the scan complete as indicated by the progress bar. *Tip: Do a first turbo scan image for a large volume to locate the specimen then re-draw a new grid more precisely. Erase the mosaic image before capturing the new one.*

2.   Change magnification

1) Rotate your objective to a higher magnification and on the joystick window select the corresponding magnification.

2) Each time you change magnification adjust exposure, white balance, and shading as described above.

3) Focus the specimen using either a joystick or microscope. (Tip: on the joystick window as the image becomes more out of focus, the bar will turn blue. This is a helpful tool in determining the best focal plane for an image.)

4) Like before, draw the area you wished to scan with the tool.

5) To position the scan area more accurately, you can use the Position Scan Tool

6) Delete existing image with tool.

7) Press Scan button . Select Turboscan Stepped for flat specimens. Zstack can be selected for more 3 dimensional specimens (instruction below).

3. Zstack instructions

1) Open the ZStack Plus module

2) Set the value for top and bottom of your specimen.

3) Setup the step size

4) Press Scan button [Scan]. Select Zstack.

5) Check the Stack result to check you have all the information required

6) Export the planes (save mosaic image and check *Save all ZStack panes,* cf Saving)and stack them into Zerene stacker
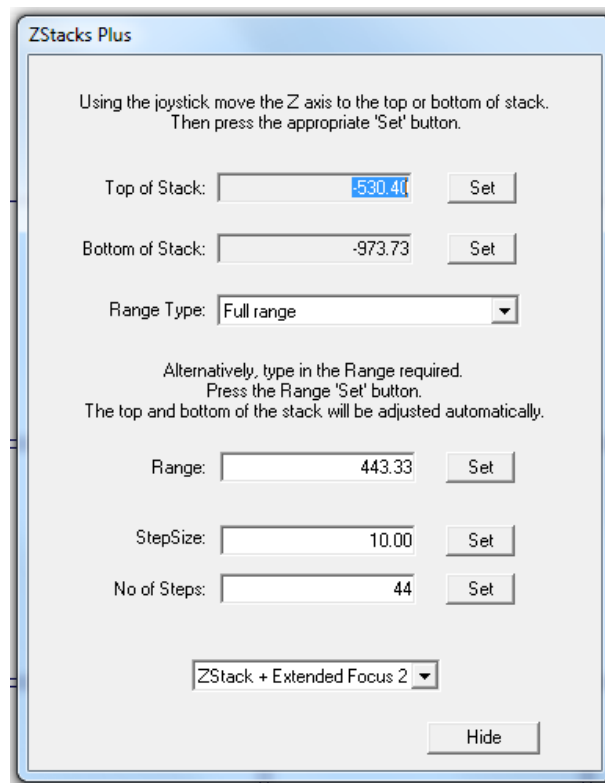
4. Saving

- "Save Workspace As" function and enables the user to save (and subsequently restore using the "Open Workspace" function) a complete mosaic. When this option is selected the scan pattern is saved together with every tile comprising the mosaic image and its spatial coordinates. This allows the entire mosaic to be subsequently reconstituted in its original form (using the "Open Workspace" function). Due to the amount of space that may be required to save a large mosaic in this way, the user has the option to apply JPEG compression when saving the workspace. There is a surveyor workspace viewer allowing to reopen your workspace on your computer: http://objectiveimaging.com/download/files/Setup_Viewer_V7.0.1.0_x86.exe
- The option "Save Mosaic Image As" enables the user to save the displayed mosaic image. This differs from "Workspace" as it only saves a pictorial version of the Microscope Slide Scanner Manual mosaic without the associated pattern or co-ordinate data. The resolution of the image saved can be selected by the user. As only the image is saved it has no functional attributes and cannot therefore be "opened" within the application. *Tip: Check the box Save all ZStack panes and stack them in Zerene or another stacking software for better results (cf. Zerene workflow in the focus stacking workflow).*
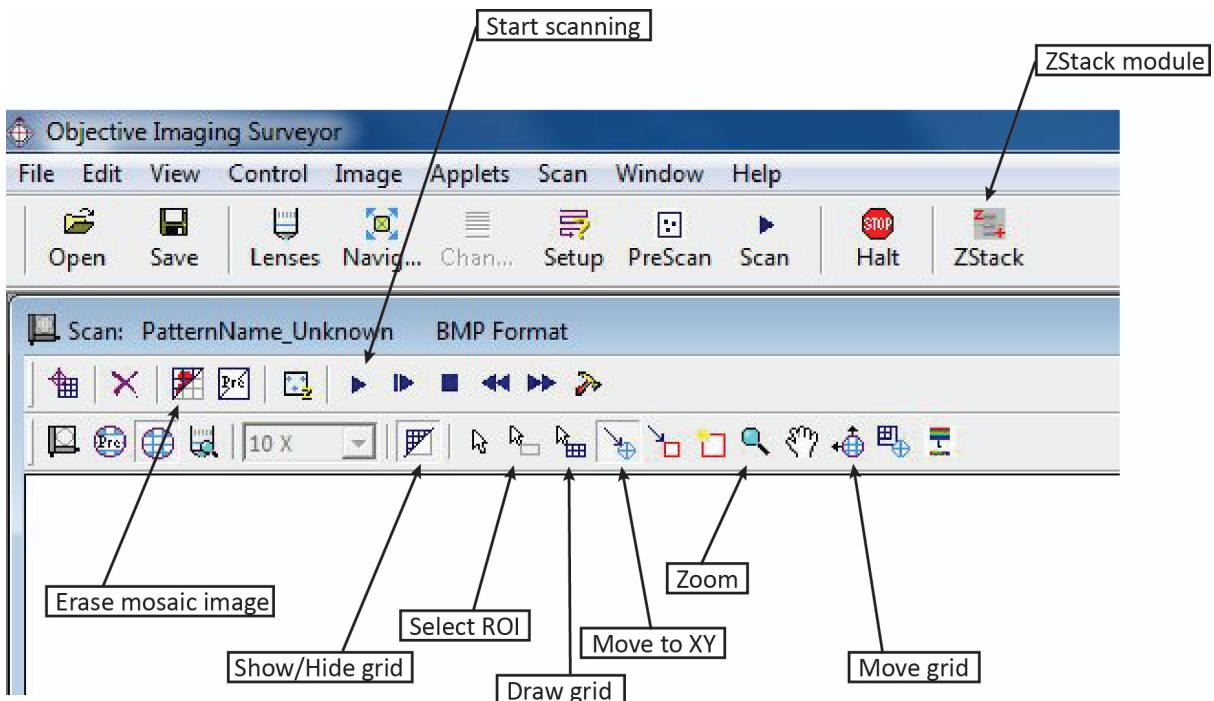
5. Other useful tools

Turn the Pattern Borders off and on (*Tip: that the button to use if you don't see your grid when you just draw it*)

Move to XY Position

Zoom (left click to zoom in, right click to zoom out)

6.   Free Surveyor workspace viewer
http://objectiveimaging.com/download/files/Setup_Viewer_V7.0.1.0_x86.exe

7.   References
- 2014. Microscope Slide Scanner Manual. Field Museum of Natural history.
- 2005. Surveyor Guide. Objective imaging.

8.   Credits
Author: Aurore Mathys
Date: December 2021
Version: 1.0
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