

PROTOCOL FOR CAPTURING IMAGES, THROUGH FOCUS VIDEO (TFV), AND MEASUREMENTS WITH VELOCITY SOFTWARE

Password for the computer: Nematode

Note: This computer is exclusively used for image capturing and **NOT** for personal use or other tasks. Also, the present protocol is designed to minimize the use of our microscope (Nikon-Eclipse E600) since most of the tasks can be directly performed on the computer using Volocity.

- 1- Turn on the microscope (orange switch on the right side of the microscope) as well as the automatic z-drive (green switch on the larger box/left side).
- 2- Place your slide on the microscope and identify the specimen of interest using lower magnification lens (*e.g.*, 4x, 10x or 20x).
- 3- Once the specimen is ready to be photographed, open Volocity (purple globe in the software shortcut bar). To visualize the nematode on the computer when Volocity is on, pull out the pin (Bino/Photo) on the right side of the ocular lens of the microscope.
- 4- When launched, Volocity will first ask if you would like to create a new library or open an existing library. Select a new library if that is the case and save it with a proper name (*e.g.*, sample ID/isolate/slide number/specimen number, etc.). Make sure you know where the file was saved (you can even create a new folder if that is helpful).
- 5- All the tasks (photos, videos, measurements, etc.) you perform on Volocity will be saved in this library. However, this library can only be opened using Volocity software. Therefore, you might have to export the files (photos, videos, tables) using a proper extension (*i.e.*, Tiff or Jpeg, Quick time, etc.). See the end of this protocol.
- 6- On Volocity you will find three main panels: 1) left panel: where all the images, videos and general tasks are displayed; 2) center panel: the microscope image focused on your specimen of interest; 3) Right panel: Volocity tools for capturing the images/videos as well as adjusting the image.
- 7- The Volocity software was already calibrated (lens/scale and exposure) when it was first installed. Therefore, the main features (tools) for photo and video capturing will be available on the right panel when the software is running. You might adjust the exposure/light intensity for 100x lens if needed (this is not recommended for the smaller lens).
- 8- On the left panel, click on Video Preview, the microscope image should appear in the main panel. If not, make sure the pin on the microscope head is on Photo. If an error occur (*i.e.*, image is dark) reinitiate Volocity.
- 9- The light intensity has also been calibrated (light switch on the left lower side of the microscope should be on the black arrow mark). However, you should readjust it using

the menu Video -> Auto White Balance. This must be done when you switch microscope objective lenses. For reference, you can also display the scale bar (also calibrated) using the menu Video -> Display -> Show scale.

- 10- Photos and TFF for each nematode specimen can be captured at different magnifications (10x to 100x) for use as a morphological voucher as well as for measurements. Therefore, make good use of the frame size, trying to place the whole specimen in the focal field (when possible).
- 11- Before capturing a picture at the preferable magnification, make sure you have the setting for the correct lens on Volocity, so the scales will make sense. On the upper part of the right panel, we have settings for all four lenses (10x, 20x, 40x, 100x, and 4x). For example, if you are using a 20x lens, then the setting should be on 20x.
- 12- To capture a picture of the image displayed in the center panel, simply click on the camera icon (capture single frame) on the right panel. You will immediately see the picture on the left panel as part of your library. You might rename this frame if you are planning to capture pictures from multiple specimens/slides and save them in the same library (you might want to create a different library for each isolate/sample though).
- 13- You might wish to capture pictures using higher magnifications. If that is the case, change the objective lens on the microscope (*e.g.*, from 10x to 20x), adjust the light intensity as previously explained (step 10), make sure the lens setting is correct (step 11), and center the nematode to make full use of the focal field.
- 14- You might need to focus on a particular feature (*e.g.*, tail tip) but also would like to have a reference point for another feature (*e.g.*, anus) in the same frame for future measurements. If that is the case, you can use the pointer (arrow) that is built into the microscope; this can be used as a reference for one of the two features. Make sure to turn off the pointer when not using it.
- 15- On higher magnification (*i.e.* 40x and 100x), you might also wish to readjust the position of the specimen (moving the x and y controls in the microscope) as well the rotation (moving the base of the microscope) before capturing a picture or a TFF. If you need to move the base of the microscope, do it so carefully. **NOTE: 40X and 100x lenses can ONLY be used with immersion oil.**
- 16- TFF are usually captured at high magnifications such as 40x or 100x. These objective lenses both require oil and should be carefully used. Make sure to reduce the amount of oil used on the slides with these lenses. Also, make sure to clean the lenses with the proper tissue and solution (always on side of the microscope). To capture a TFF make sure the settings are correct according to the lens in use. Also, readjust the light intensity as previously explained. On the lower part of the right panel (Ludl Controller 1) you will find the Z-axis control. Click in the arrow to set up the different levels (0, bottom and top). Instead of using the focus control on the microscope, you can use the bar on

Volocity software. After setting these three levels, double click on the icon with arrows up/down in the upper part of right panel (right side of the freeze icon).

- 17- An acquisition setup window will pop up. Based on the distance you recorded from bottom to top, a number of frames will be captured. The frames can be either capture by a z-spacing (μm distance) or based on total number of frames pre-determined by the user. For example, you might decide you wish to capture 40 frames. If so, then type this value on the -> Capture this many slides option. Then, click OK. **NOTE: Due to memory issues, Volocity might crash if the number of frames is higher than 40.**
- 18- After defining the acquisition setup, click on the red button to capture all the frames. TFV are very large files and therefore timing consuming. You might crop the area of interest (only the nematode specimen) reducing the size of the file. This should be done after setting the number of frames to be captured and before the real acquisition (*i.e.*, before clicking on the red button).
- 19- As previously mentioned all the files captured are saved on your library. To save a photo as tiff or jpeg format, simply select the file and click on the menu -> File -> Export. Another window will pop up with options for file format. You can either select Item as TIFF or Item as JPEG for photos. You can also rename the file if you wish. Make sure you save your files on the correct path (your own folder). **NOTE: only photos saved as TIFF will retain the scale bar.**
- 20- If you want to save a series of images (frames) as a TFV, then select the file of interest on your library and use the menu -> File -> Export format option set as Item as Quicktime. The options button in this window can also allow you to define the speed/length of the video (how many frames per second). You might wish to save as real time (very fast movie).
- 21- Measurements can be performed directly on images saved in the library. Simply select the line tool, click on the starting point (*e.g.*, head of the nematode) and with the shift key pressed click at the ending point (*e.g.*, tail tip). If the nematode is curved, you might want to include more points to accurately measure overall length. Different measurements (lines) will be saved with different colors/names. This can be seen when the tab **Measurements** of the central panel is highlighted.
- 22- To save these measurements on the current library, use the menu Measurements -> Make a measurement item. This data can also be exported as a Tab delimited text file for further analysis on Excel.
- 23- After finishing your image capturing and measurements, close/quit Volocity. Also, make sure that all the files (library, photos, videos, tables, etc) are saved into your own folder. Then, move this folder to your personal hard drive to avoid file storage in this computer.
- 24- Finally, make sure to properly turn OFF the microscope and z-drive control. Also check to be certain the arrow on the microscope is separately turned off. Clean the 40x and

100x lenses if you used them with the proper paper tissue/solution. Also, clean the area and cover the microscope before you leave!

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