



Z - Stack Acquisition Properties Window

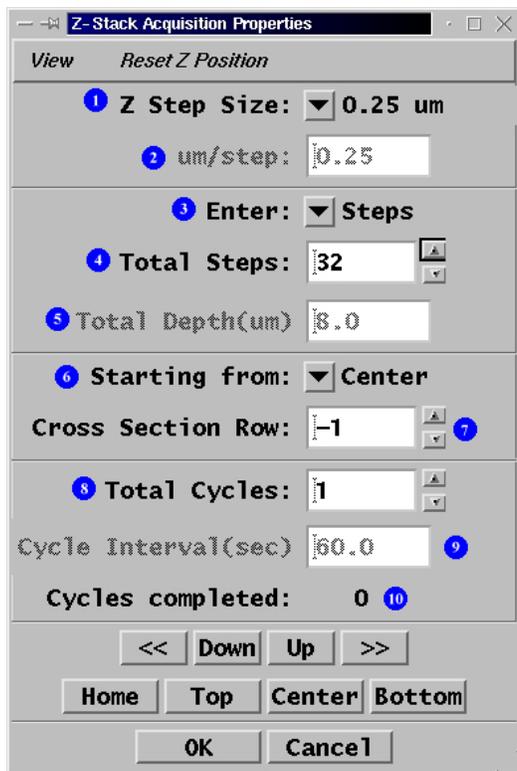
View Function

Z Stack Crosssection - allows the user to view a crosssection row of the Z stack as it is being acquired. The row is selected in 7

Reset Z Position Function

Current Position as 0 - sets the current z position to 0 or home
Starting Position - sets the start point of the z focus routine to the current z position

selected



is required.

1.

- 1 **Z Step Size** - allows the user to select the incremental Z setup.
- 2 **um/step** - highlighted when user defined step size is in 1
- 3 **Enter: Steps or Depth** - allows the user to select either the number of z steps or the total depth of the optical section.
- 4 **Total Steps** - highlighted when the user selects "Steps" in 3. The "Z Step Size" and the "Total Steps", will determine the total depth of the optical section.
- 5 **Total Depth(um)** - highlighted when the user selects "Depth" in 3. Allows the user to input the total distance of the optical section.
- 6 **Starting from: Top or Center or Bottom** - allows the user to determine where the optical sectioning will start.
- 7 **Cross Section Row** - allows the user to select the row the cross section to be displayed when View Cross Section is selected under the "View Function".
- 8 **Total Cycles** - allows the user to set the number of time-lapse cycles when 4D optical sectioning is required.
- 9 **Cycle Interval (sec)** - highlighted when the number of cycles in 8 is set to greater than 1. Allows the user to select the time interval between Z cycles.

⑩ **Cycles Completed** - displays the number of time-lapse cycles that have been completed.

Z - Stack Acquisition Properties Window



<< - move the Z focus motor down by the increment specified in either ① or ②

DOWN - move the Z focus motor down by the increment specified in either ① or ②

UP - move the Z focus motor up by the increment specified in either ① or ②

>> - move the Z focus motor up by the increment specified in either ① or ②

HOME - moves the Z focus motor to the position specified as 0 or home using the "Reset Z Position function"

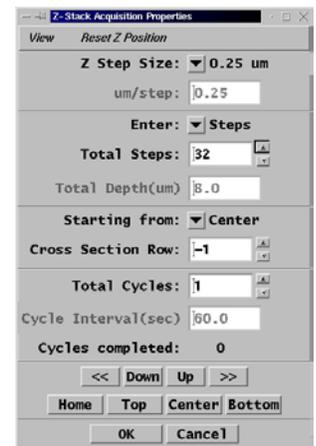
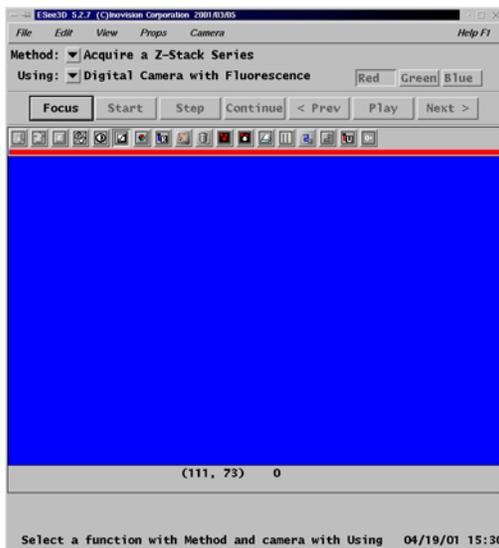
TOP - moves the Z focus motor to the top of the optical section

CENTER - moves the Z focus motor to the center of the optical section

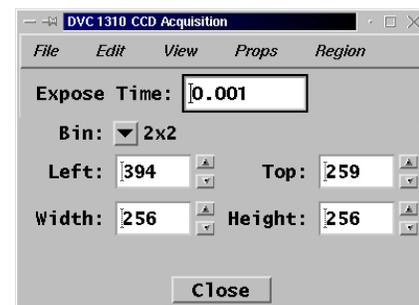
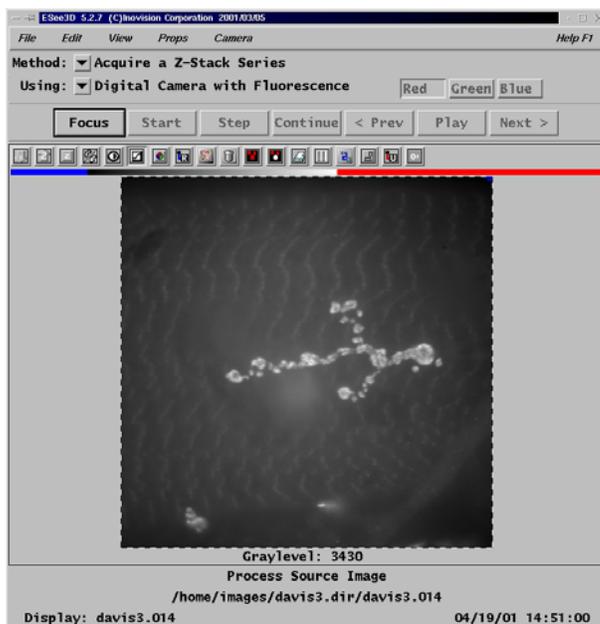
BOTTOM - moves the Z focus motor to the bottom of the optical section

Acquiring a Z Stack of Images

1. Place the specimen on the stage and visually focus on the specimen.
2. Adjust the microscope so that the image is now being sent to the camera port.
3. From the ESee Graphical Interface Screen,
 - a. Set **Methods** to *Acquire a Z Stack Series*
 - b. Set **Using** to *Digital Camera with Fluorescence* if a excitation filter wheel is being used
or
Set **Using** to *Digital Camera Only* if a filter wheel is not being used
4. The Camera Setup Screen and the Z Focus Setup Screen will appear.

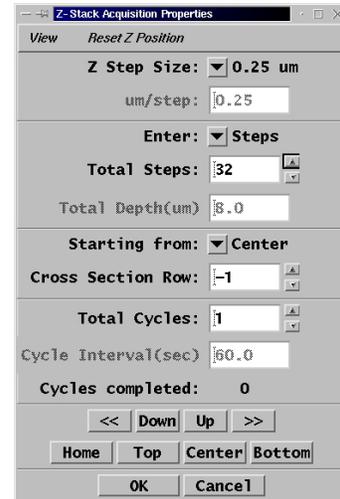


5. From the ESee Main Screen, click on the Focus button, Using the Z Focus's Digipot, bring the image into focus on the screen. At the same time, set the camera for the best exposure time. Then click the Close button.

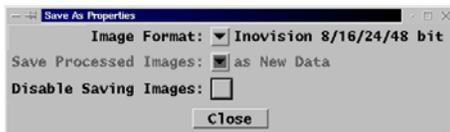


Acquiring a Z Stack of Images

6. Setup the Z Stack Acquisition Screen
 - a. Select the **Step Size**
 - b. Select the **Total Steps**
 - c. Set **Starting from** to *Center*
 1. If the image is in focus, then you are at the center of the image
 - d. Under the **Reset Z Position** function at the top of screen,
 1. Click on the *Current Position as 0*
 2. Click on *Starting Position*
 - e. Go to the bottom of the screen and click on **OK** .



7. On the ESee Graphical Interface Screen, click on **Start** .
8. The following two screens will appear. They will ask you to:
 - a. Select the image format. Leave as *Inovision 8/16/24/48* .
 - b. Input a *List Name* that the image will be stored under. The click on *Create Folder* .



9. Click on the **Start** button on more time and the system will start acquiring a Z Stack.