## Topic: Using the Olympus IX81 ZDC with Volocity Acquisition

Discussion

Ensure that the IX81 ZDC has been installed according to the instructions in the manufacturer's manual, and that the objective lenses that you intend to use with the ZDC are approved by Olympus. Please see the ZDC manual or contact your local Olympus representative for further information. Ensure that the IX81 ZDC has been connected to Volocity and configured according to the instructions in the Volocity manual.

Volocity Preferences

If you are using the IX81 with an automated stage and a second piezo focus drive, ensure that the microscope's focus drive is selected in the XY Stage preferences, not the piezo focus drive. You can access the XY Stage preferences by selecting **Preferences...** from the **Volocity** menu (Apple Macintosh) or the **Edit** menu (Windows) and selecting the X-Y stage icon.
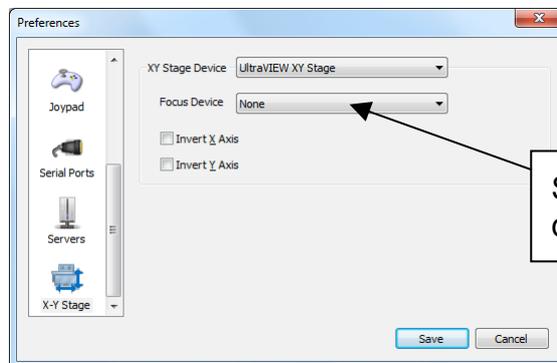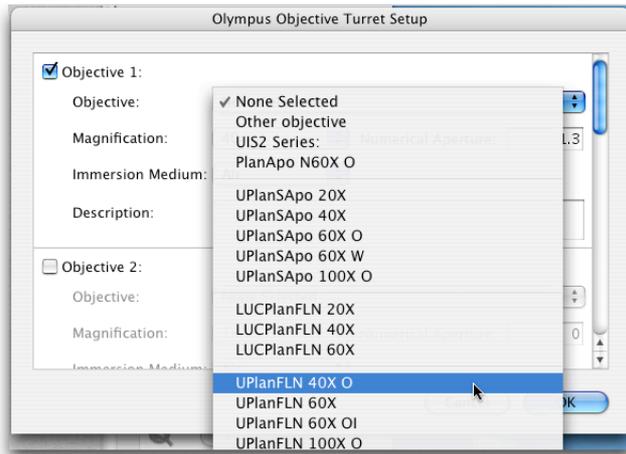

Select the Microscope's focus drive in this drop down menu.

Configuring the objective turret

Select **Show Video Preview** from the **Window** menu.

Double click on the Olympus objective turret device icon, the **Olympus Objective Turret Setup** dialog opens.

For each objective position, select your objective lens from the drop down menu. If any of your lenses is not present in the list it is not recommend for use with the ZDC. For lenses not in the list you may select the **Other Objective** option. **N.B.** If this option is selected the ZDC may not function correctly or may function in an unpredictable manner. Improvision recommends that you avoid attempting to use lenses not listed in the drop down list with the ZDC.

Setting ZDC limits

Select **Set Autofocus zone limits…** from the **Video** menu.

Follow the Wizard instructions.
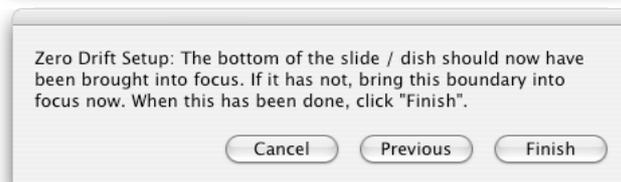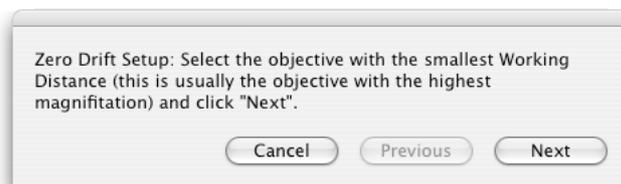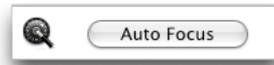

The ZDC is now ready for use.

Using the ZDC from the video preview

1. Focus on your plane of interest using the IX81 focus control.

2. Click on the "Set Offset" button on the Video Preview Window.

3. Click on the "Auto Focus" button whenever you wish Volocity to automatically reset the focus to your plane of interest.

Acquiring a single plane, single point over time

1. Focus on your plane of interest using the IX81 focus control.

2. Click on the "Set Offset" button on the Video Preview Window.

3. When setting your acquisition protocol in the acquisition setup dialog set "Autofocus using" to "Olympus Zero Drift".
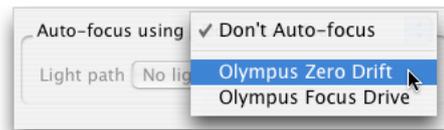
   N.B. The "Using light path" field may be set to "None", however, if you want to ensure that the specimen is not illuminated during auto-focussing you should select a light path that closes the appropriate shutter.

4. Select "Capture".

5. Volocity will activate the ZDC briefly prior to acquiring each time point to restore your focal plane.

Multiple planes, single point over time

6. Set the top and bottom limits of your focus drive. If you are using your Olympus focus drive for both focussing and acquisition you must set the top and bottom either side of zero. If you are using a second Z axis for the acquisition this is not necessary.

7. Focus on the mid-point of your volume using the IX81 focus control.

8. Click on the "Set Offset" button on the Video Preview Window.

9. When setting your acquisition protocol in the acquisition setup dialog set "Autofocus using" to "Olympus Zero Drift".

N.B. The "Using light path" field may be set to "None", however, if you want to ensure that the specimen is not illuminated during auto-focussing you should select a light path that closes the appropriate shutter.

10. Select "Capture".

11. Volocity will activate the ZDC briefly prior to acquiring each time point to restore your focal plane. Each time the focal plane is found Volocity will treat the plane as zero, regardless of its actual Z position. If top is set to 10 and bottom to -10, the top and bottom will always be 10 above and below the focal plane that Volocity finds.

Single plane, multipoint, over time

1. Add your acquisition points, ensure that you set the focal plane correctly for each point.

2. Go to any acquisition point.

3. Click on the "Set Offset" button on the Video Preview Window.

4. When setting your acquisition protocol in the acquisition setup dialog set "Autofocus using" to "Olympus Zero Drift".

N.B. The "Using light path" field may be set to "None", however, if you want to ensure that the specimen is not illuminated during auto-focussing you should select a light path that closes the appropriate shutter.
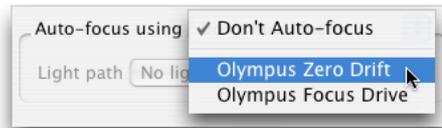
5. Select "Capture".

6. Volocity will activate the ZDC briefly prior to acquiring each acquisition point to restore the correct focal plane.

Multiple planes, multipoint, over time

1. Add your acquisition points, ensure that you set the focal plane correctly for each point.

2. Set the top and bottom limits of your focus drive. If you are using your Olympus focus drive for both focussing and acquisition you must set the top and bottom either side of zero. If you are using a second Z axis for the acquisition this is not necessary.

3. Go to any acquisition point.

4. Focus on the mid-point of your volume using the IX81 focus control.

5. Click on the "Set Offset" button on the Video Preview Window.

6. When setting your acquisition protocol in the acquisition setup dialog set "Autofocus using" to "Olympus Zero Drift".

N.B. The "Using light path" field may be set to "None", however, if you want to ensure that the specimen is not illuminated during auto-focussing you should select a light path that closes the appropriate shutter.

8. Select "Capture".

9. Volocity will activate the ZDC briefly prior to acquiring each acquisition point to restore the correct focal plane. Each time the focal plane is found Volocity will treat the plane as zero, regardless of its actual Z position. If top is set to 10 and bottom to -10, the top and bottom will always be 10 above and below the focal plane that Volocity finds.