

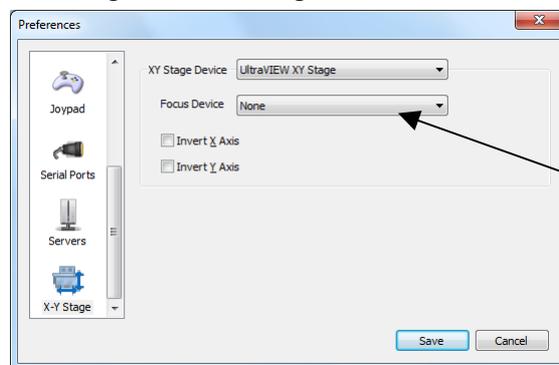
**Topic: Using the Zeiss Axio Observer Definite Focus with Volocity Acquisition**

**Discussion**

Ensure that the Zeiss Axio Observer.Z1 has been installed according to the instructions in the manufacturer’s manual, that you have fully defined your objective lenses (using the TFT key pad) and installed the beam combiner module as described in the Definite Focus users manual. Ensure that the Axio Observer.Z1 has been connected to Volocity and configured according to the instructions in the Volocity manual.

**Volocity Preferences**

If you are using the Axio Observer 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.

**A. Continuous Auto-Focus**

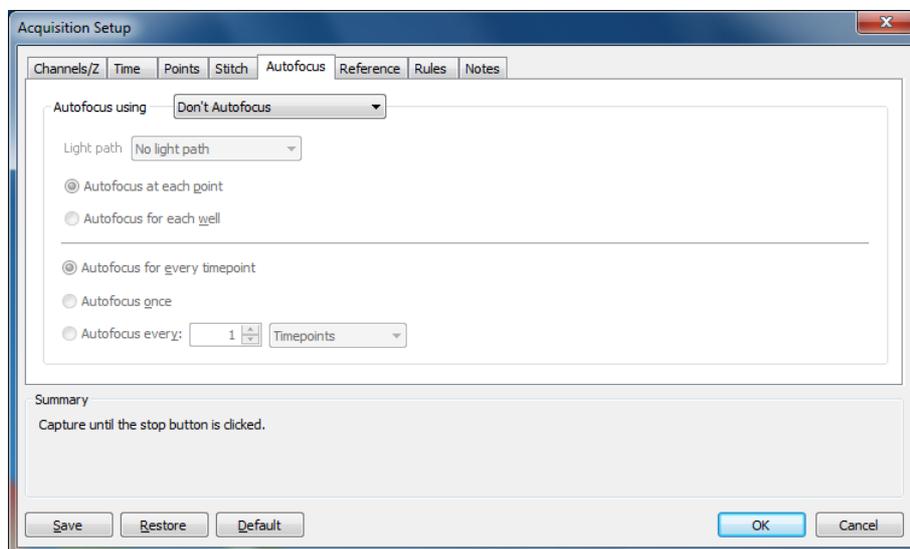
**Activating the Definite focus from the TFT touch screen**

1. Focus on your plane of interest using the Axio Observer focus control.
2. Press **Home/Microscope** on the TFT touch screen
3. Select the **Definite Focus** Tab
4. Press **Stabilization On** to activate the Definite focus



Alternatively, select the Stabilization check box in the video preview.

Acquisition protocols can be executed with the Definite Focus active. Ensure that the **Autofocus using** in the **Autofocus** tab of the acquisition setup dialog is set to **Don't AutoFocus**.



The Definite Focus will be deactivated whilst the protocol makes changes to the X, Y or Z axes, but will be re-activated in the interval between time points.

## **B. Auto-Focus as part of an acquisition protocol**

The ZDF can set to auto-focus prior to the acquisition of time points by adding an auto-focus action to the acquisition protocol.

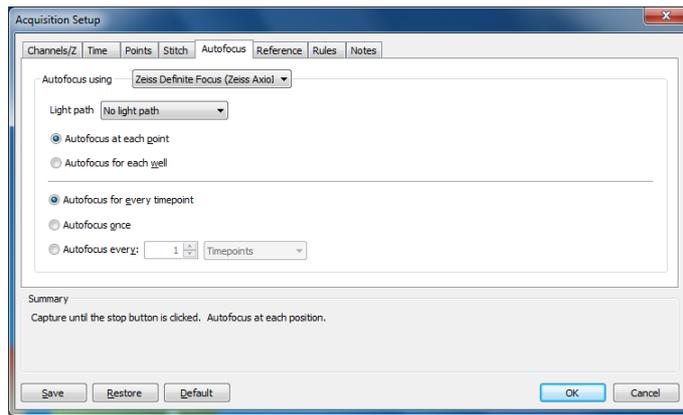
### **Single Acquisition point**

Ensure that the Definite Focus is deactivated from the TFT touch screen or video preview.

1. Focus on your plane of interest using the Axio Observer focus control.
2. Click on the "Set Offset" button on the Video Preview Window.



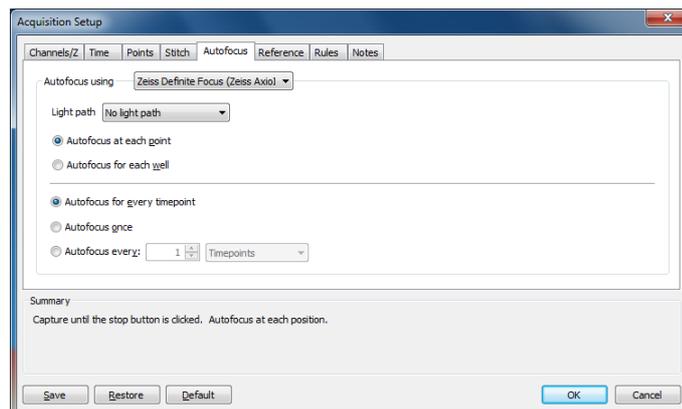
3. Set your acquisition protocol. Select the **Autofocus** tab, set **Autofocus using** to **Zeiss Definite Focus**.



4. N.B. The “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 Definite Focus briefly prior to acquiring each time point to restore your focal plane. If your protocol has been set to acquire multiple Z planes, the plane restored by the ZDF will be treated as zero by Volocity, 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 the ZDF restores.

### Multiple Acquisition points

1. Add your acquisition points. If you wish to set a different Z plane for each point you must ensure that you set the focal plane correctly before adding each point.
2. Set your acquisition protocol. Select the **Autofocus** tab, set **Autofocus using** to **Zeiss Definite Focus**.



3. Go to any acquisition point.
4. Ensure that the specimen is correctly focussed.
5. Click on the “Set Offset” button on the Video Preview Window.



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.

6. Select “Capture”.
7. Volocity will activate the Definite Focus briefly prior to acquiring each acquisition point to restore the correct focal plane. If your protocol has been set to acquire multiple Z planes, the plane restored by the ZDF will be treated as zero by Volocity, 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 the ZDF restores.