

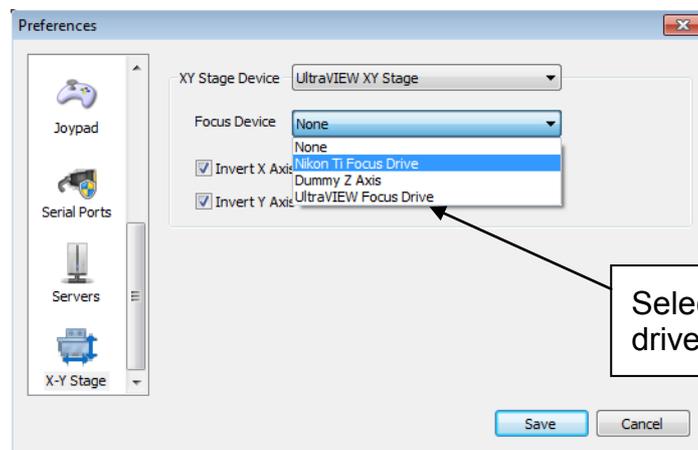
Topic: Using the Nikon PFS with Volocity Acquisition

Discussion

Ensure that the Perfect Focus System (PFS) has been installed according to the owners' manual, the TiE drivers are installed correctly, and that the TiE has been connected to Volocity and configured according to the instructions in the Volocity manual.

Volocity Preferences

If you are using the Nikon TiE with an automated stage and a secondary piezo focus drive, ensure that the microscope focus drive is selected in the XY Stage preferences as shown below. 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.



How to use the PFS in the video preview

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

Microscope focus control



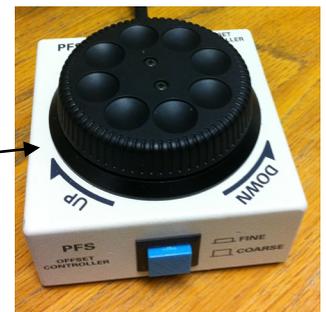
2. To enable the PFS either:

- a. Select "ON" from the PFS button array (found on the front of the microscope stand the "ON" button should be illuminated).
- b. Select "Continuous AF" in the video preview controls.



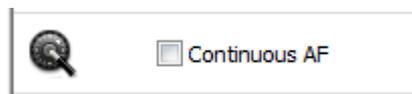
3. Adjust the focus as required by using the PFS Offset controller.
4. With the PFS activated the current focal plane will be maintained.

PFS Offset controller

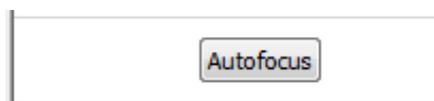


5. To disable the PFS either:

- a. Select "ON" from the PFS button array (the "ON" button will be unilluminated).
- b. Deselect "Continuous AF" in the acquisition controls.



6. To perform a 'one-shot' method of auto focusing i.e. turning the PFS on allowing it to auto focus then turning it off automatically, click the 'Auto focus' button in the video preview controls.



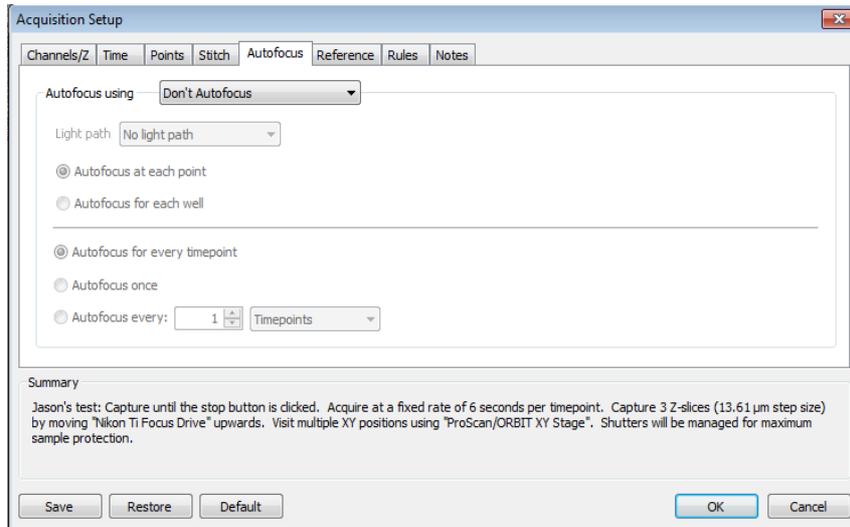
Going forward in this document we will refer to the above as enabling and disabling the PFS.

Single plane over time – Continuous Auto Focus

Use this method if you would like the PFS to continually maintain focus throughout the course of the acquisition.

1. Enable the PFS
2. Adjust the focus as required by using the PFS Offset controller.

- When setting your acquisition protocol in the acquisition setup dialog set “Auto-focus using” to “Don’t auto-focus”.

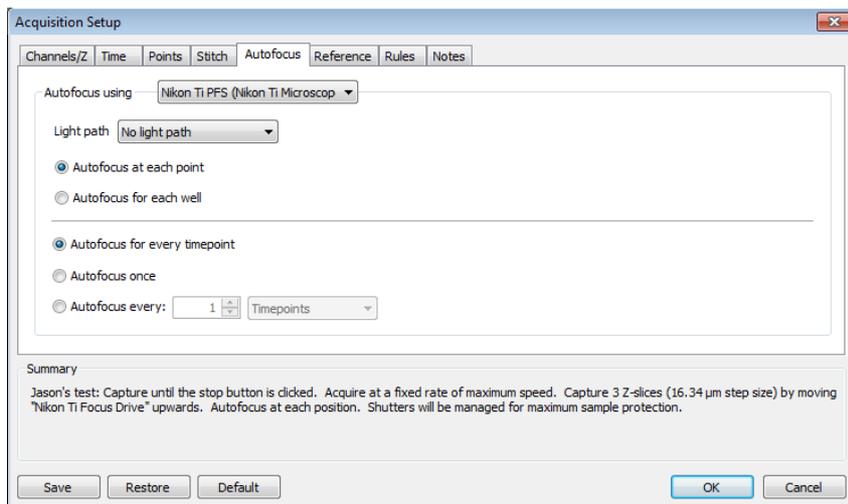


- The PFS will continually maintain your focal plane throughout your acquisition.

Single plane over time – Auto Focus in Acquisition setup

Use this method if you would like to only enable the PFS prior to acquiring each timepoint.

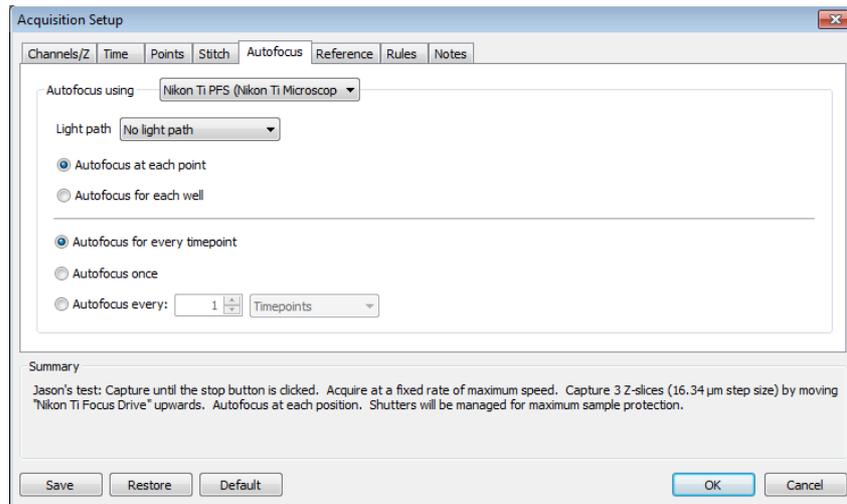
- Enable the PFS.
- Adjust the focus as required by using the PFS Offset controller.
- Disable the PFS.
- When configuring your acquisition protocol in the acquisition setup dialog set “Auto-focus using” to “Nikon Ti PFS”.



- Start the experiment.
- Volocity will activate the PFS briefly prior to acquiring each time point to restore your focal plane.

For acquisition setups that involve multiple planes or multiple points we do not recommend the use of Continuous Auto Focus. Please ensure this is disabled prior to starting an acquisition that involves multiple planes or multiple points. For the

further workflows in this technical note please ensure that the acquisition setup dialog is set “Auto-focus using” to “Nikon Ti PFS”.

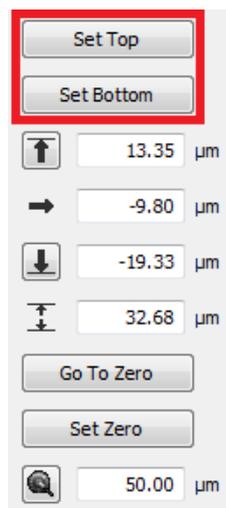


Multiple planes over time

1. Enable the PFS
2. Find the focus point of interest in your sample (typically the middle of the sample) by adjusting the PFS Offset controller.
3. Set the focus drive position that you are using to acquire the Z stack to zero.



4. Disable the PFS
5. Set your sample top and sample bottom positions either side of the zero



position.

6. When configuring your acquisition protocol in the acquisition setup dialog select “Auto-focus using” to “Nikon Ti PFS”.
7. Start the experiment.
8. Volocity will activate the PFS 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 (the position of the correct focus plane can change if drift in Z occurs).

Single plane, multipoint, over time

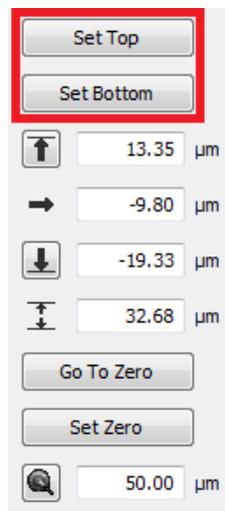
1. Disable the PFS
2. Add your XY points, adjusting the microscope focus control as required to maintain focus point of interest.
3. Return to any XY point you have previously made.
4. Enable the PFS.
5. Set your focal plane by adjusting the PFS Offset controller.
6. Disable the PFS
7. When configuring your acquisition protocol in the acquisition setup dialog select “Auto-focus using” to “Nikon Ti PFS”.
8. Start the experiment
9. Volocity will activate the PFS briefly prior to acquiring each acquisition point to restore the correct focal plane.

Multiple planes with microscope focus control, multipoint, over time

1. Disable the PFS.
2. Move to the position where you plan to add your XY first point
3. Set the microscope focus control to zero.



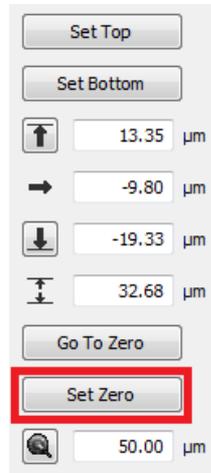
4. Add your first XY point
5. Using the microscope focus control set your sample top and bottom positions either side of the zero position.



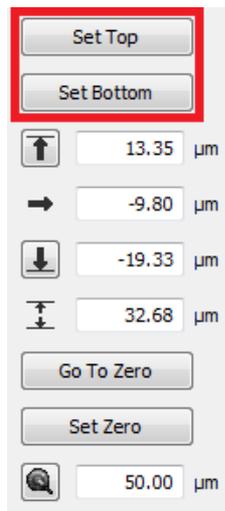
6. Continue to add further XY points as required adjusting the microscope focus control to maintain focus position of interest.
7. Go to any XY point you have previously made.
8. Enable the PFS
9. Set your focal plane by adjusting the PFS Offset controller.
10. Disable the PFS
11. When configuring your acquisition protocol in the acquisition setup dialog select "Auto-focus using" to "Nikon Ti PFS".
12. Start the experiment
13. Volocity will activate the PFS 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.

Multiple planes with secondary drive (piezo), multipoint, over time

1. Disable the PFS.
2. Set the secondary drive's (piezo) focus position to zero.



3. Add XY points as required adjusting the Nikon TiE focus to maintain focus position of interest.
4. Using the secondary drive (piezo) set your sample top and bottom positions either side of the zero position.



5. Go to any XY point you have previously made.
6. Move the piezo to zero.



7. Enable the PFS
8. Set your focal plane by adjusting the PFS Offset controller.
9. Disable the PFS
10. When configuring your acquisition protocol in the acquisition setup dialog select "Auto-focus using" to "Nikon Ti PFS".
11. Start the experiment
12. Volocity will activate the PFS briefly prior to acquiring each acquisition point (the piezo will be move to zero to insure the PFS is within range) 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.