

PerkinElmer UltraVIEW VoX Spinning Disc Confocal Microscope

Standard Operating Procedure

A. Basic Operation

1. Turning ON the system

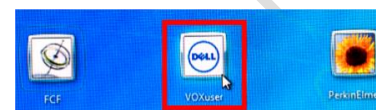
- 1.1. Switch on the power supply ① and ② mounted on the wall.
- 1.2. Switch ON the modular laser system 2.0 ③.
- 1.3. Switch ON Mercury lamp ④.
- 1.4. Turn ON computer system ⑤.
- 1.5. Log onto "VOXuser" account.
Please use password 'Voxuser[year]'



...A.1.1



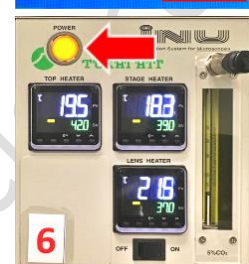
...A.1.2



...A.1.5

2. ** (Optional) When live cell imaging apply

- 2.1. Turn on temperature and CO₂ control module ⑥.
- 2.2. Turn on CO₂ tank (Anti-clockwise).
- 2.3. Turn the valve of regulator (clockwise), check if the pressure reach indicated level (blue line) on gauge.
DO NOT change regulator setting if gauge is showing 17psi
- 2.4. Install Objective heater on the objective lens.
- 2.5. Mount your sample onto sample frame.
- 2.6. Place sample frame (with sample mounted) on the stage (label towards the top right corner).
- 2.7. Add Milli-Q water to water bath (follow instructions during introductory training seek assistance if you are not sure).
- 2.8. Replace incubator cover.
- 2.9. Bring back condenser column.



...A.2.1



...A.2.2



...A.2.3

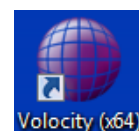
**** Very important step. Watch to check the condenser is not touching top plate of the incubator. Otherwise push back condenser and seek technical assistance. DO NOT PROCEED. ****



...A.2.4

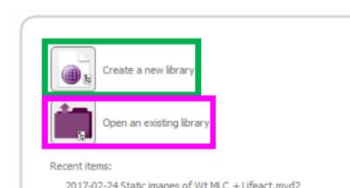
3. Volocity Software start up

- 3.1. Launch the Volocity (x64) software on desktop.



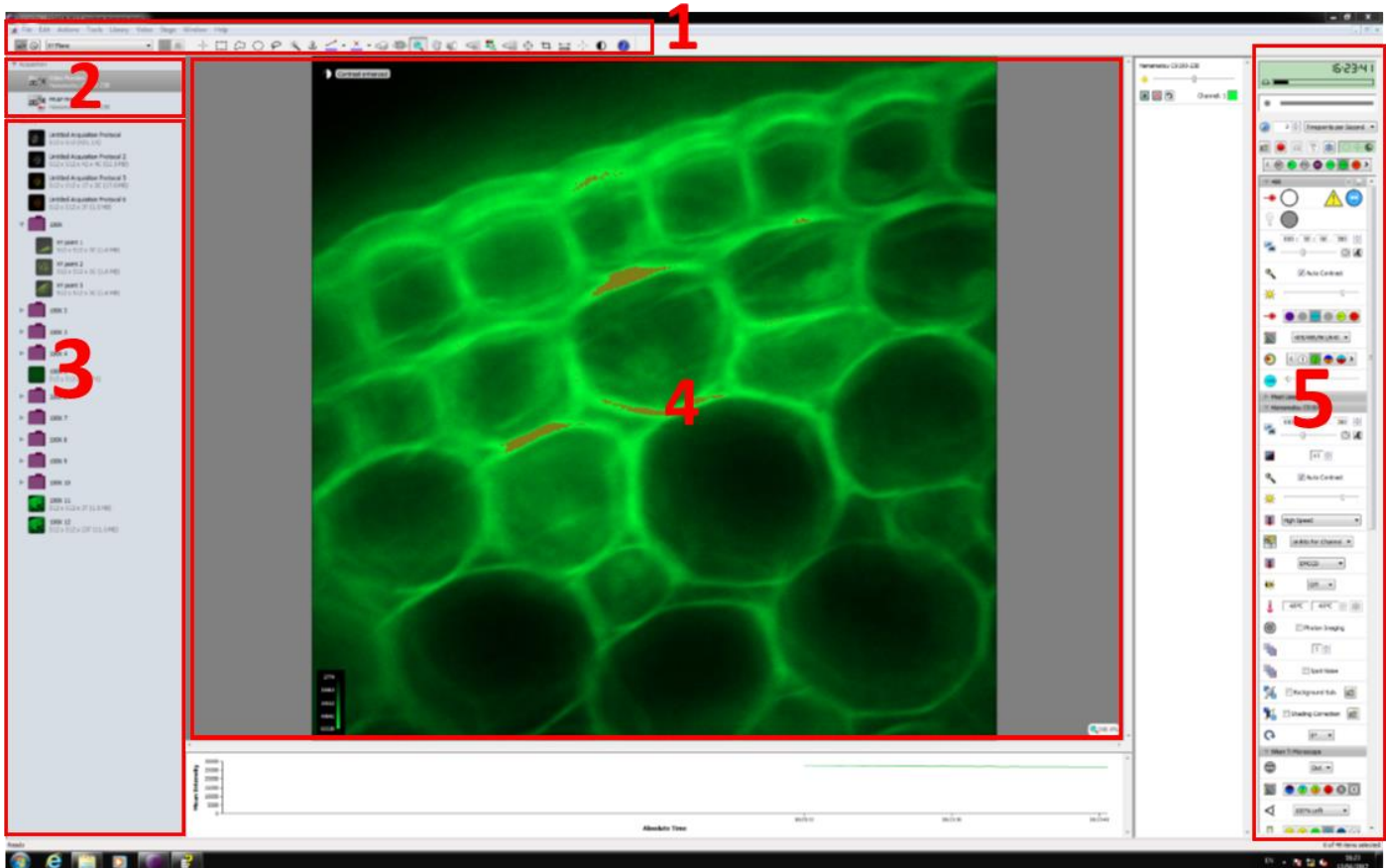
...A.3.1

- 3.2. Create a new library or choose Open an existing library if you want to append to existing image set.
- 3.3. Create your folder under:
C:\Data\PI\your name




...A.3.2

B. Overview of the Volocity User Interface



- 1 Menu bar
- 2 Acquisition
- 3 Library
- 4 Video Preview window
- 5 Device Controls

C. Microscope setup and locating your specimen

1. Select suitable lens for your sample.
2. (Optional) apply immersion oil if 60X or 100X lens is selected.
3. Mount your sample onto the stage with suitable sample holder (select between slide or Petri dish holder)
4. Click on 'Video Preview'  button. And select 'BF' light path on the 'Device Control' to view your specimen under bright field via eyepiece.
5. And select 'FL' light path to view your specimen under fluorescence.
- 5.1. You can select suitable filter set on the TI-RCP controller.



...C.4



...C.5



...C.5.

1

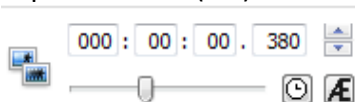


...C.6

6. Select 'SD' on light path selector to prepare for image acquisition.

7. Change your light path to suitable wavelength then click 'unfreeze' and ensure the laser emission is 'ON'.
8. You should now be able to view fluorescent image in the video preview window.
9. Toggle between different light paths according to the excitation wavelengths of labelling dye used on your specimen. Adjust the following parameters for each channel:

9.1. Exposure time (ms):



9.2. Sensor sensitivity: (maximum value = 255)



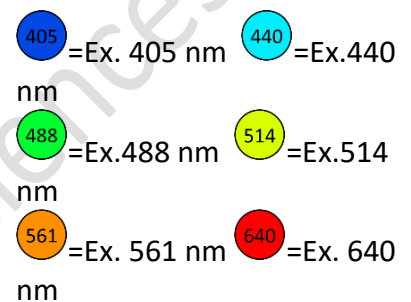
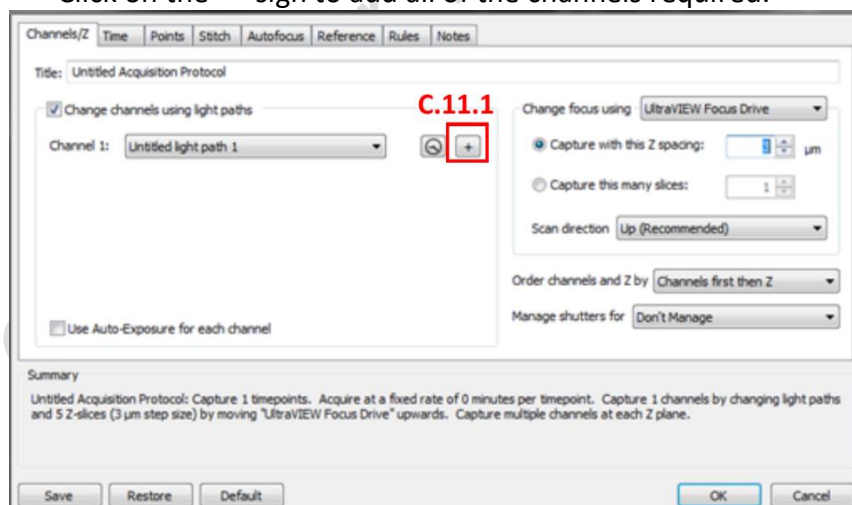
9.3. Laser power: (typical range 3-10%)



10. Click 'Save' after you have adjusted your imaging parameters for each channel.

11. Right click on the acquisition control panel to view the acquisition setup window.

Click on the '+' sign to add all of the channels required.




12. Click on 'Record button'





D. Setting up z-stack confocal scan

Prerequisite:

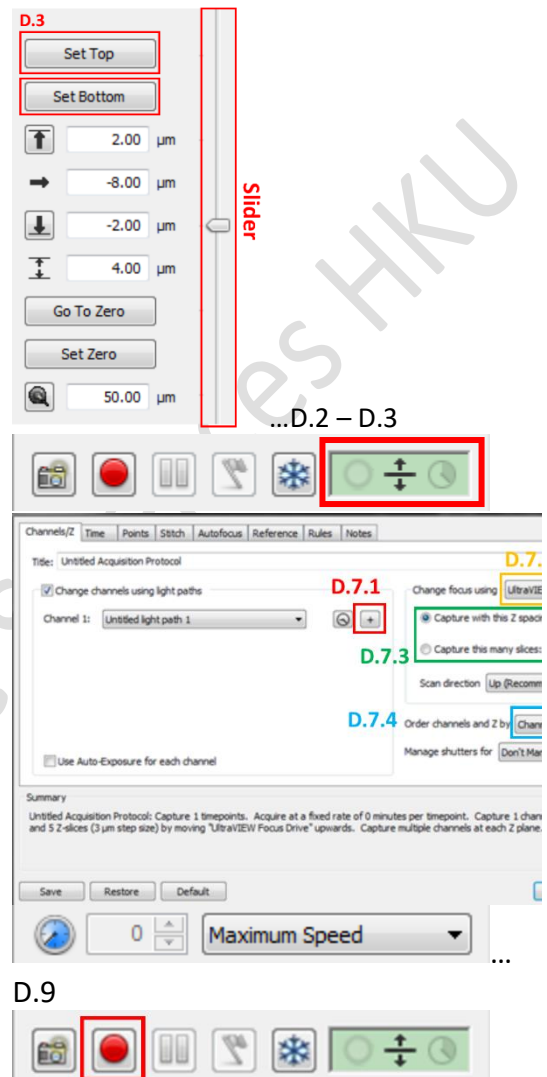
Follow Part C (p.2) to have the specimen in focus and visible in the video preview window.

1. Click on the z adjust button  under 'UltraVIEW' to view z control panel
2. Use the slider on right side of the panel to adjust focal plane position (z)
3. 'Set Top' and 'Set Bottom' z-positions as desired.
 - 3.1. You can preview the stack by clicking:

Go to top: 

Go to bottom: 
4. Right click on the acquisition control panel to view the acquisition setup window.
5. Adjust the following parameters in the 'acquisition setup' menu:
 - 5.1. Click on the '+' sign to add all of the channels required.
 - 5.2. Select 'UltraVIEW focus Drive' for changing focus
 - 5.3. Adjust axial increment per optical slice
 - 5.4. Choose z-scan priority:

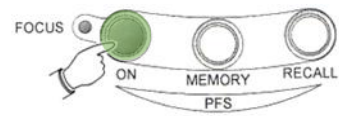
Channels / z-direction
 - 5.5. Click 'OK'
6. Set the time-point option to 'Maximum speed'
7. Click on 'Record button'



E. Setting up multi-timepoint experiments

Prerequisite:

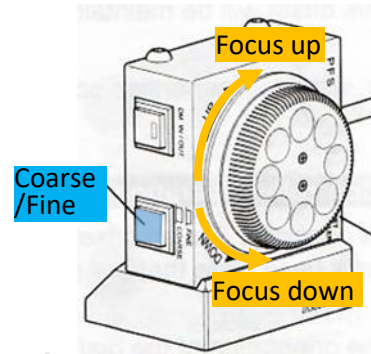
- Follow Part C (p.2) to have the specimen in focus and visible in the video preview window.
 - Follow Part D step 5-7 to view 'acquisition setup' window.
1. After you have your specimen in focus press 'ON' on the microscope to activate the Perfect Focusing System (PFS). This option will enable the microscope to lock onto your sample over long imaging sessions.



...E.1

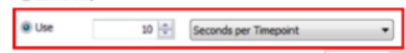
2. **Fine-tune the focus with PFS Offset controller**

Note: This will work best for medium filled coverslip-bottom Petri dishes or chamber slides.

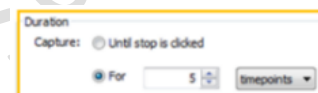


...E.2

3. In the Acquisition setup dialog, go to 'Time' tab
 - 3.1. Adjust duration for each timepoint
 - 3.2. Adjust how many timepoints to be imaged.
4. Click 'OK'

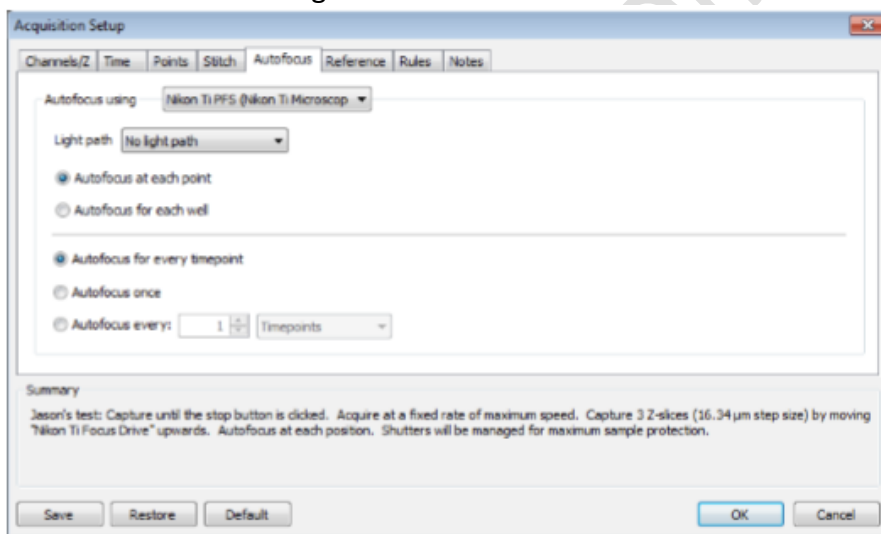


E.3.1

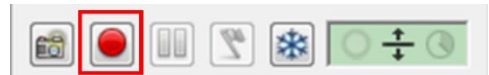


..E.3.2

5. In the Acquisition setup dialog, go to 'Autofocus' tab
6. set "Auto-focus using" to "Nikon Ti PFS".



7. Turn off PFS function then click on 'Record button'



F. Setting up multi-point experiments

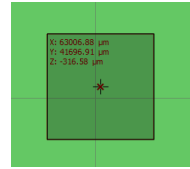
To setup multipoint scans position have your specimen in focus.

1. At your desired location, add point by:
2. Stage\Add point (or press Ctrl+Shift+A)

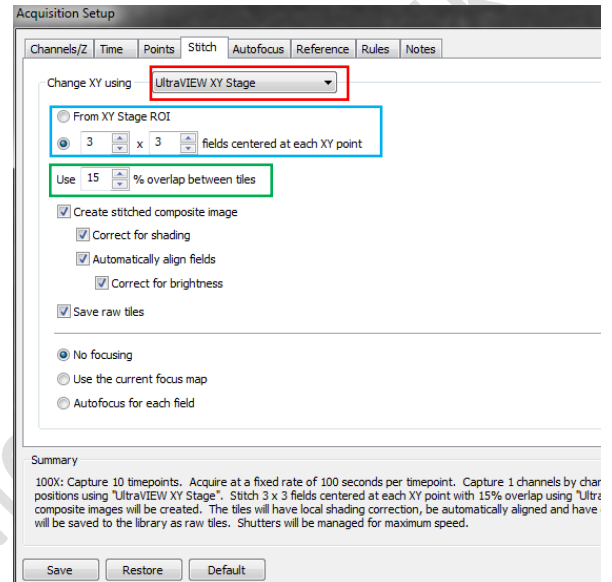
3. To view the coordinates of different points saved, right click the preview image and choose 'XY stage'

Under this option, the image area and coordinates (x,y,z) is displayed.

4. **Review point**
5. Go to acquisition setup to verify imaging parameters then click on 'Record button'
6. Multi-point scans is compatible with z-stack (Part D), multiple time-point experiments (Part E) and tile scan function.
7. Tile scan function can be accessed in the 'Stitch' tab in acquisition window.
 - 7.1. **Select** UltraVIEW XY stage as control.
 - 7.2. **Choose** number of fields to be imaged per coordinate.
 - 7.3. **Determine** the % of overlap between tiles.
 - 7.4. Click 'OK' and then start recording.









...F.3



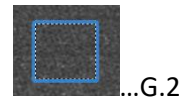
G. FRAP function

FRAP function can be accessed under the **UltraVIEW** device panel.

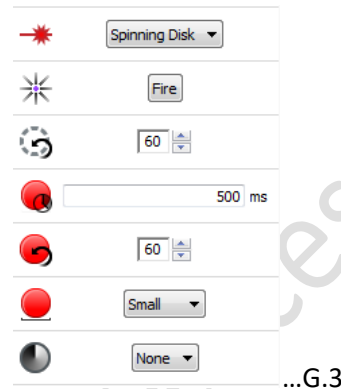
1. Select 'FRAP Preview'
2. Draw an ROI around the area you want to administer the treatment.
3. Adjust FRAP parameters:
 - 3.1. Double click on the  to choose laser wavelength
 - 3.2.  UltraVIEW PK cycles (determine the number of rounds the laser will go through the ROI)
 - 3.3.  Spot period
 - 3.4.  Spot cycle
 - 3.5.  Spot size
 - 3.6.  Attenuation (decrease FRAP strength)
4. Right click on the acquisition control panel to view the acquisition setup window.
5. Set the number of
 - 5.1. images to be acquired BEFORE bleaching.
 - 5.2. images to be acquired AFTER bleaching.
 - 5.3. cycles of treatment to be performed.



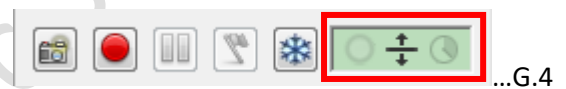
...G.1



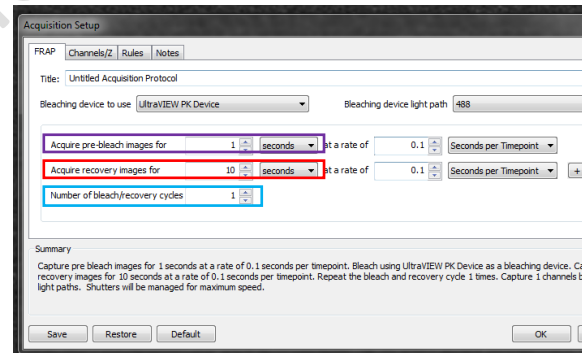
...G.2



...G.3

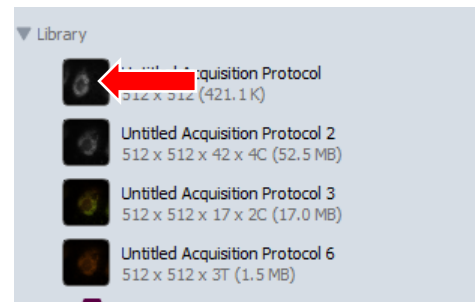


...G.4



H. Viewing and exporting acquired Images

1. Double click the icon in library panel to open up a new window containing the images.



...H.1

2. Choose between different viewing modes on menu bar. On the right listed a few useful ones of the viewing modes.

XYZ = 2D view with orthogonal slices

3D Plane = manually rotate the z-stack

3D Opacity = render images into 3D objects

Extended Focus = combine the z-stack into a deeper depth-of-view image.

3. To export the images, right click on the image and select 'Export'

Alternatively, go to File\Export\

4. Choose from different file types.

5. Export:

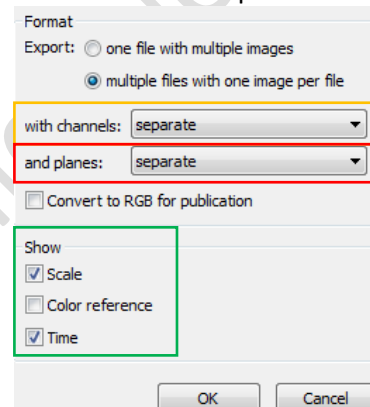
5.1. Separate channel

5.2. Separate z-planes

5.3. Label images with scalebar, color reference and time stamp.

'Item as': more options available

'View as': direct export of image view



...H.5

6. Save to C:\Data\PI\your name"
7. Upload to your own server as soon as possible.

I. Turning OFF the microscope

1. Exit Volocity software
2. [Optional] If incubation is used follow these steps during power down:
3. Turn OFF CO₂ supply

****Do not change gas regulator setting as in A.2.3.****



...i.3

4. Turn on temperature and CO₂ control module (6).



...i.4

5. Remove incubator cover, clean with 70% ethanol
6. Use tissue paper to soak up excess water from water bath
7. Remove sample frame from incubator and place the frame on air-table before dismounting your sample chamber.
8. Turn off computer (5).
9. Switch OFF Mercury lamp (4).
10. Switch OFF the modular laser system 2.0 (3). Wait until light and fan is off
11. Clean oil objective (with lens cleaning tissue only but NOT Kimwipe)
12. Remove residue oil from the objective lens with a dry lens cleaning tissue
13. Clean the lens with a new lens cleaning tissue UNTIL no more oil residue can be observed on the tissue.
14. Switch OFF power supply mounted on the wall (1) and (2).
15. Replace microscope cover.

Next section is supplementary material from PerkinElmer regarding piezo stage operation with Nikon PFS system in Ti-E microscopes.

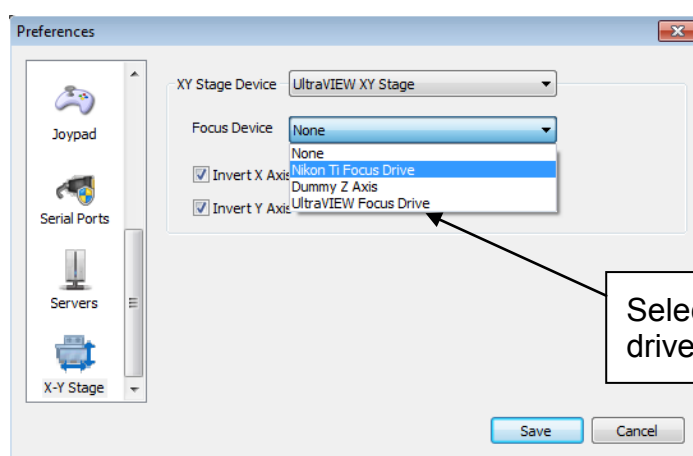
Topic: Using the Nikon PFS with Volocity Acquisition

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.



Select the microscope focus drive in this drop down menu.

How to use the PFS in the video preview

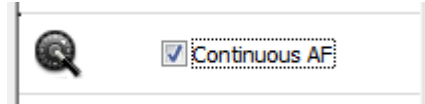
1. Focus on your plane of interest using the microscope focus control

Microscope focus control



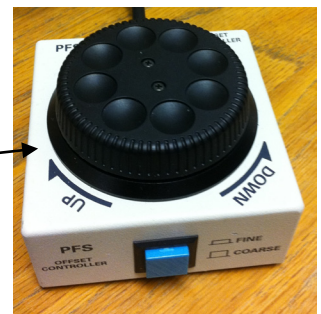
2. To enable the PFS either:

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



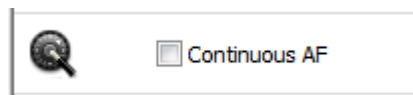
- Adjust the focus as required by using the PFS Offset controller.
- With the PFS activated the current focal plane will be maintained.

PFS Offset controller



5. To disable the PFS either:

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



- 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.



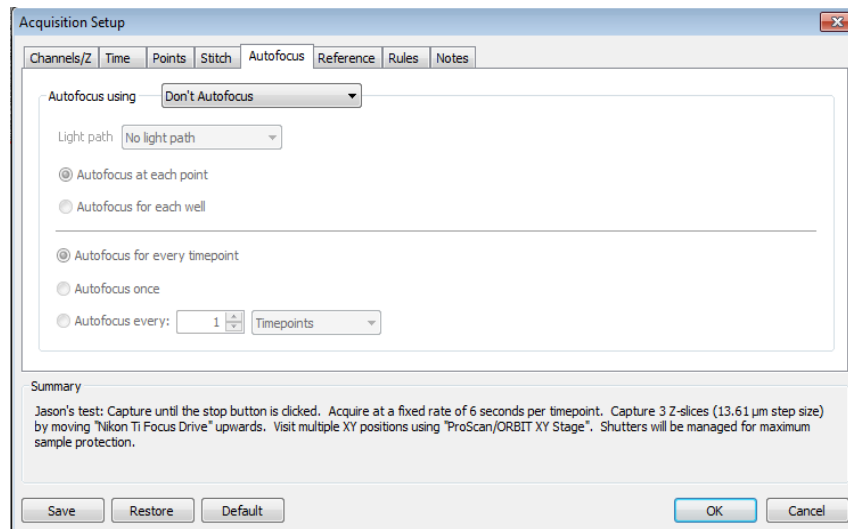
Going forward in this document we will refer to the above as enabling and disabling the PFS.

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.

- Enable the PFS
- 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”.

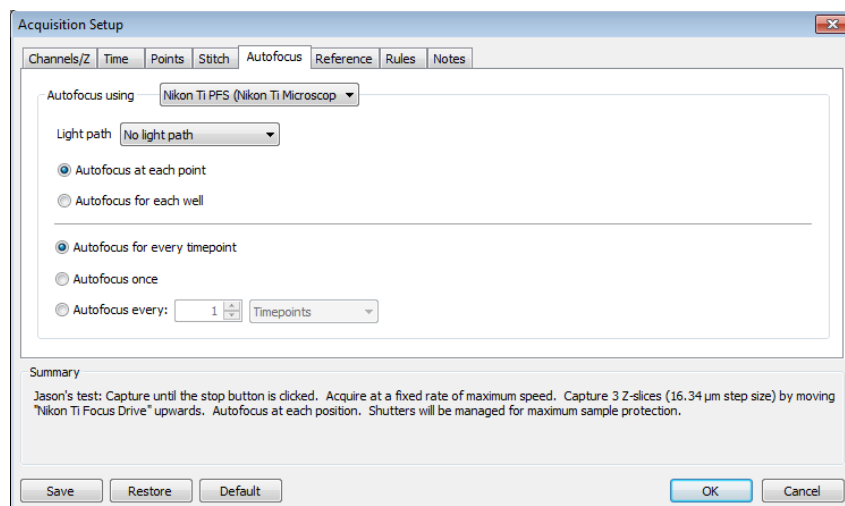


- The PFS will continually maintain your focal plane throughout your acquisition.

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.

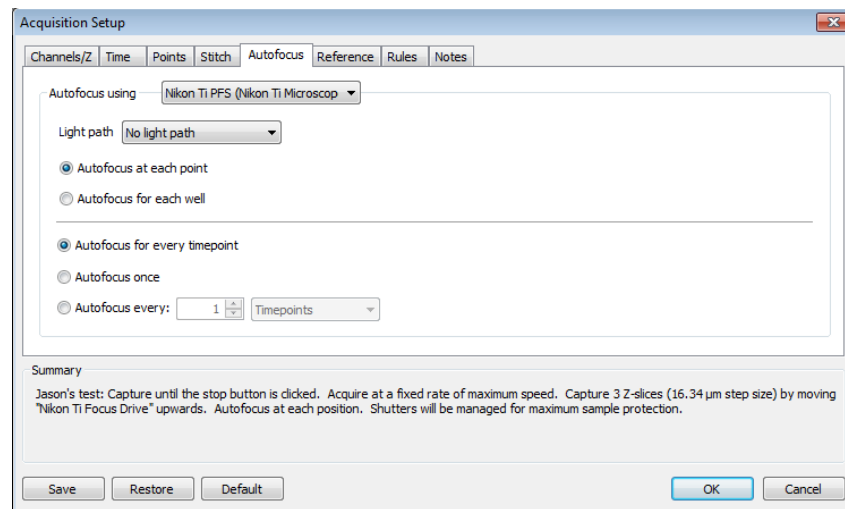
- Enable the PFS.
- Adjust the focus as required by using the PFS Offset controller.
- Disable the PFS.
- When configuring your acquisition protocol in the acquisition setup dialog set “Auto-focus using” to “Nikon Ti PFS”.



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

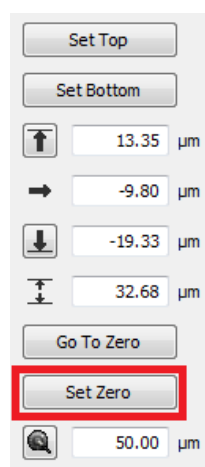
For acquisition setups that involve multiple planes or multiple points we do not recommend the use of Continuous Auto Focus. Please ensure this is disabled prior to starting an acquisition that involves multiple planes or multiple points. For the

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

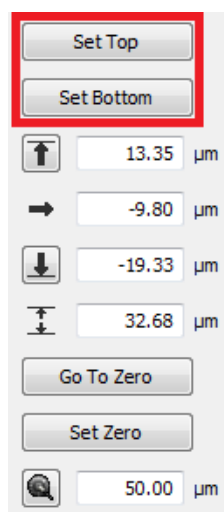


Multiple planes over time

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.

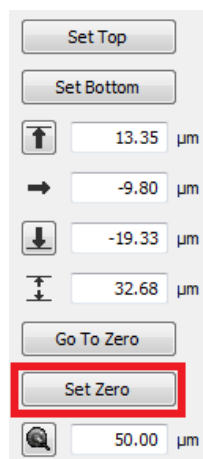
6. When configuring your acquisition protocol in the acquisition setup dialog select “Auto-focus using” to “Nikon Ti PFS”.
7. Start the experiment.
8. Volocity will activate the PFS briefly prior to acquiring each time point to restore your focal plane. Each time the focal plane is found Volocity will 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

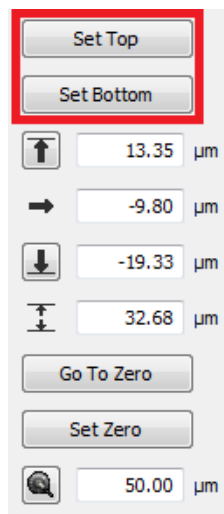
1. Disable the PFS
2. Add your XY points, adjusting the microscope focus control as required to maintain focus point of interest.
3. Return to any XY point you have previously made.
4. Enable the PFS.
5. Set your focal plane by adjusting the PFS Offset controller and update the point.
6. Move to another point and repeat the step 5 until all the points are updated.
7. Disable the PFS
8. When configuring your acquisition protocol in the acquisition setup dialog select “Auto-focus using” to “Nikon Ti PFS”.
9. Start the experiment
10. 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.



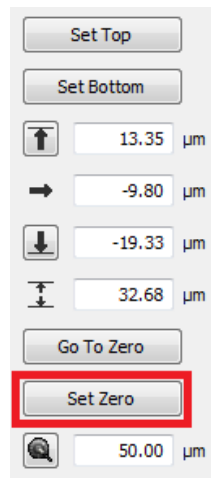
4. Add your first XY point
5. Using the microscope focus control set your sample top and bottom positions either side of the zero position.



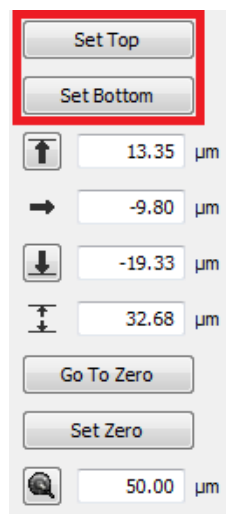
6. Continue to add further XY points as required adjusting the microscope focus control to maintain focus position of interest.
7. Go to any XY point you have previously made.
8. Enable the PFS
9. Set your focal plane by adjusting the PFS Offset controller and update the point.
10. Move to remaining points and repeat step 9 until all the points are updated.
11. Disable the PFS
12. When configuring your acquisition protocol in the acquisition setup dialog select "Auto-focus using" to "Nikon Ti PFS".
13. Start the experiment
14. 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.