

### Discussion

Ensure that the Perfect Focus System (PFS) has been installed according to the instructions in the manual, and that the TE2000 has been connected to Volocity and configured according to the instructions in the Volocity manual.

#### Using the PFS from the video preview

1. Select “AF On” on the PFS keypad to activate the PFS (the “AF ON” L.E.D. should be on).
2. Set your focal plane by turning the “FOCUS OFFSET” control on the PFS keypad.
3. Select “AF On” again to deactivate the PFS (the “AF ON” L.E.D. should be off).



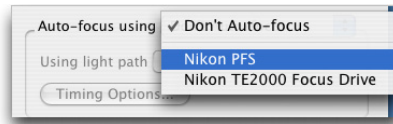
4. Every time you select “AutoFocus” on the video preview the PFS will be briefly activated and the focal plane that you have set will be restored.

#### Single plane, single point over time - Option A

1. Select “AF On” on the PFS keypad to activate the PFS (the “AF ON” L.E.D. should be on).
2. Set your focal plane by turning the “FOCUS OFFSET” control on the PFS keypad.
3. When setting your acquisition protocol in the acquisition setup dialog set “Autofocus using” to “Don’t auto-focus”.
4. The PFS will continually maintain your focal plane throughout your acquisition. Volocity does not need to interact with the PFS.

#### Single plane, single point over time - Option B

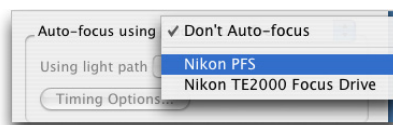
1. Select “AF On” on the PFS keypad to activate the PFS (the “AF ON” L.E.D. should be on).
2. Set your focal plane by turning the “FOCUS OFFSET” control on the PFS keypad.
3. Select “AF On” again to deactivate the PFS (the “AF ON” L.E.D. should be off).
4. When setting your acquisition protocol in the acquisition setup dialog set “Autofocus using” to “Nikon PFS”.



5. Select “Capture”.
6. Velocity will activate the PFS briefly prior to acquiring each time point to restore your focal plane.

### **Multiple planes, single point over time**

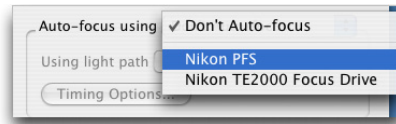
1. Set the top and bottom of your focus drive. If you are using your Nikon focus drive for both focussing and acquisition you must set the top and bottom either side of zero. If you are using a second Z axis for the acquisition this is not necessary.
2. Select “AF On” on the PFS keypad to activate the PFS (the “AF ON” L.E.D. should be on).
3. Set your focal plane by turning the “FOCUS OFFSET” control on the PFS keypad.
4. Select “AF On” again to deactivate the PFS (the “AF ON” L.E.D. should be off).
5. When setting your acquisition protocol in the acquisition setup dialog set “Autofocus using” to “Nikon PFS”.



6. Select “Capture”.
7. Velocity will activate the PFS briefly prior to acquiring each time point to restore your focal plane. Each time the focal plane is found Velocity 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 Velocity finds.

### **Single plane, multipoint, over time**

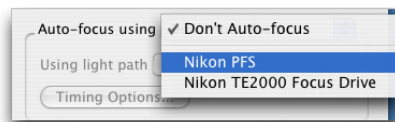
1. Add your acquisition points. If the acquisition points have different Z positions they can be set with the microscope’s focus drive or with the PFS offset (if the PFS AF function is active).
2. Go to any acquisition point.
3. Select “AF On” on the PFS keypad to activate the PFS (the “AF ON” L.E.D. should be on).
4. Set your focal plane by turning the “FOCUS OFFSET” control on the PFS keypad.
5. Select “AF On” again to deactivate the PFS (the “AF ON” L.E.D. should be off).
6. When setting your acquisition protocol in the acquisition setup dialog set “Autofocus using” to “Nikon PFS”.



7. Select "Capture".
8. Volocity will activate the PFS briefly prior to acquiring each acquisition point to restore the correct focal plane.

### **Multiple planes, multipoint, over time**

1. Add your acquisition points. If the acquisition points have different Z positions they can be set with the microscope's focus drive or with the PFS offset (if the PFS AF function is active).
2. Set the top and bottom of your focus drive. If you are using your Nikon 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. Select "AF On" on the PFS keypad to activate the PFS (the "AF ON" L.E.D. should be on).
5. Set your focal plane by turning the "FOCUS OFFSET" control on the PFS keypad.
6. Select "AF On" again to deactivate the PFS (the "AF ON" L.E.D. should be off).
7. When setting your acquisition protocol in the acquisition setup dialog set "Autofocus using" to "Nikon PFS".



8. Select "Capture".
9. 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. 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.