

## Using the Nikon PFS with Meta Imaging Series 7

### ABSTRACT

This document describes how to use the Nikon PFS continuous-focus device with MetaMorph version 7.

### OVERVIEW

The Nikon PFS is a continuous-focus mechanism for the Nikon TE-2000 inverted microscope. The PFS can be controlled through the Meta Imaging Series 7 software.

**NOTES:** The Nikon PFS can only be used with certain objectives. See document M383 E from Nikon Corporation, titled **Eclipse TE2000 Inverted Microscope Perfect Focus System** for details. Read the manual in its entirety, but especially chapter 1, sections 4.1 “Suitable specimen”, and 4.2 “Suitable objectives”. The Nikon PFS must be calibrated before use. See the document mentioned above, chapter VI, section 9 “Adjusting the focusing function” on page 37.

ARTICLE #  
T20058

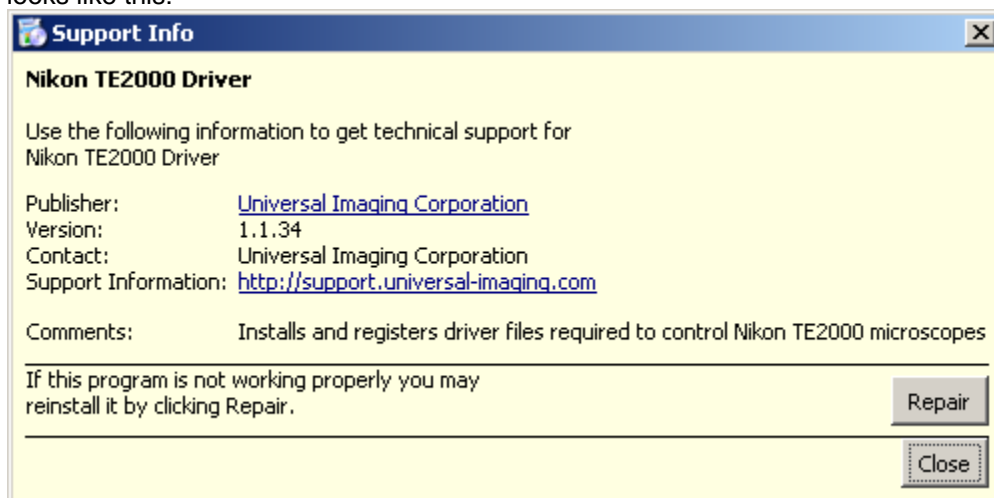
PRODUCTS  
MetaMorph®

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7-Jun-2006

LAST UPDATED  
7-Nov-2006

In order to use the Nikon PFS, the following steps must be taken:

1. When the PFS module is installed onto the microscope you power on the transmitted light and microscope and wait for the microscope to finish initializing. Then you can power on the PFS Controller. Make sure that when you power off the microscope, you power off the PSF Controller first before you power off the stand and transmitted light.
2. The appropriate Nikon TE2000 driver files must be installed on the computer. At the time of this writing, the driver files are version **1.1.34**. To verify that this driver is installed, go to the **Add or Remove Programs** Control Panel, and find “Nikon TE2000 Driver” in the list. If found, highlight it. Then click on the line that says “Click here for support information”. The dialog that appears, looks like this:



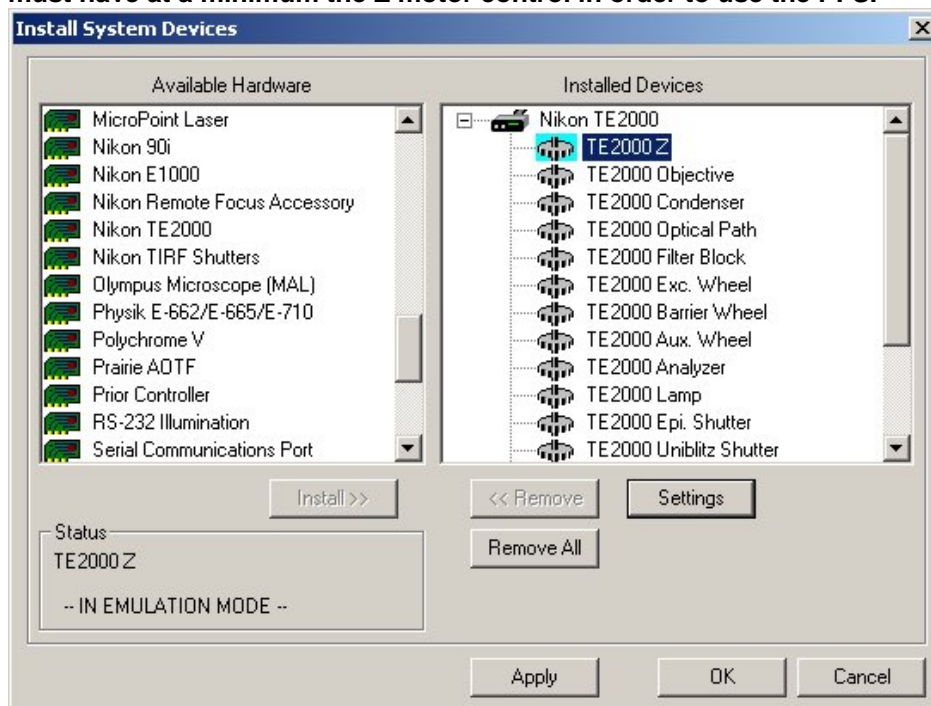
Make sure the Version is at least 1.1.34.

3. If either the Nikon TE2000 Driver is not in the Add or Remove Programs Control Panel, or it is there but is earlier than 1.1.34, then uninstall the current version and run the file:

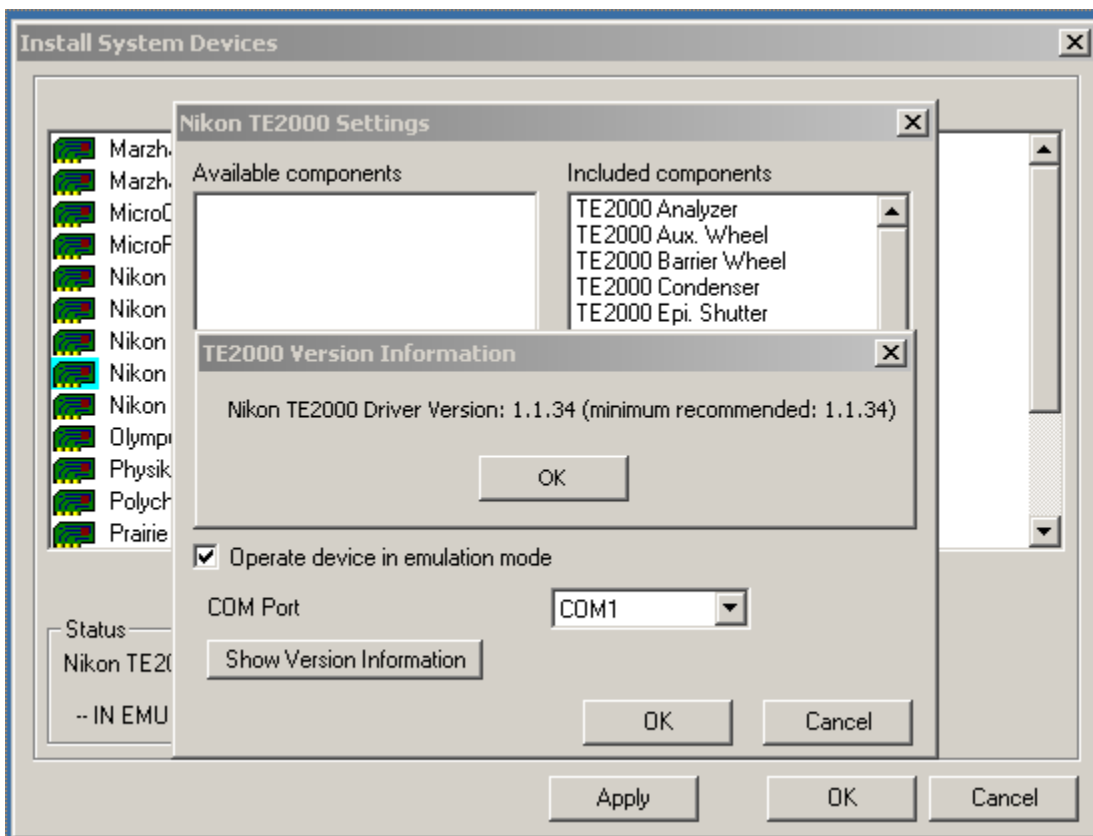
**c:\mm\Nikon-TE2000.msi**

This will install the proper file (c:\mm being the directory where Meta Imaging Series 7 is installed). You need a minimum of **Meta Imaging Series version 7.0r4** to use the Nikon driver files version 1.1.34.

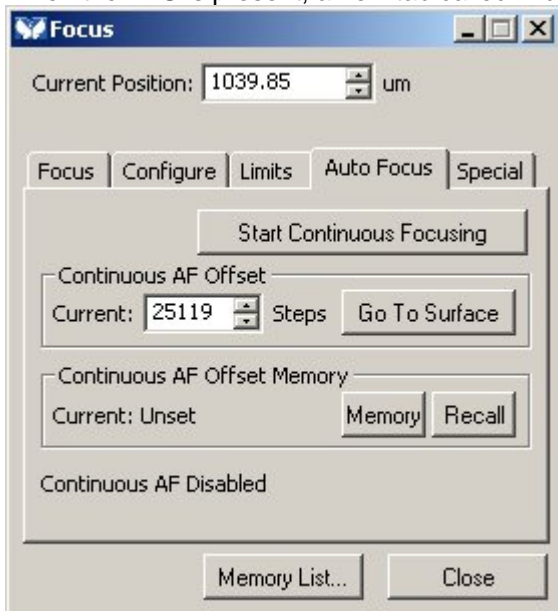
4. Install the Nikon TE-2000 driver using the Meta Imaging Series Administrator program. **You must have at a minimum the Z motor control in order to use the PFS.**



5. You can highlight the “Nikon TE2000” entry and press the Settings button, and then press the “Show Version Information” button to confirm that the Nikon driver is the correct version (at least 1.1.34).



- Open MetaMorph. To control the PFS, open the Focus dialog box under the Devices Menu. When the PFS is present, a new tab called "Auto Focus" appears in the Focus dialog, like this:



7. Bring the sample into focus with the focusing knobs on the microscope.

8. The button "Start Continuous Focusing" will enable the PFS - when pressed, the microscope will use the IR diode to find the bottom of the slide - this is called the "surface". Then, it will move the Z motor additional steps using the "Current AF Offset" step position. These steps are not in calibrated units. After pressing the "Start Continuous Focusing" button, the AF On light will be on.
9. The absolute range of the PFS is from 0 to 100,000 steps. The step size is not in calibrated units, and the units will vary for each objective. Each objective will have a subset of the absolute range that it can use for travel. Typically, the surface will be somewhere in the middle of the objective's range, such as 20,000. The surface is usually not at zero. The surface location will differ for each objective.
10. You can adjust the Current AF Offset in the "Focus" dialog or use the knob on the PFS Controller so that the cells are in focus, since the cells are usually not at the surface, but are some distance away from the surface. The Current AF Offset will update as the offset knob is used on the PFS control box.
11. You can store one Offset position using the Memory button, and move back to that stored Offset using the Recall button. These buttons are similar to the ones on the PFS control box.
12. The Start Continuous Focusing button can be recorded in a journal, as can the stop focusing action.
13. From a journal there is access to variables so that you can determine whether the system is focusing, and can set the offset

#### JOURNAL COMMANDS

Here are the Journal Commands available from MetaMorph:

1. MM Journal function - Start Continuous Focusing
2. MM Journal function - Stop Continuous Focusing
3. MM Journal function - Continuous AF Offset to Surface
4. MM Journal function - Continuous AF Offset Memorize
5. MM Journal function - Continuous AF Offset Recall

#### JOURNAL VARIABLES

Here are the Journal Variables available from MetaMorph:

1. Device.Focus.ContinuousAF.Offset (get and set the PFS Offset)
2. Device.Focus.ContinuousAF.IsSearching (get only whether PFS is searching for focus = 1 or not = 0)
3. Device.Focus.ContinuousAF.IsOn (get whether the PFS currently is enabled/maintaining focus = 1, or not = 0).
4. Device.Focus.ContinuousAF.IsOffsetMemorized (get whether an offset position is memorized = 1, or not = 0).
5. Device.Focus.ContinuousAF.MemorizedOffset (get the current memorized offset position).

#### USE FROM METAFUOR

The PFS is also available in MetaFluor: On the MetaFluor Z Motor Dialog Auto Focus tab there is a Start/Stop continuous focus button plus continuous focus offset (note - only updates if Poll Z is checked)

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**NOTES**

1. The normal Z motor (stepper motor) of the TE2000 microscope can NOT be used when the autofocusing is enabled. This is because the PFS "gets control" of the stepper motor. So, you cannot do a z-series or other action of the z stepper motor while the PFS continuous focusing is enabled. This is why you will need to stop the PFS continuous autofocus if you want to do a z-series.
2. When recording Start Continuous AutoFocus in a journal, the PFS offset is NOT saved in the journal. Instead it will use the current PFS offset value. You can use the variable Device.Focus.ContinuousAF.Offset to set a specific offset if this is necessary to do in the journal.
3. You can NOT change microscope objectives (example: move from 10x turret position to 20x turret position) while the PFS continuous auto focus is enabled. You must first turn off the continuous auto focus, then move the turret to the new objective, and then you can turn continuous auto focus on if needed.
4. MetaMorph will crash/lock-up if the PFS controller power is turned off during use of MetaMorph.
5. The PFS step size, usable step range, and surface location are different with each objective. As a result, the surface-to-sample offset may not be compatible between different objective models, so the sample must be brought into focus for each objective to find the offset for that objective.
6. The PFS manual specification states focusing time as "700 msec or less (near the boundary surface)".
7. There is a Nikon procedure for adjusting how quickly the PFS adjusts the Z to maintain focus. If a PFS is constantly searching for focus (too sensitive) a dial can be adjusted on the PFS controller to slow it down.
8. There is a separate document from Nikon called Adjusting the PFS Focus Positioning Sensitivity dated May 23, 2006 that describes the procedure for calibrating the PFS and it requires a specific 40x .75 NA WD .72 objective.
9. There is a lever on the back left of the PFS module that slides the PFS dichroic in/out of the optical path.
10. There should be IR Cut filters for both the transmitted (45mm) & EPI (25mm) illumination paths

**SAMPLE JOURNAL - WAIT FOR PFS TO ACHIEVE FOCUS**

Following is a sample journal for MetaMorph which waits for the PFS to achieve focus.

```
// wait maximum of 1 second, testing every 50ms, for PFS to achieve focus
RepeatCount = 20
DelayLength = 50 ms
While RepeatCount > 0 and Device.Focus.ContinuousAF.IsSearching != 0
    Delay(DelayLength)
    RepeatCount = RepeatCount - 1
End While

// error if still searching
If Device.Focus.ContinuousAF.IsSearching != 0
    // ContinuousAF never finished
    // warn user and/or end journal
End If
```

**USING MDA WITH MULTIPLE STAGE POSITIONS, USING THE SAME PFS OFFSET**

1. Before using MDA, make sure the PFS continuous AF is enabled.

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2. Create a journal that loops until the variable `Device.Focus.ContinuousAF.IsSearching = 0`.
3. Configure MDA to run the journal (2) after the stage is moved.
4. Do the MDA. MDA will move to each stage position, and run your journal (2) waiting for the PFS to complete the AF, and then will acquire the image.

**USING MDA WITH MULTIPLE STAGE POSITIONS, WITH A DIFFERENT PFS OFFSET FOR EACH STAGE POSITION**

1. It is much easier to have a fixed number of stage positions, example = 5.
2. For each stage position, the user has to determine the correct PFS offset.
3. You store that offset in a variable, for example `stage1offset`, `stage2offset`, and so forth.
4. Create a journal that uses the variable `MDA.Status.StagePosNum` to determine which stage position is in use. Then set the variable `Device.Focus.ContinuousAF.Offset` to the user's offset for that stage position from step (3), for example `stage1offset`. Then loop until the variable `Device.Focus.ContinuousAF.IsSearching = 0`.
5. Configure MDA to run the previous journal after the stage is moved.
6. Do the MDA. MDA will move to each stage position, and run your journal (4) which sets the proper offset, and waits for the PFS to complete the AF, and then will acquire the image.

**USING MDA TO DO A Z-SERIES**

**NOTE:** The Z motor cannot be moved when the PFS continuous AF is enabled. So you must turn off the PFS before moving the Z motor, and then turn it back on afterwards.

1. Use one of the methods as described above, as applicable.
2. In one of the above journals, turn off the PFS continuous AF by using the command "Stop Continuous Focusing".
3. MDA does the Z-series.
4. After acquisition, have MDA run another journal that uses the command "Start Continuous Focusing" to re-enable the PFS continuous AF.

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