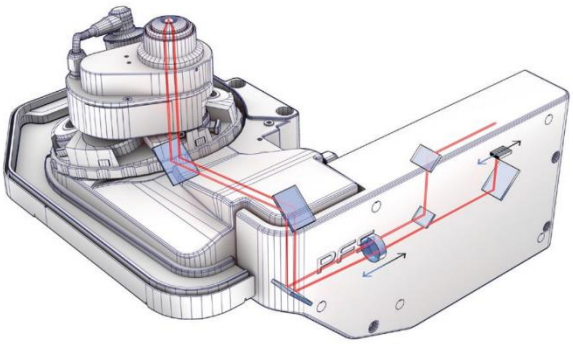
Nikon Practical Tips 1 – Ti2 PFS and WID

1 Introduction

The Nikon Perfect Focus System (PFS) is designed to track the position of a reflecting surface to stabilize the optical focus during long time experiments. This document presents strategies to combine the PFS focus tracking with multi-point time-lapse experiments and the motorized Water Immersion Dispenser (WID).

2 PFS Basics



The IR beam of the PFS LED reflects on the top or bottom of the coverslip or sample carrier bottom (dish, chamber slide, multi-well plate). For dry objectives this is the bottom surface of the glass/plastic bottom. For oil immersion objectives this is the top surface of the glass/plastic bottom. For water immersion objectives there are two reflections: from the top and bottom. The reflection is projected on a line-CCD-sensor. When the reflection peak is off-center, the microscope focus drive is used to move the nosepiece until the reflection is centered again. This strategy ensures that the distance between the objective and the sample is constant.

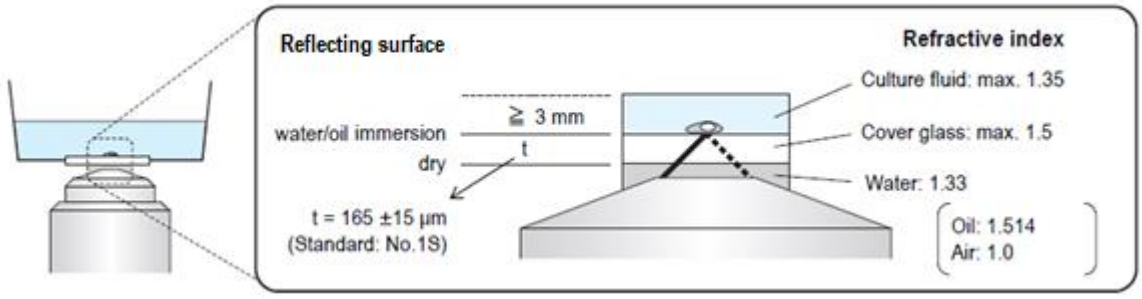


Figure 1: PFS Basics: the reflection of the IR beam on the coverslip/glass bottom.

The position of the 'PFS Offset Lens' determines the distance between the reflecting surface and the optical focus. Changing the PFS Offset Lens position allows for moving the optical focus away from the reflecting surface – deeper into the sample while maintaining perfect focus. Watch the MicroscopyU tutorials for more information on the PFS:
Nikon Perfect Focus System (*PFS*)
The Nikon Perfect Focus System (*PFS*)
Perfect Focus Offset System Mechanics
Correcting Focus Drift in Live-Cell Microscopy

3 PFS Key Points

When using the PFS, the following key points must be kept in mind:

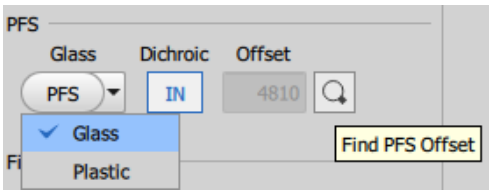
- The Ti2 features PFS support for a limited range of objective models. All PFS objectives work with glass bottom plates/coverslips #1.5 (170 um). For a small number of low magnification objectives, support for plates with a plastic bottom (thickness < 1.3 mm) is implemented.
- The strength of the PFS IR reflection depends on the difference of refractive index on the reflecting surface. Correct operation is only guaranteed with the specified RI gradient between the bottom/coverslip and the medium. The PFS is designed for live cell experiments, the RI after fixation is too high for a clear IR reflection.
- The PFS IR reflection deteriorates when the reflecting surface is not clean or not flat. Only use high quality sample carrier with a flat bottom and clean the surfaces thoroughly. Level the insert for best performance.
- Fill the Water Immersion Dispenser (WID) with Type I pure water* only. Clean the vessel, tubes and nozzle regularly with ethanol and vinegar, at least once a month. Using standard demineralized water instead of Type 1 pure water will leave a (calcium) residue on the plate bottom and the objective front lens which causes the PFS to fail after some time. * https://en.wikipedia.org/wiki/Purified_water

4 PFS Controls

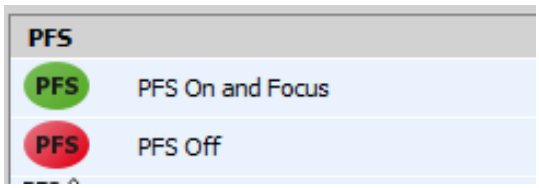
The PFS is operated using the following controls:

The PFS button on top of the body focus dial:
a short press enables/disables the PFS
a long press moves the PFS mirror in/out
two short presses moves the PFS offset lens to the center position.
When the PFS is enabled and locked, rotating the focus dial changes the PFS Offset Lens position.

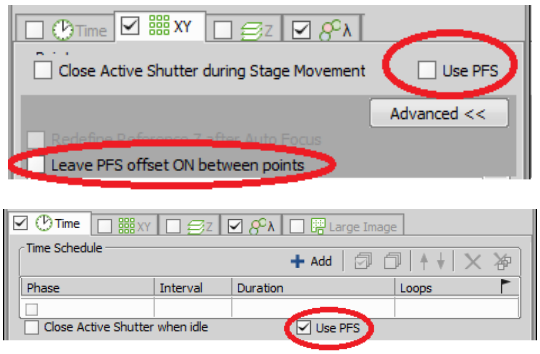
The PFS button next to the Remote Control Pad focus dial:
a short press enables/disables the PFS.
When the PFS is enabled and locked, rotating the focus dial changes the PFS Offset Lens position.



The PFS section of the Acquisition or Ti2 Control pads. Pressing the PFS button enables/disables the PFS. This button is green when the PFS is enabled. Some objectives For objectives that support a plastic bottom, the drop-down menu allows for selecting the bottom material. The Dichroic button moves the mirror in/out. The offset button shows the position of the PFS Offset Lens. The button with the magnifier glass enables the PFS and changes the Offset Lens to return to the current focus position.



The PFS Job tasks.



The PFS options in the ND Acquisition XY and Time Panels.

5 PFS Indicators and Sounds

The green PFS LED on the front of the microscope gives information on the PFS state. In the off state, the LED is dark if no reflection peak is detected. When a reflection is detected, the LED blinks with short pulses. In the on state, the LED shines continuously if the peak is detected and locked to the center position. In case of an error (no reflection peak, mirror not in, objective not supported), the LED blinks with longer pulses.

LED	On/Off	Reflection Peak	Status
off	off	not detected	switching on will not lock
short blink	off	detected	switching on will lock
long blink	on	not detected	error, not locked
on	on	detected	locked

By default, the Z-drive / PFS is programmed to generate the following sounds:

Sound	Meaning
one beep	a reflection peak is detected
two beeps	an error occurs: run into range limit, PFS on but no peak detected etc.

Note: the PFS button on the Ti2 panel in NIS-Elements never blinks. The error state is only signaled by the flashing of the LED on the microscope front, not on the PC screen.

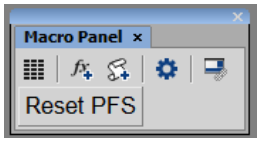
6 Basic PFS Operation

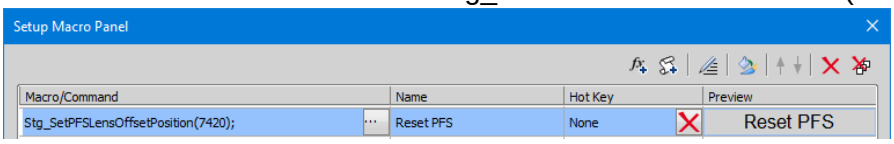
The standard way to focus with the PFS is:

- disable the PFS*
- optionally**: reset the PFS Offset Lens to the default position by double-clicking the PFS button on top of the microscope focus dial.
- move the z-drive manually towards the focus position
- when the PFS detects a reflection peak***, the PFS will beep and the LED starts to blink
- enable the PFS: the PFS will center the reflection peak by moving the z-drive
- fine focus with the PFS

* When the PFS is enabled from the beginning, the operation mode suddenly switches from normal to PFS mode when a peak is detected. As a result, rotating the dial will start to move the PFS offset lens, which might not be desired.

** When the PFS Offset Lens is too much off-center, no peak is ever detected and locking always fails. This happens e.g. when accidentally focusing to a wrong surface using the PFS. The only standard way to reset the PFS Offset Lens Position is to double click the PFS button on the body focus dial. To reset the PFS Offset Lens to another default position you can create a macro toolbar button with the command Stg_SetPFSLensOffsetPosition(7420);:





*** For water immersion objectives, there are two reflections: a strong reflection from the bottom of the glass, a weaker reflection from the top of the glass. The 40x and 60x water immersion objectives require locking to the top of the glass, for these objectives you must continue to focus up until you hear a second beep.

The focus position where the beep is heard depends on the objective. These are typical values:

objective	from below	from above
MRD00105 Plan Apo λ 10x	-240 um	+90 um
MRD07650 Plan Apo IR 60x WI DIC N2	-170 um (1st)	-110 um (1st)
	-11 um (2nd)	+5 um (2nd)

7 PFS Focus Range

When the PFS is locked, the PFS offset lens can be used to change the optical focus position. When moving the PFS offset lens away from the center position, the height of the reflection peak on line detector will decrease. When the peak is not detected anymore, the PFS enters error state: a double beep is heard and the LED starts to blink. Moving the focus back will usually recover the PFS operation. If not, the PFS Offset Lens must be reset (see § 6), The PFS focus range depends on the objective characteristics and on the properties of the sample holder. To determine the PFS focus range in your situation follow these steps:

- focus on the top of the glass bottom with the PFS on.
- on the Remote Control Pad (RCP) (Joystick display ?), select the relative position (‘Rel Pos’) page.
- press the ‘Reset Z’ button on the RCP.
- focus up until the PFS goes into error state*.
- read the relative Z position on the RCP.

* at systems with a motorized water dispenser, the stage is higher and the focus drive might run into the range limit (10000 um) before reaching the end of the PFS Focus Range. Check this on the RCP absolute position (‘Abs Pos’) page.

Typical values for the PFS Focus Range are:

objective	down	up
MRD00105 Plan Apo λ 10x	-1200 um	+1280 um (z-drive limit)
MRD07650 Plan Apo IR 60x WI DIC N2	- 28 um	+140 um

8 PFS Refocus Range

During multi-point experiments, the PFS can be used to restore the focus in every point. However, before enabling the PFS, the z-drive position must be in a position where a peak is detected on the PFS line detector (within the PFS Refocus Range).

To find out what is the actual refocus range is in which the PFS can restore correct focus with the current well/dish and sample, do the following test:

1. Move to a typical scene
2. Focus with the PFS
3. Disable the PFS and move the focus up 5 um
4. Enable the PFS and verify that the focus moves back to that from step 2.
5. Repeat steps 2. to 4. with increasing distances.
6. Do the same test focussing down.

Typical refocus ranges are:

objective	down	up
MRD00105 Plan Apo λ 10x	-1200 um	+1280 um (z-drive limit)
MRD07650 Plan Apo IR 60x WI DIC N2	- 13 um	+10 um

9 Stage moves and PFS

When moving the stage with the PFS on, three effects can interfere with correct focus tracking.

9.1 Reflected surface interrupted

When using well-plates or multi-chamber slides, there might be areas between the wells/chambers that do not reflect the PFS IR light well. When moving from one well or chamber to another with the PFS on, two things can happen:

a) no peak is detected and the PFS is in error state until arriving in the next well. If the focus difference between the well is not larger than the PFS refocus range, the PFS will lock in the new well correctly. If the focus difference is larger, a small manual focus change will make it lock again.

b) another peak is detected from reflection from a completely different surface and the PFS runs the focus drive up or down to center this peak. The objective moves up and might even push the plate up.

To find out if your plate is affected by one of these effects move the stage between several wells and validate that the PFS does not beep, the PFS LED is constantly on and the focus is tracked correctly. If the PFS tracking is interrupted, try to reduce the stage speed. When you observe that the PFS makes the focus run away between the wells, the PFS should be switched off when moving between wells (see § 12).

9.2 Immersion medium effects

When moving the stage with high speed, the mechanical stress on the immersion medium is large. With water immersion, the forces will create air bubbles that interfere with correct PFS operation. To prevent the formation of air bubbles in the water, the stage speed must be reduced to 10 mm/s or lower. This effect is more prominent when the sample is tilted. Leveling the sample holder will help as well.

9.3 Surface curvature

The sample bottom tilt might be stronger than the PFS can track. The bottom of wells and dishes are curved in different degrees. When the curvature is too strong, the stage speed must be adjusted. To find out what is the maximal speed with which the PFS can track your sample, do the following test:

- Focus on one side of the well/dish using the PFS – add this point the ND XY point list
- Move the stage to the opposite side of the well/dish – add this point to the ND XY point list.
- Move the stage several times from the first point to the second point by activating each point in turn (with the both the option ‘move to active point’ and ‘leave PFS on between points’ checked).

- When the PFS beeps during the move and the PFS LED starts to flicker, the stage speed is too high. Reduce the stage speed (Device Manager | Ti2 | Properties | XY Stage | Speed) until the PFS does not beep and the PFS LED is on during the full move.

An example of these effects is illustrated by the PSF status during a muti-well large-image experiment with the 60x WI objective. Without leveling and with a stage speed of 10 mm/s, the PFS state was OK (status 1) at the wells on the left, but in the wells on the right the PFS failed to lock (status 0). The z-drive position map reveals that the plate was not level: the left-top was 160 um over the right-bottom. The curvature of the plate and the tilting of the plate added up and as a result the tilt of the right-hand wells was too large for the PFS: air bubbles were generated in the water when moving the stage in one direction.

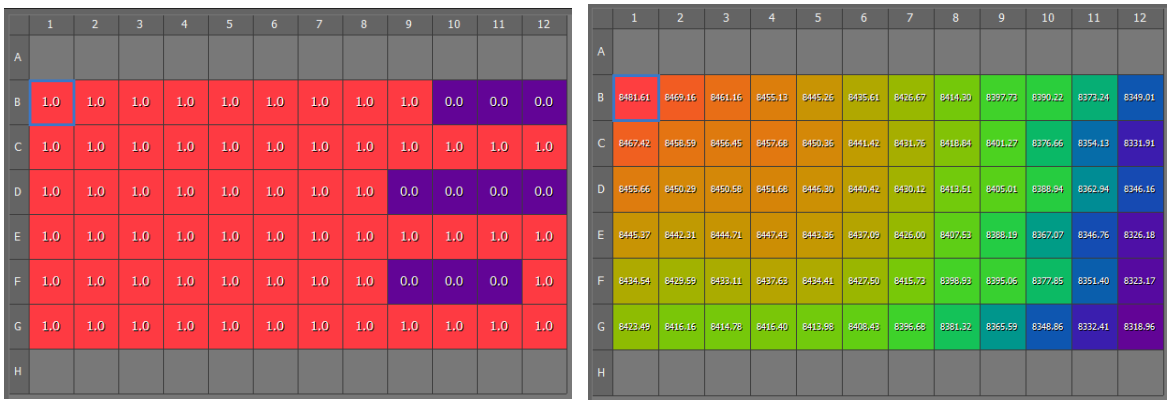


Figure 2: Left: PFS state in every well (1: on focus, 0: error). Right: z-drive position in every well: there is a focus difference of 160 um between the right-top and left-bottom corners.

After improving the leveling of the plate, the PFS locked correctly moving across all wells:

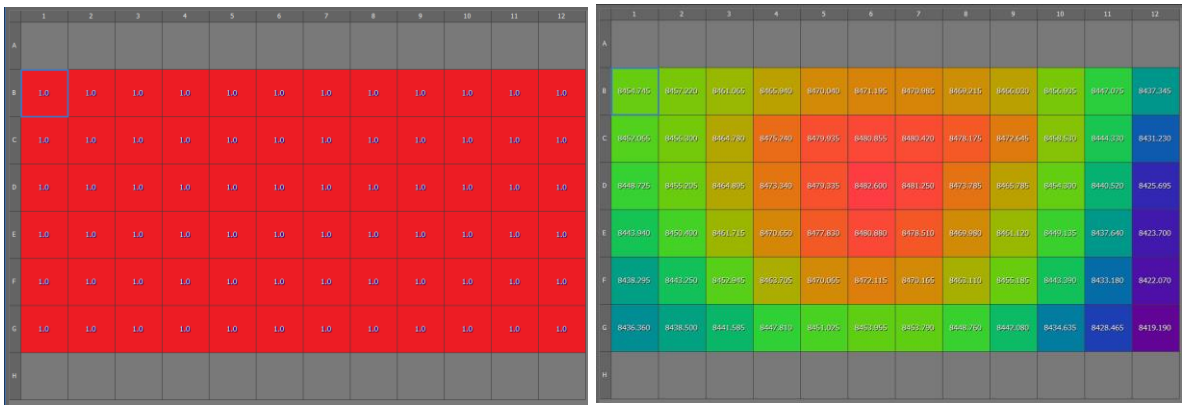


Figure 3: Left: PFS state in every well (1: on focus, 0: error). Right: z-drive position in every well: after leveling the focus difference between the plate corners has been reduced to 26 um.

10 Making a Focus Map

For selecting the best focusing strategy, it is important to get an idea of the variations in the focus position across a well and the full plate. For example, the focus map below was created by running autofocus on the center of each well. This plate is in a plate holder that supports the plate on the left- and right-hand side only. The weight of the medium makes the plate bend down in the middle.
The map shows that there is hardly any focus difference between adjacent wells in the middle. When the PFS moves to the next well in the middle of the plate and is enabled after the move, the refocus will succeed. In contrast, the focus difference is ~ 20 um on the left and right side of the plate. This focus difference might be larger than the refocus range. When there is a significant focus difference between the sides of the plate you can use the leveling screws of the insert to improve the leveling. For this plate insert, we noticed that the bending decreased during long term observation due to evaporation of the medium from the wells. The change in focus position could be tracked correctly using focusing scenario 2.

	1	2	3	4	5	6	7	8	9	10	11	12
A	3649.950	3640.810	3625.990	3610.020	3600.230	3602.240	3605.200	3622.020	3637.950	3656.010	3674.630	3687.850
B	3650.010	3641.270	3624.870	3611.580	3600.380	3598.450	3607.410	3621.600	3638.880	3655.260	3675.910	3692.330
C	3651.160	3642.520	3623.890	3610.340	3602.910	3601.270	3608.100	3619.870	3635.110	3656.330	3676.670	3687.670
D	3649.080	3644.730	3624.370	3609.850	3601.220	3600.240	3609.100	3622.240	3635.020	3656.630	3675.810	3690.080
E	3649.780	3641.850	3627.410	3608.980	3603.190	3600.330	3605.470	3621.300	3638.690	3656.480	3675.000	3691.950
F	3648.110	3644.710	3623.480	3610.780	3602.630	3601.710	3605.470	3620.150	3634.750	3657.360	3677.470	3687.630
G	3648.040	3644.210	3624.070	3612.200	3600.930	3599.180	3605.200	3621.160	3638.670	3658.080	3675.500	3691.230
H	3649.770	3641.060	3622.570	3610.260	3600.070	3598.550	3608.760	3621.530	3634.800	3654.500	3673.220	3688.760

Figure 4: Focus Map of Well Plate

The focus map below shows the focus position within one well. The variations within each well were measured by doing an autofocus at several positions across the well. The focus map below shows that there is about a 40 µm focus difference between the center of the well and the periphery.

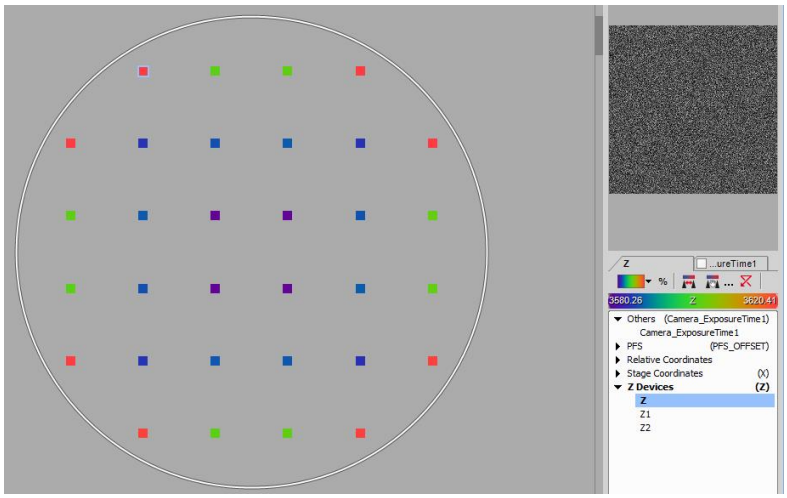


Figure 5: Focus Map of Well

11 Long term focus drift

The focus position depends on the room temperature. When the focus strategy requires the use of absolute focus positions, it is important that the focus changes stay within the PFS refocus range. To get an indication of the focus drift due to temperature, you can do a long term single point experiment with the PFS on. The PFS will correct for the focus drift by moving the microscope z-drive. The z-drive position reflects the actual mechanical focus drift. The graph below shows that when the room temperature changed 0.5 degree, the focus position moved 2.5 µm. Note that the temperature dependency is determined by many components: the objective, stage, nosepiece, body etc.

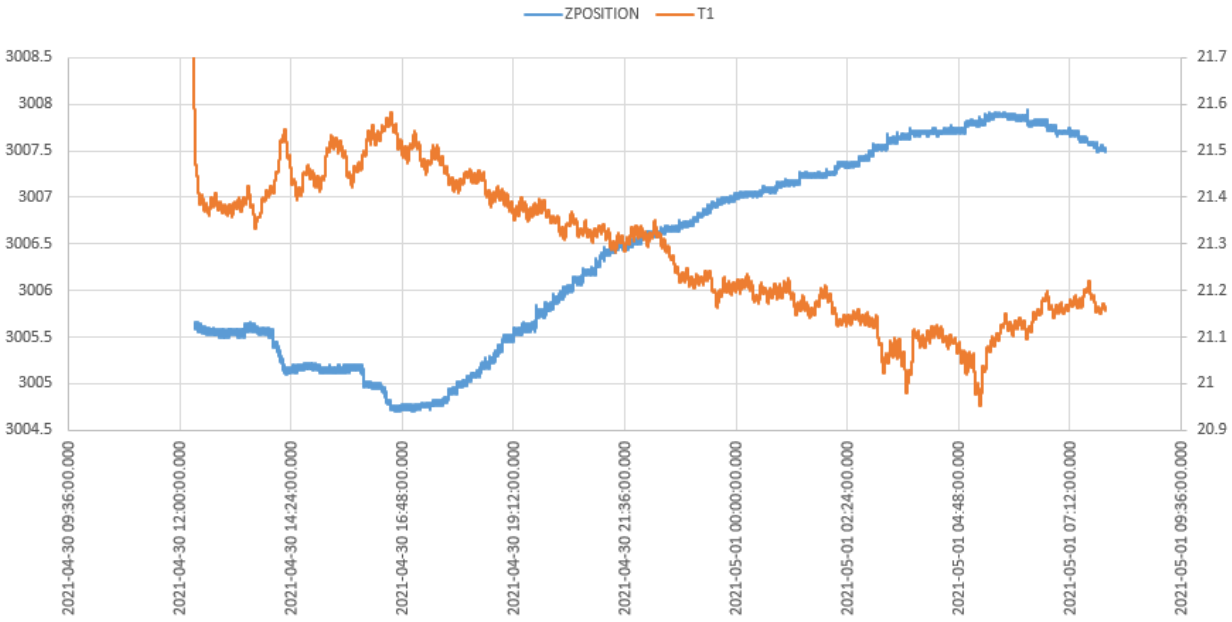


Figure 6: Focus Drift and Room Temperature over Time

12 Focusing Stategies

This section describes several PFS focusing strategies for multi-point time-lapse experiments. For all strategies, a key point is that the PFS should be on when waiting between rounds. During the waiting, the PFS will actively track the optical focus position and adjust the microscope focus drive to compensate mechanical focus drift. Absolute focus positions used during rounds should be updated for the focus shift that occurred during the waiting period.

12.1 PFS always on

The simplest focusing strategy is keeping the PFS on during all stage moves. This is only possible when the PFS can correctly track the focus during all (long and short distance) moves. Occasionally the PFS might switch off due to rare reflections. To recover from this situation, it is best to enable the PFS before every capture. The positive point of this strategy is that no absolute focus positions are used. The PFS will correct any focus drift in time.

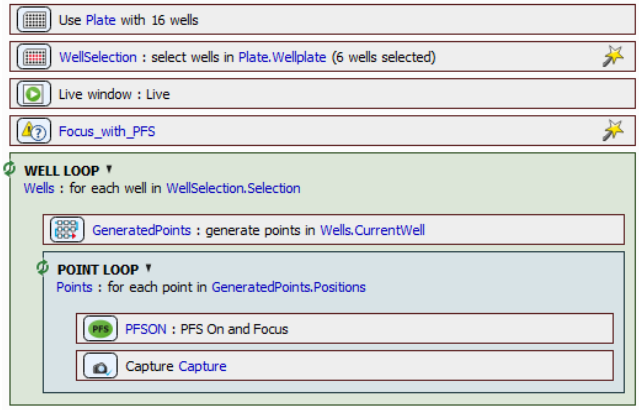


Figure 7: Focusing Strategy PFS always ON

When the PFS loses track during long moves, try if decreasing the stage speed resolves this. The total experiment time will increase. If this is a problem, one of the focusing strategies described below must be used.

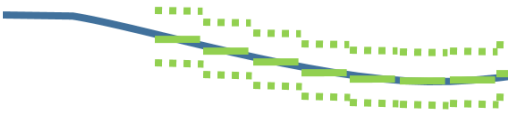


Figure 8: The PFS is on during the stage moves and can track the tilted surface correctly.

Figure 9: Stage moves (red) when the PFS is OFF and focus moves (purple) when PFS is switched on at the new position. When the new focus position is within the PFS refocus range, the PFS will lock (left). When the PFS is outside the PFS refocus range, a run-away might occur (right).

12.2 PFS Off during capture

When the PFS is On, the closed loop control strategy will move the z-drive over small distances when keeping the focus on position. For imaging contrast techniques that are sensitive for small focus the image will temporary appear slightly out-of-focus.. When capturing phase contrast image from very thin samples, it is best to switch off the PFS

before capture and switch it on after. This guarantees that the focus does not change during capture.

12.3 PFS on within a well, off when moving between wells

This strategy is used when the PFS can track movements correctly within one well, but loses the track when moving between wells. The latter can be due to confusing reflection between wells, or due to the combination of high stage speed and bottom curvature. Before moving to the next well, the PFS is switched off. For switching on the PFS at the new well, there are scenarios to consider:

Strategy 1. The focus difference between the wells is within the PFS refocus range.
In this case the PFS is simply switched on and will find the correct focus. See Figure 10.

Strategy 2. The focus difference between the wells is larger than the PFS refocus range.
In this case the Ti2 z-drive focus must be moved first within the PFS refocusing range before the PFS is switched in. The z-position per well can be set during the first round manually or by autofocus. To accommodate for focus drift in time, the stored z-position must be updated after the PFS locked. See Figure 11.

The scenario depends on the shape of the bottom of the plate and the pattern in which the wells are visited. If the pattern is a mosaic, most moves are between adjacent wells with a small focus difference. But for the move from the last well back to the first well the focus difference is much larger. For this move refocus will fail and requires strategy 2. When two regions with adjacent wells are selected, the PFS refocus after the movement within each region can succeed so strategy 1 can be used, but the refocus after the move between regions fails and requires strategy 2.

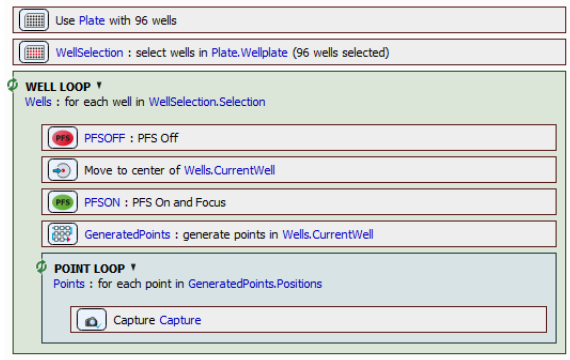


Figure 10: Strategy 1: Switch PFS Off before moving to the next well. Switch PFS On before the point loop.

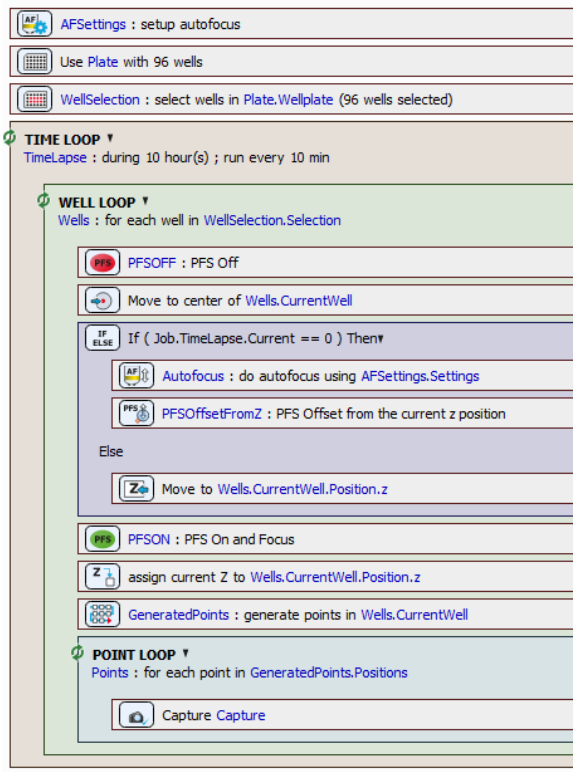


Figure 11: Strategy 2: Switch PFS Off when moving to the next well, restore focus before PFS On.

12.4 PFS off during moves within a well

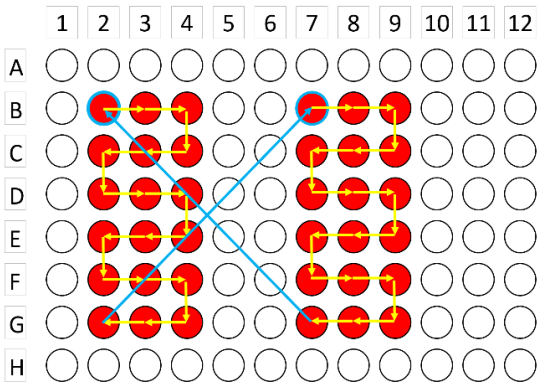
In general, best focusing is realized with the PFS on during movements within the well. The PFS will refocus during each move and no time is lost ‘refocusing’ when arriving at the new position. If the PFS loses track during movements within a well, this might be indication that the IR reflection is not strong enough or is scattered by the scene (high cell confluency or cells with high RI). Usually this also prevents correct refocusing at each position. In such cases the strategy is to identify in each well one location with a clear PFS signal. Before the point loop in the well; move to this location; enable the PFS and wait for focus; disable the PFS and make the point loop with the PFS off.

12.5 PFS Off on long distance moves

Strategy 2 is used when the PFS loses track during long or fast moves and the focus difference between the start and end points of the move is larger than the PFS refocus range. In this scenario, the PFS is switched off during the move to avoid a ‘run-away’. On the new stage position, the focus must be moved to within the PFS refocus range before enabling the PFS. This requires an absolute focus position for these wells. There are several options to implement this strategy:

- During wizard phase, move the stage to all ‘focus control points’; ask the user to focus and store the focus for this well
- During wizard phase, move the stage to all ‘focus control points’; perform an auto-focus and store the focus for this well
- perform the autofocus at ‘focus control points’ in the run phase.

‘Focus Control Points’ can be the end-point of long moves; a point set determined manually by the user or the same point set used to define a region.



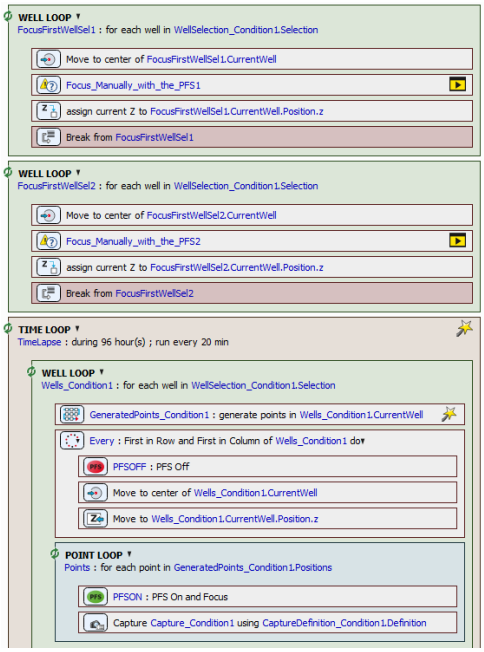


Figure 12: Well loop over two Well Selections and Job fragment that illustrates how to ask the user to focus at every first well; store the focus for that well and restore the focus at that location at every round.

Figure 12 shows a typical path for looping over two well selections. During the short moves (yellow) the PFS can stay on. During the long moves (blue), the PFS might lose track. In that case, the PFS must be switched off during the long moves and enabled again at the first well of each selection (wells labeled blue). If the focus difference is larger than the PFS refocus range, the focus must be moved to the stored position for each well before enabling the PFS.

12.6 Lock to the bottom during moves

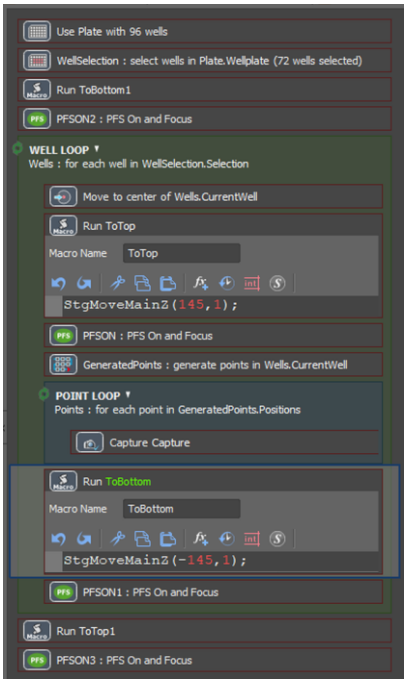
The PFS IR beam is reflected on two surfaces when used with a Water Immersion objective: on the top- and bottom-surface of the glass bottom. The reflection on the bottom surface is stronger than that from the top surface. For the 40x and 60x WI objectives, the PFS must lock the top-surface to allow the optical focus to reach the sample plane.
There are several reason why the PFS might inadvertently jump to locking to the bottom-surface:

- The reflection of the top-surface is compromised due to high confluence of cells with a high RI
- During a stage move, the focus difference between start and end position is larger than the top-surface refocus range

Because the reflection from the bottom-surface is stronger, locking to the bottom-surface is more stable. In addition, the IR reflection on the bottom-surface is not influenced by the cells growing on the top-surface.

Strategy 3 relies on the reliable locking to the bottom during large distance stage moves. Before the point loop, the focus is moved 145 um* up and the PFS is switched on again to lock to lock to the top-surface. Even when locking fails, the optical focus will be correct.

* due to the virtual depth phenomenon, the thickness of a 170 um glass bottom will appear to be 145 um when using a water immersion objective.



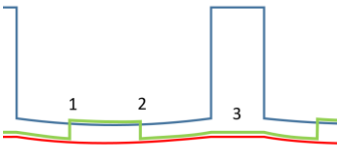


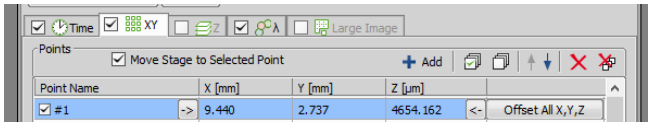
Figure 13: Strategy 3: Locking to the bottom surface during long distance moves. Left; Macro commands implement the relative focus move up (+145 um) and down (-145 um). Top: The surface the PFS locks to (green), the sample holder bottom surface (red) and top surface (blue). Before imaging, the z-drive is moved up to the top surface (1). After imaging, the z-drive is moved back to the bottom surface (2). During long distance moves, the PFS locks to the bottom surface (3).

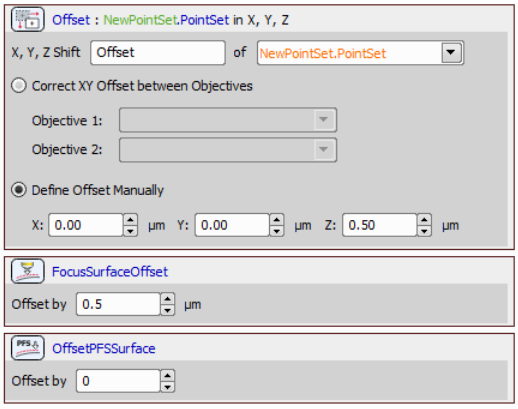
12.7 Combination of strategies

The sample characteristics or timing needs might require a combination of above strategies. For example, when absolute focus positions are stored to bring the focus within the PFS refocus range, thermal focus drift might render them invalid. A strategy to solve this is:

- define a ‘trusted’ sample location where PFS or autofocus works well
- on every round or every ‘N’th tile, move to this ‘trusted’ sample location
- enable the PFS or do an auto-focus
- compute the focus drift by comparing the found focus position with that of previous round
- adjust all stored focus positions for the focus drift.

NIS has some standard functions to adjust the focus position of an absolute point set for the focus drift. Examples are the ND Acquisition XY ‘Offset X,Y,Z’ button and the Jobs tasks ‘Offset Point Set’; Offset Focus Surface and ‘Offset PFS Surface’. The offset parameter of these tasks must be set by a Macro task.





13 (PFS) AutoFocus

Instead of asking the user to focus, an auto-focus can be done using the microscope focus drive or the PFS. The range of the auto-focus must be larger than the expected focus difference. For larger ranges, it is usually faster to do a coarse auto-focus first with the focus drive. If this brings the focus within the PFS refocus range, enabling the PFS next will restore the focus position. Alternatively, a second auto-focus can be done over a smaller range and smaller steps.

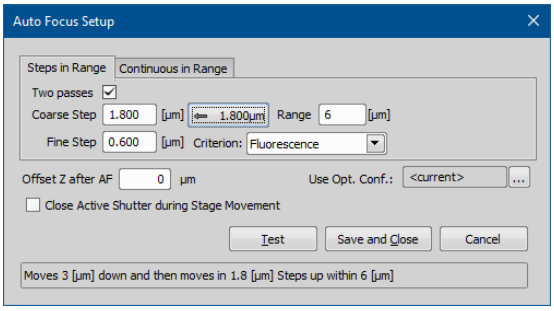


Figure 14: Auto Focus Setup

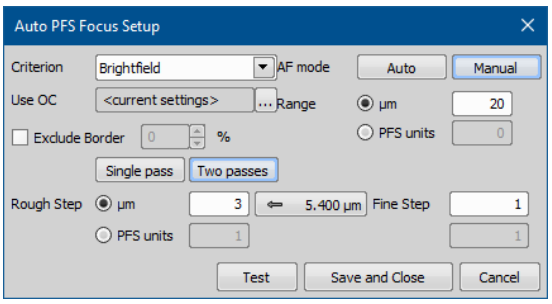


Figure 15: Auto PFS Focus Setup

14 Water Immersion Dispenser (WID)

Long-term multi-point experiments with a water immersion objective require replenishment of the immersion water with the Water Immersion Dispenser. Operation of the WID is described in instruction manual M695.

The WID tubing and nozzle are made from durable material and designed not to wear out during normal operation. The correct operation depends on the exact internal diameter of the tubes and nozzle and exact position of the nozzle tip. If you observe any small anomaly on the tubes or nozzle, these parts should be replaced.

There is one common problem that happens after installing a new nozzle. When brand-new, the nozzle tip is very clean and hydrophilic. This makes that when the nozzle moves side-

ways after replenish, the full drop of water is dragged off the top of the objective. This problem is solved by gently touching the nozzle with your fingers. Touching the nozzle with your fingers will apply sufficient fat to make it hydrophobic.

14.1 Schedule

By default, the replenishment is scheduled at 30 minutes interval. In our experience this is sufficient even at 37 degrees. To make sure there is sufficient water during a round, usually the replenishment is initiated at the beginning of the round at the first well position.

14.2 Type I pure water / air bubbles

The immersion water is part of the optical light-path. For high quality imaging and correct operation of the PFS, it is crucial to use Type I pure water. When a different grade of water is used, the PFS will stop to work correctly after a few rounds. The well/point loop will smear water over the bottom of the plate/dish. During the waiting time this water will evaporate, leaving a trace of residue of impurities on the plate/dish bottom. This residue is an anchor point for air bubbles. The air bubbles will obscure a part of the field-of-view and interfere with correct PFS operation.

When the supply tube is not air-tight, the supply water will also introduce air-bubbles in the water. When running the ‘replenish’ sequence, visually check that the supply tube does not have any air bubbles. When bubbles are seen, unmount the tube; cut a small part of the end and remount it. When that does not resolve the problem, the tube must be replaced.

14.3 WID Spare Parts

The following spare parts are available for the Water Immersion Dispenser:

2K170-985-1	Supply Silicon Tube
2K170-986	Efflux Silicon Tube
2K170-991	Bottle
MEV54006-A002	Nozzle assembly

15 Leveling

Leveling is a quick action that improves the focus during multi-point experiments. The PFS can be used to speed up the procedure. The instructions for leveling are:

- release all pulling and pushing screws of the insert until the insert lays flat on the stage
- reset the PFS Offset lens
- on the RCP (Joystick), select the Relative Position page (Rel Pos).
- visit each corner of the sample holder, enable the PFS* and identify the highest corner
- in the highest corner, press the RCP ‘Reset Z’ button

- visit each corner of the sample holder, enable the PFS, screw the push screw until the relative z-position is around 0 and slightly tighten the pull screw.
- revisit the highest corner, enable the PFS, slightly tighten the pull and push screw and reset the z-position again.
- visit each corner of the sample holder, enable the PFS and slightly tighten either the pull screw or the pull screw to make the relative z-position 0
- repeat the previous step until all screws are sufficiently tight.

* when using a water immersion objective, make sure to lock to the correct surface.

Colofon

Date: December 1, 2023
Authors: European Support Team <instruments-support.eu@nikon.com
Volodymyr Nechyporuk-Zloy, Isabelle Delias, Frank van den Boom, Kees van der Oord
Applies to: Nikon Ti2 inverted microscope with Perfect Focus System 4 and optional motorized Water Immersion Dispenser
Version: 1.0
File: https://nikonglobaleu.sharepoint.com/sites/Teams_0003701/Shared Documents/General/HowTos/Published/Nikon Practical Tips 1 - Ti2 PFS WID - 20231115.docx
Date: 01-12-2023 12: