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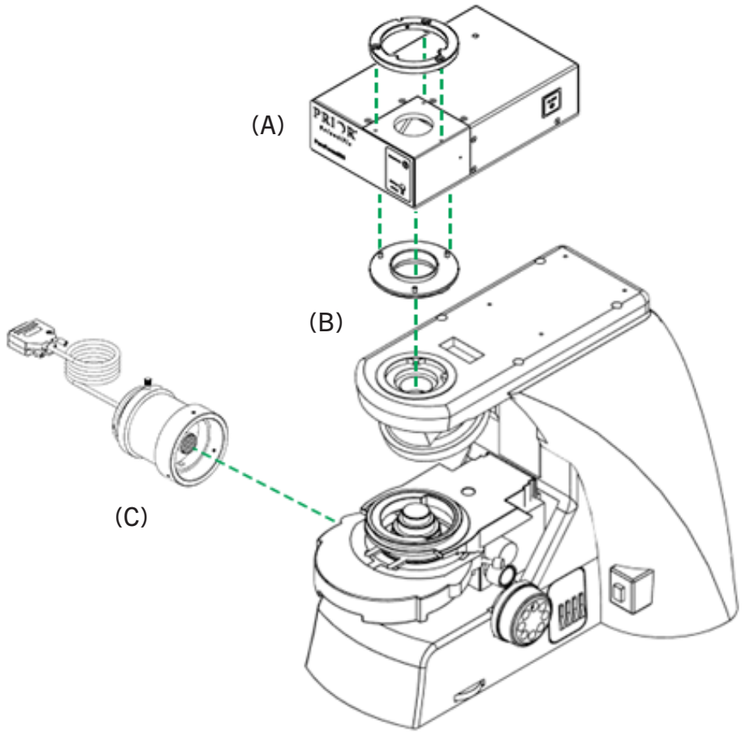
PureFocus850 |
Quick Start Guide

Laser Autofocus System



1. Download the latest software package from www.prior.com/download-category/operating-manuals.
2. Copy the folder onto the PC desktop and unzip the file.
3. Install the flange set onto the PureFocus850 head as shown (A).

For inverted microscopes, you can find full installation instructions at www.prior.com/download-category/help-sheets-installation-instructions.

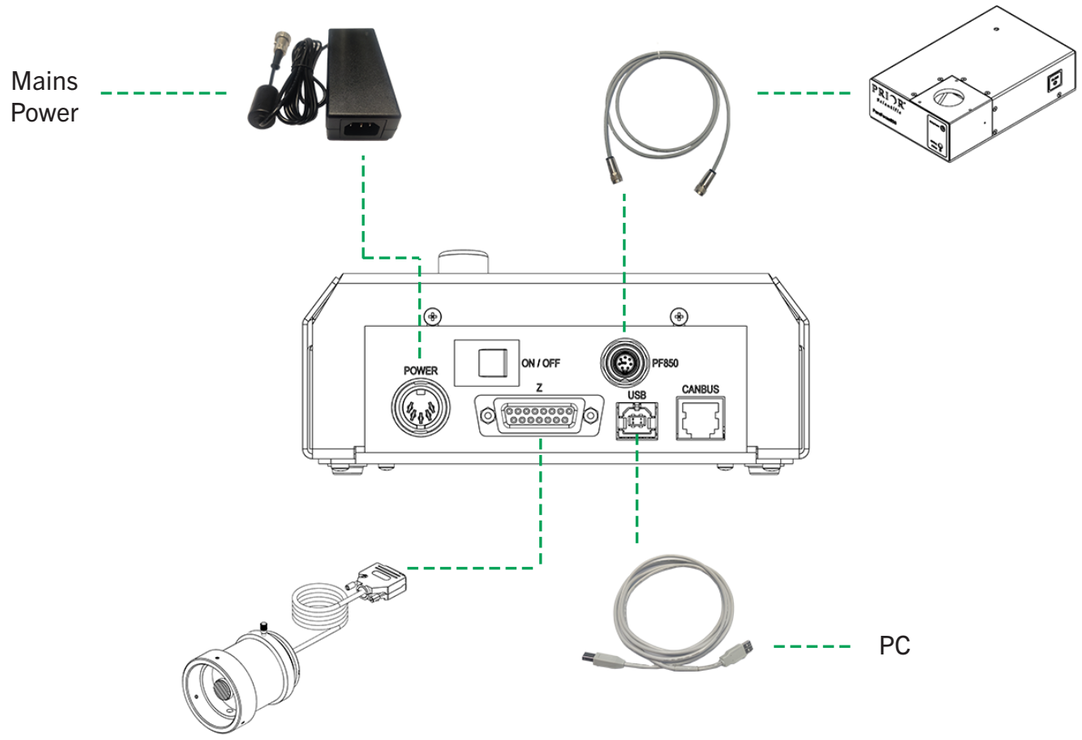
4. Install the PureFocus850 head onto the microscope (B).

The PureFocus850 can be installed above or below the epi-illuminator, depending on the microscope.

Prior recommends that the PureFocus850 is installed above the epi-illuminator on reflected darkfield microscopes, except when an analyzer is installed.

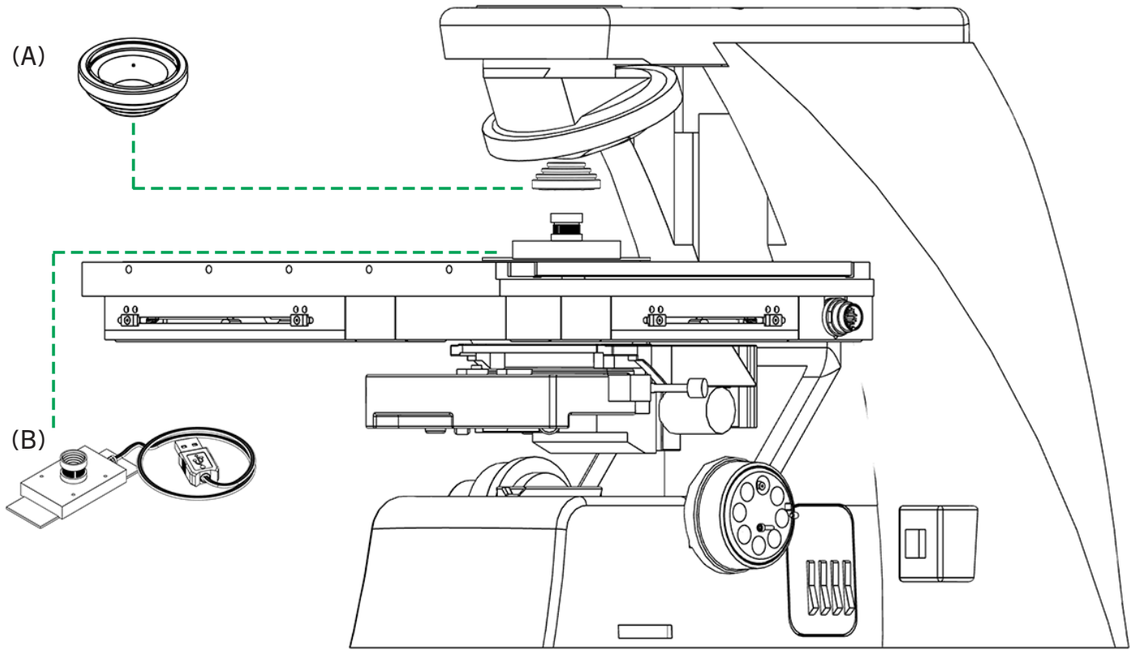
The PureFocus850 must always be installed below the epi-illuminator if an analyzer is installed.

5. Install the PS3H122R focus drive on the left-hand focus knob (C). See full installation instructions for the focus drive at www.prior.com/download-category/help-sheets-installation-instructions.



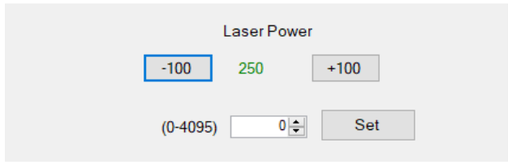
1. Connect the PureFocus850 controller to the PC, mains power, PS3H122R and PureFocus850 head.

If using a piezo device, connect the PF404 cable provided to the analog input of the piezo device's controller.

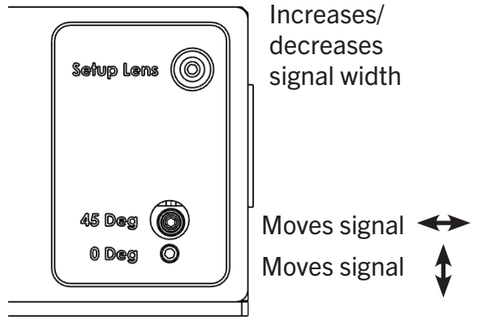


1. Install all the objectives you intend to use with the PureFocus850 onto the microscope, leaving two positions free.
2. Install the PF200/PF201 target (A).
3. Install a dustcap into the remaining position.
4. Place the PF300 onto the stage (B). The orientation of the camera does not matter.
5. Plug the PF300 into the PC, using the extension cable provided if necessary.

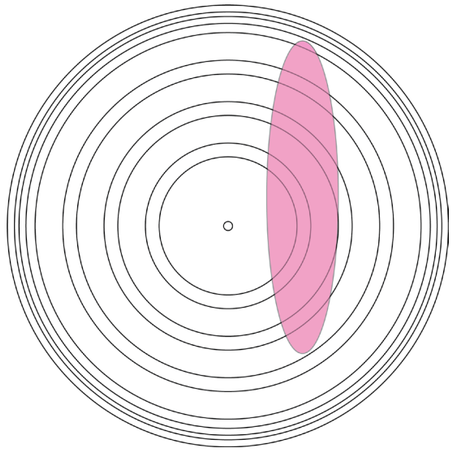
(A)



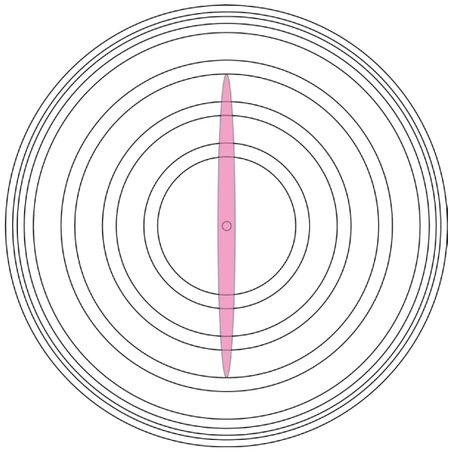
(C)



(B)



(D)



1. Switch on the PureFocus850 controller. In the software download, open the folder labelled ‘gui’.
2. Open camera.exe. Select ‘USB 2.0 camera’ from the dropdown menu in camera.exe. Focus on the target using the microscope focus and/or by rotating the camera lens.
3. Open PureFocus850.exe and select the correct COM port number. In PureFocus850.exe click ‘Laser’, then increase the laser power (A) until a clear pink signal appears on the target (B). The orientation of the signal does not matter.

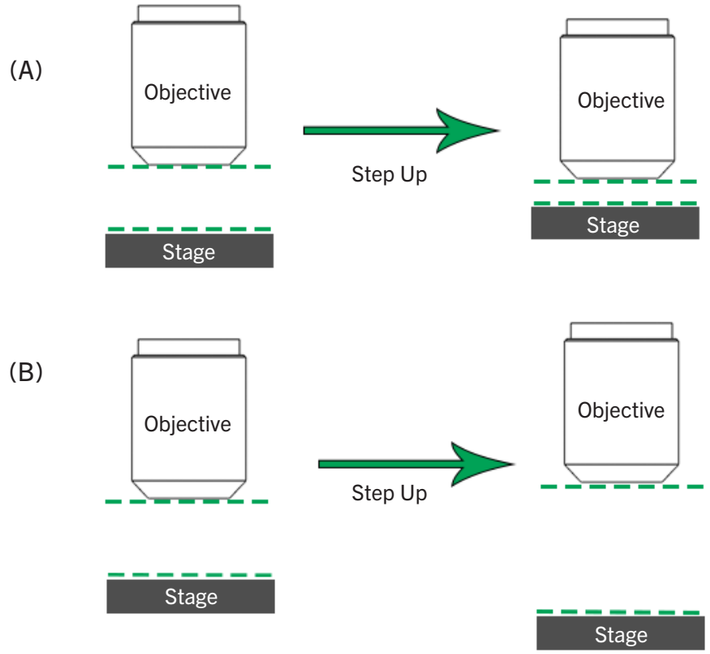
The PureFocus850 COM port will appear under ‘Ports (COM & LPT)’ in Device Manager as ‘USB Serial Port’.

4. Adjust the setup screw, 45 Deg screw and 0 Deg screw (C) until the signal is aligned with the target as per (D).

If the pink signal is too long or too short to complete step 4, click ‘offset’ in the digipot function area of the user interface. The controller will display MAN OFF. Use the digipot on the controller to adjust the length of the pink signal.

If reinstalling a PureFocus850, rotate the blade screw (see page 8) clockwise until resistance is felt.

5. Close camera.exe and remove the PF300 and PF200/PF201 from the microscope.



1. In PureFocus850.exe:
   a. Click ‘Mode’ and select the slice (PF850) or line (PF850M).
   b. Click ‘Mode’ and check the microns per revolution setting is set to 100.

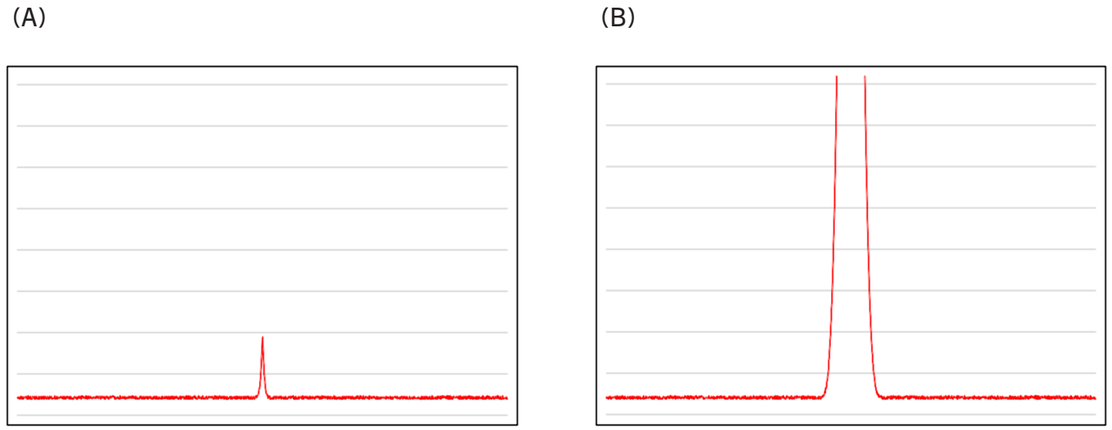
If using a piezo drive, instead of step 1b click ‘Mode’ > ‘Piezo’ and set the piezo travel to match the range specified by the manufacturer.

If using a stepper drive that is **not** a PS3H122R, do not attempt step 3 and consult the full PureFocus850 manual. A different value is required during step 1b. Failure to determine the correct value will cause damage to the microscope objective and/or sample during step 3.

   c. Enter 1000 in the step size box.
2. Bring the 10X objective, or alternatively your longest working distance objective, into the light path. Move the objective far away from the stage.
3. Click ‘Step Up’ and observe if the objective gets closer to the stage (A). If you cannot see any motion, increase the step size value in increments of 1000 until movement can easily be observed.

If during step 3 you observe the objective and stage getting further apart, instead of performing step 4 click ‘PID’ > ‘Negative’.

4. Once satisfied the objective and stage are getting closer together, under ‘Mode’ click ‘Focus Direction Inverted’.
5. Click ‘Step Up’. The objective and stage should now move further apart (B).



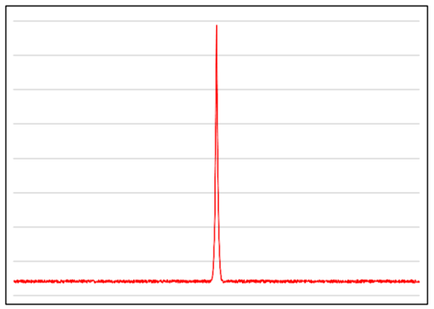
1. Place the PF209 test sample on the microscope stage.
2. Check the controller display reads MAN FOC mode.
3. Using the 10X objective (or next lowest magnification objective greater than 10x), bring the mirror sample into focus using the PureFocus850 controller controller.
4. Set the laser power to 500.
5. In PureFocus850.exe click on ‘Setup’.
6. Adjust focus using the PureFocus850 controller until a sharp peak is present on the graph (A).

Additionally, for line mode (PF850M) units:

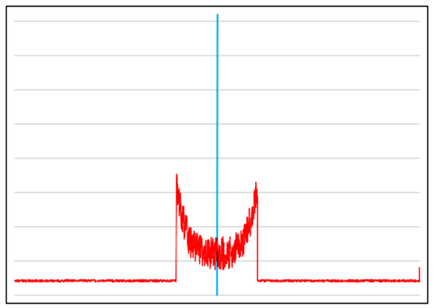
7. Adjust the 45 Deg screw to maximize the height of the peak. Adjust the screw as much as necessary but not more than 20% of a turn. The peak may oversaturate the sensor (B).
8. Switch to your highest magnification objective. Verify that a peak can still be observed. Click ‘Laser’ and increase the laser power or integration time if necessary. Adjusting focus slightly may also be required to observe the peak.

If during step 8 you cannot observe a peak or it is very small at maximum laser power and integration time, adjust the 45 Deg slightly (5-10% of a turn) to maximize the size of the peak. If a peak can still not be observed, contact Prior.

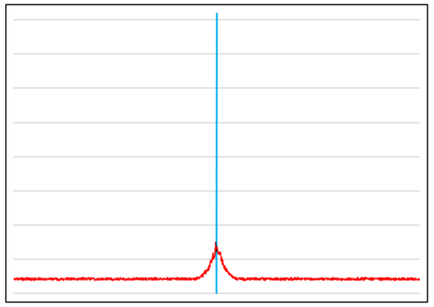
(A)



(B1)

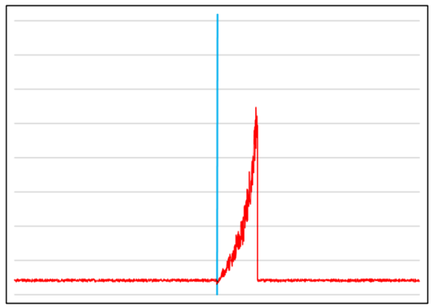


(B2)

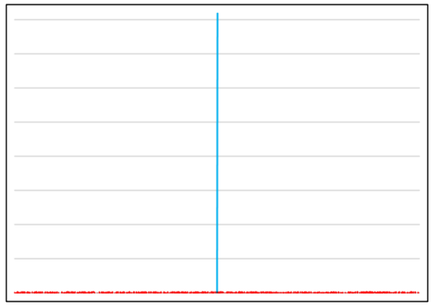


(C)

(D)



(E)

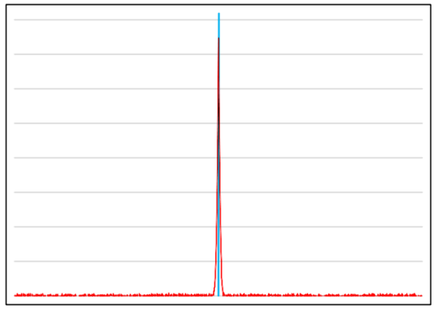


1. Switch back to your 10X (or alternative) objective. Reduce the laser power such that the top of the peak is within the Y axis of the graph (A).
2. Click ‘Auto-set Centre’. A blue pinhole centre line will appear on the graph.
3. Adjust focus using the PureFocus850 controller until the peak spreads out (B1). If the peak gets smaller without spreading (B2), adjust focus in the opposite direction.
4. Click ‘Laser’ and increase the laser power so that the signal is clear. Turn the blade screw on the side of the PureFocus850 head. (C) anticlockwise until most of the signal on the left of the graph can no longer be seen (D).

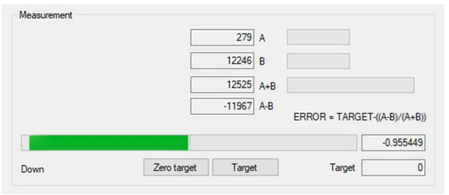
Proceed to step 5 even if the spread out signal does not have two peaks as shown in B1.

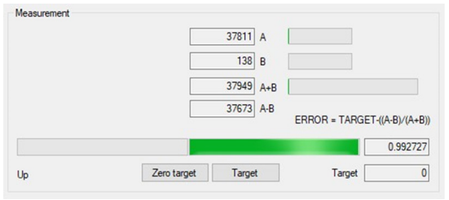
5. Bring the dust cap into the light path.
6. Click ‘Measure Average Background and Set’. The background signal will drop to the bottom of the graph (E). Remove the dust cap from the microscope. Fit any additional objectives into the free positions left by removing the dust cap and PF200/PF201.

(A)



(B)





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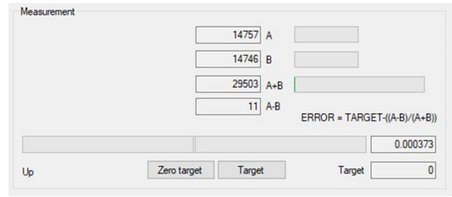
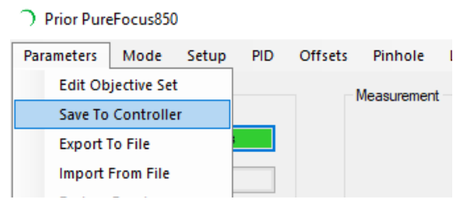


(D)



1. Switch back to your 10X (or alternative) objective.
2. Adjust focus using the PureFocus850 controller until the sharp peak is present on the graph at the pinhole centre (A).
3. Close the setup graph.
4. Adjust focus in both directions and verify that the error signal swings from +0.9 to -0.9 (B).
5. Adjust focus until the error value is approximately +0.3.

Ensure the setup graph is closed before attempting step 6.

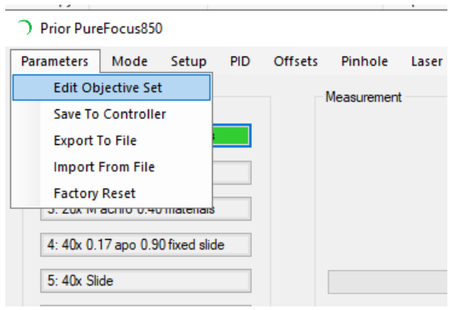
6. Click ‘Servo On’. The error value should decrease to and be held at approximately zero (C).

The focus movement during step 6 should be short and controlled. Be prepared to click ‘Servo Off’ if focus oscillates or the objective may crash into your sample. If focus oscillates, proceed to the next page.

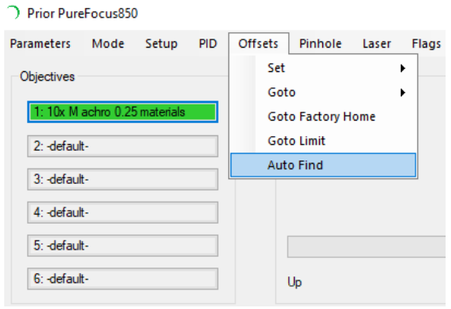
If the objective drives towards, or away from, the sample, verify the settings on page 6 of this document, then consult the full PureFocus850 manual.

7. Click ‘Servo Off’.
8. Click ‘Parameters’ > ‘Save to controller’ (D).

(A)



(C)



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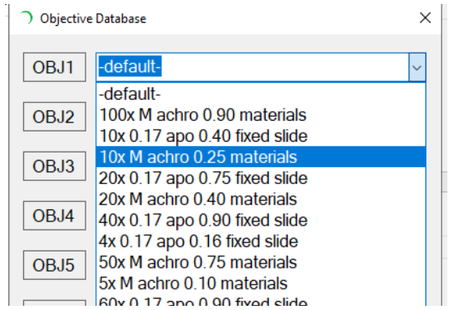


(D)

1. Load a sample onto the stage. This can be the PF209 or a sample of your choice. The sample used should resemble the type of sample that will be used with the microscope.

2. Click ‘Parameters’ > ‘Edit Objective Set’ (A).

3. Select up to six objective presets that most closely match your objectives (B).

4. Edit the names of the objectives to match the specifications of your objectives and click ‘Create Set’.

5. Click on the first objective in your set so that it is highlighted green (C) and ensure that objective is in the light path of the microscope.

6. Ensure the controller display reads MAN FOC. Bring the sample into focus using the PureFocus850 controller.

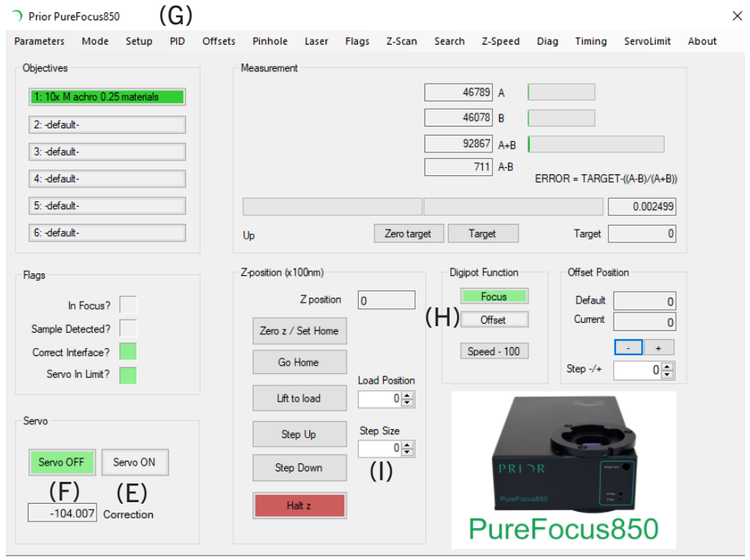
Ensure the setup graph is closed before attempting step 7. The setup graph must not be open when clicking ‘Servo On’.

7. Click ‘Offsets’ > ‘Autofind’ (C). The recommended speed value is 20%. A message will appear when an offset is found (D).

If during step 7 an offset is not found, increase laser power by 50% and reduce the speed value to 5% and repeat. If an offset is still not found, refer to the full PureFocus850 manual or contact Prior.

The offset value is dependent on sample type and objective type. Offsets greater than 10,000 are not typical for non-coverslipped samples. Review the full PureFocus850 manual for guidance.

(G)



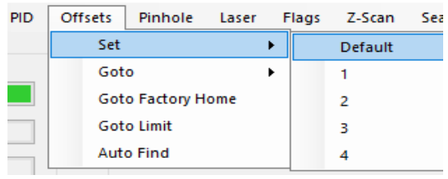
(H)

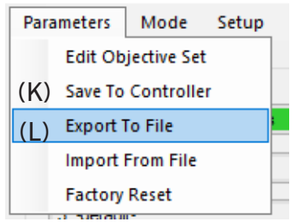
(I)

(F)

(E)

(J)





(K)

(L)

8. Click ‘Servo On’ (E). If focus oscillates, click ‘Servo Off’ (F), click ‘PID’ (G), and reduce P gain by a factor of 2. Check focus stability in different areas of the sample. Repeat until focus is stable with the servo on.

If using a piezo device, during step 8 reduce P gain and I gain by a factor of 2 if focus oscillates. Repeat until focus is stable.

9. Check that the sample is in focus. If it is not, click ‘Offset’ (H). When the display reads SRV OFF, adjust the PureFocus850 controller digipot until the sample is in focus. When satisfied the sample is in focus, click ‘Servo Off’, then ‘Focus’ under digipot function.

10. Step Up 500 units (I). Click ‘Servo On’ to confirm that focus recovers quickly and stably.

11. Click ‘Offsets’ > ‘Set’ > ‘Default’ (J).

12. Click ‘Parameters’ > ‘Save to Controller’ (K).

13. Click on the next objective in your set. Repeat steps 6 to 12 for all objectives.

14. Once all objectives have been set up, click ‘Parameters’ > ‘Save to Controller’.

15. Click ‘Parameters’ > ‘Export to File’ to save your objective set to the PC (L). Multiple objective sets can be created and recalled for different samples and imaging modalities, or if objectives are routinely exchanged on the microscope.

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