

Single Position Time Series with Definite Focus on the LSM780

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Definite focus requires the sample to be in media of a lower refractive index than glass. This is because the definite focus reflects infrared light off the interface between the cover slip and the media. Because of Snell's law, the reflection only occurs when light is going from high refractive index to low refractive index. This is the same principle as TIRF [Total Internal Reflection Fluorescence].

This is for live cell use. Definite focus will not work with mounting media.

Definite Focus works with the following objectives:

**10x Dry
20x Dry
40x Water
40x Oil
63x Oil**

IMPORTANT INFORMATION TO AVOID FOCUS DRIFT

In order to stabilize the focus one must:

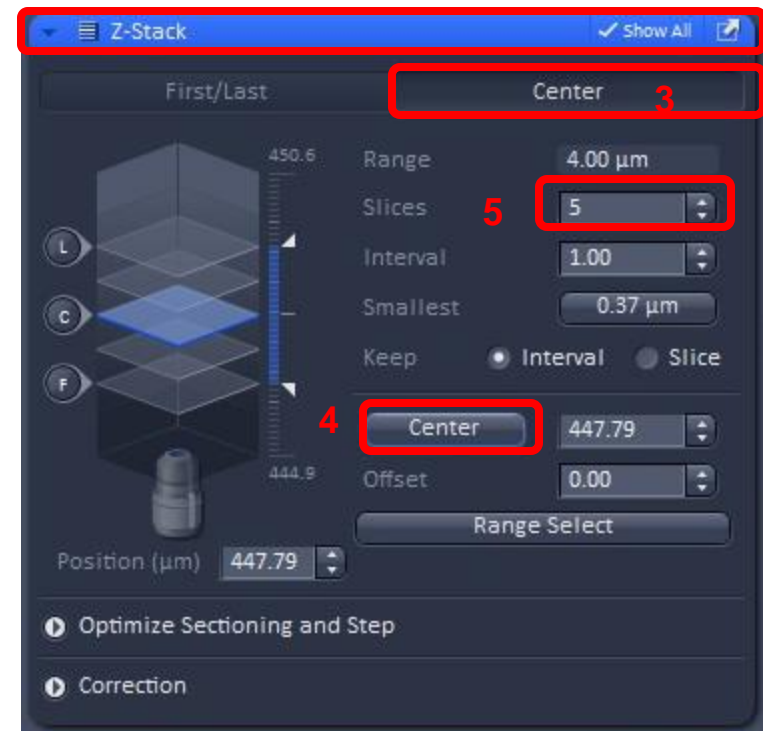
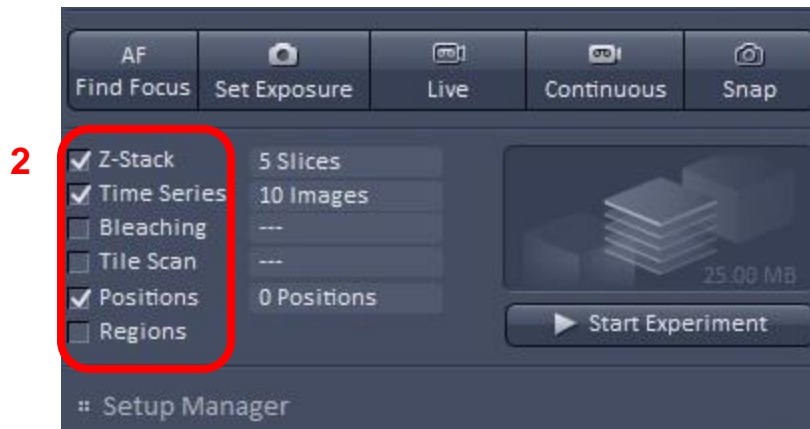
- 1. Have whole microscope on for 2 hours before starting any experiment.**
- 2. The sample should sit on the microscope for 15 minutes before you start imaging.**

If you are going to do a live cell experiment in the morning then it would be best to keep the microscope on from the day before.

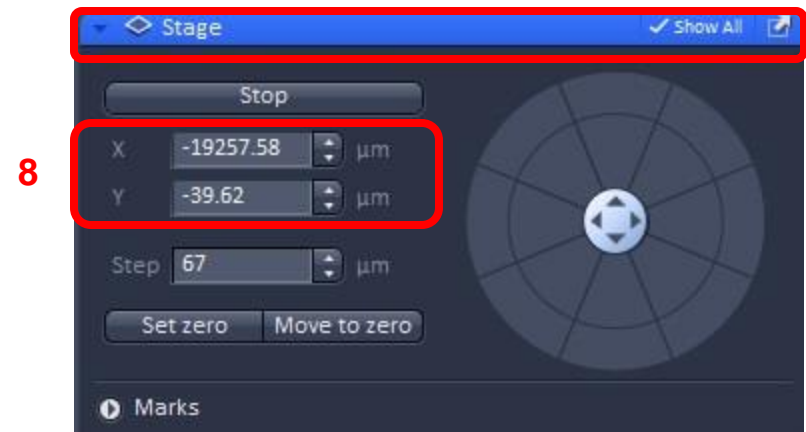
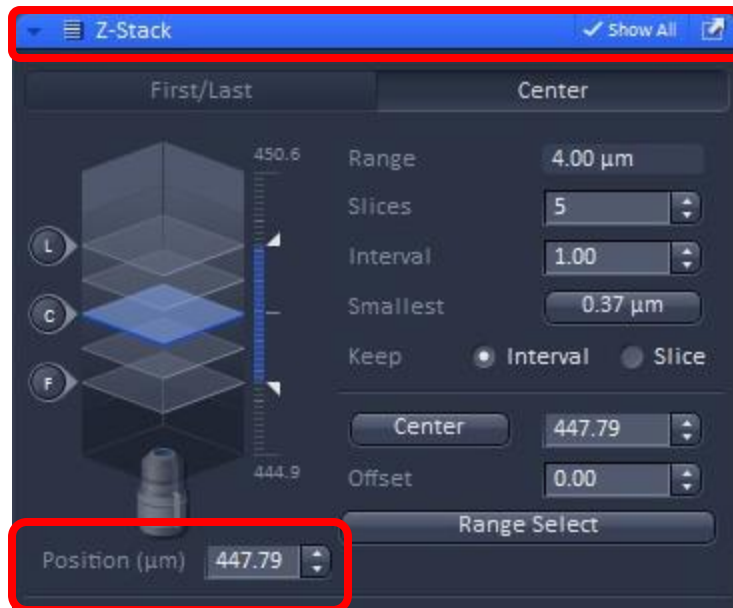
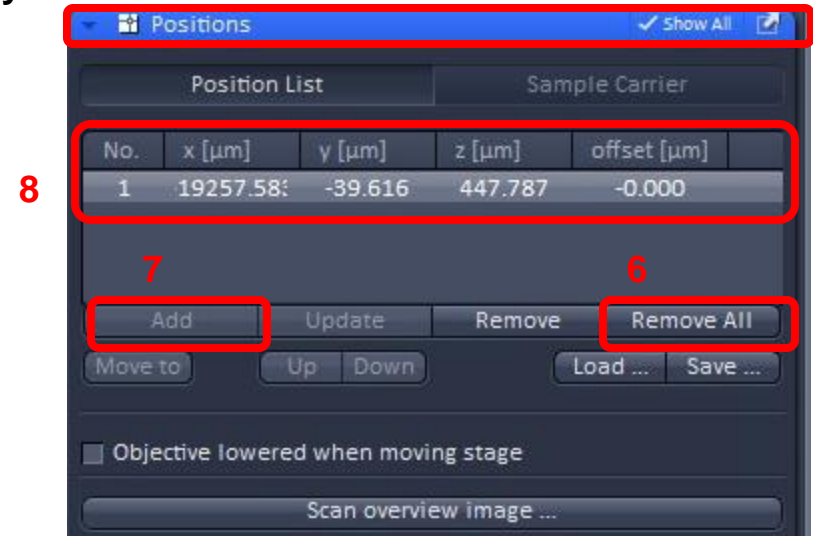
The following items that are critical to turn off in this scenario:

- 1. X-Cite Lamp power switch.**
- 2. Lasers turned off via software.**
- 3. Argon Laser toggled and key turned off.**
- 4. Computer turned off.**

1. Set up your sample as you normally would for imaging.
2. Check Z Stack, Time Series and Postions Boxes.
3. In the Z stack box, set the Z stack by selecting Center.
4. Click Center.
5. Select the number of slices you want. It must be an odd number.



6. In Positions Box check if any positions are listed, if so click the Remove All button.
7. Click Add to add your current position.
8. Double check x, y and z numbers in Positions window with x, y and z numbers in the Stage [x and y] and Z-Stack [z] windows. They should match.

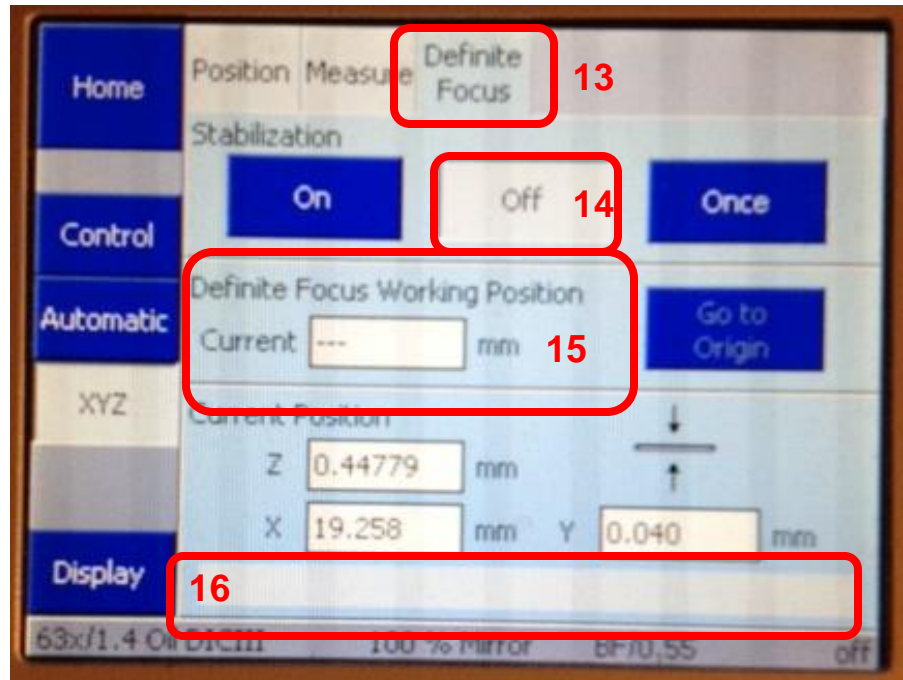
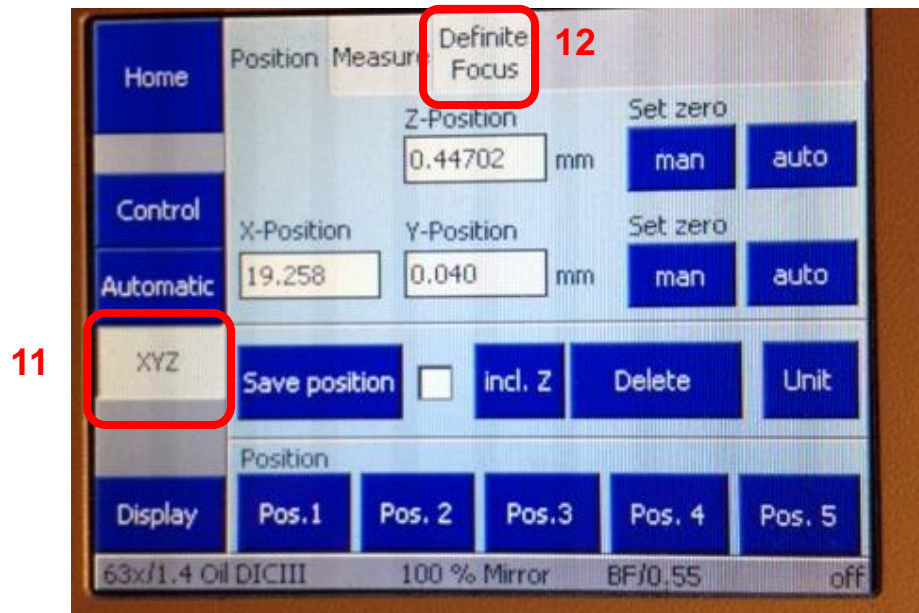




On the touch pad:

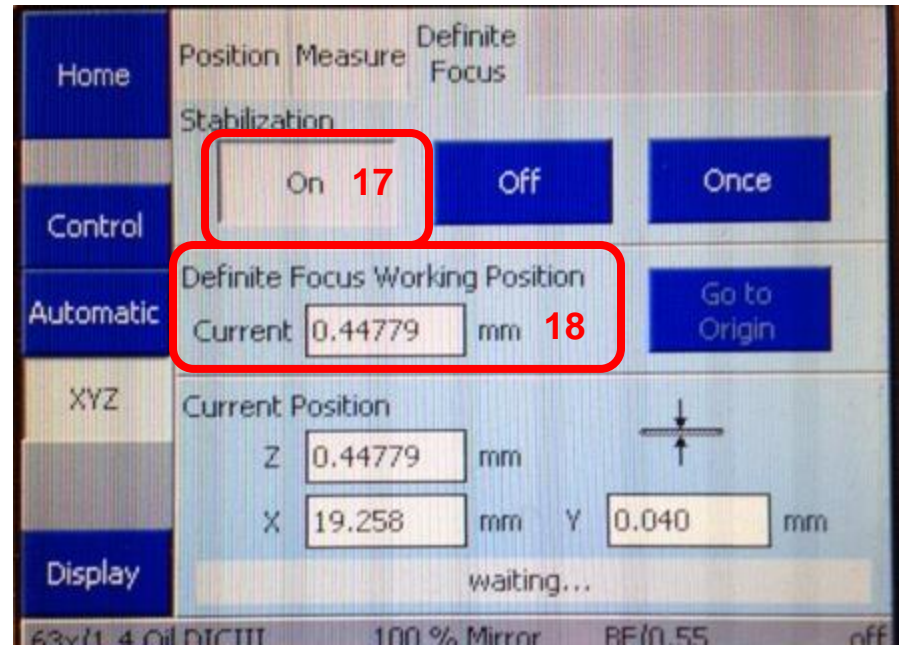
9. Select the Home screen.
10. Select Microscope.
11. Select XYZ.
12. Select Definite Focus.
13. This is what the Definite Focus Screen looks like.
14. The current position is off. Lighter Color.
15. You will also note that under Definite Focus Working Position in the box next to Current you see no numbers. Just -----.
16. You will also see this box is blank.

NOTE: If you do not see the Definite Focus tab that indicates the Definite Focus Power Supply is turned off. Please see the appendix for instructions on how to troubleshoot this.

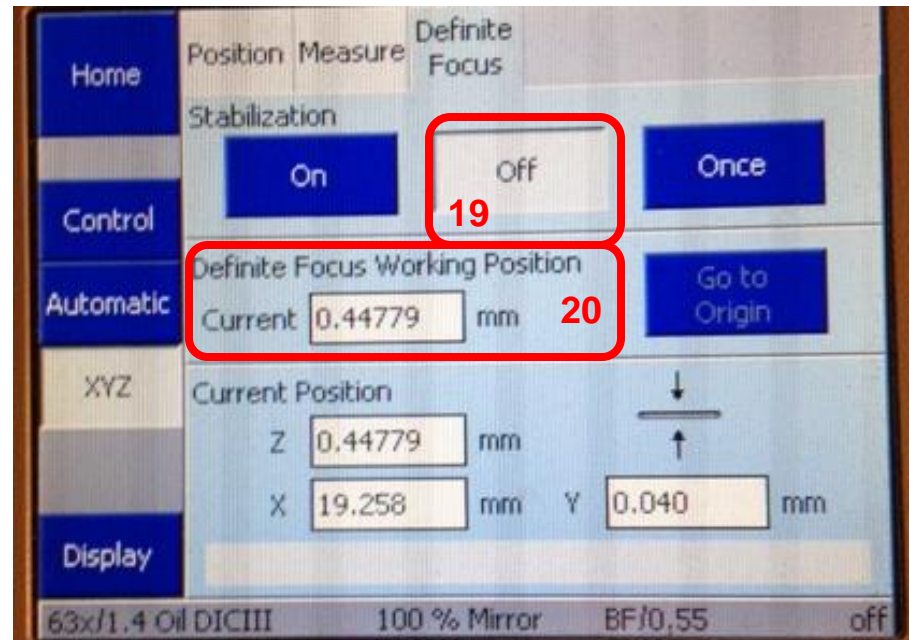
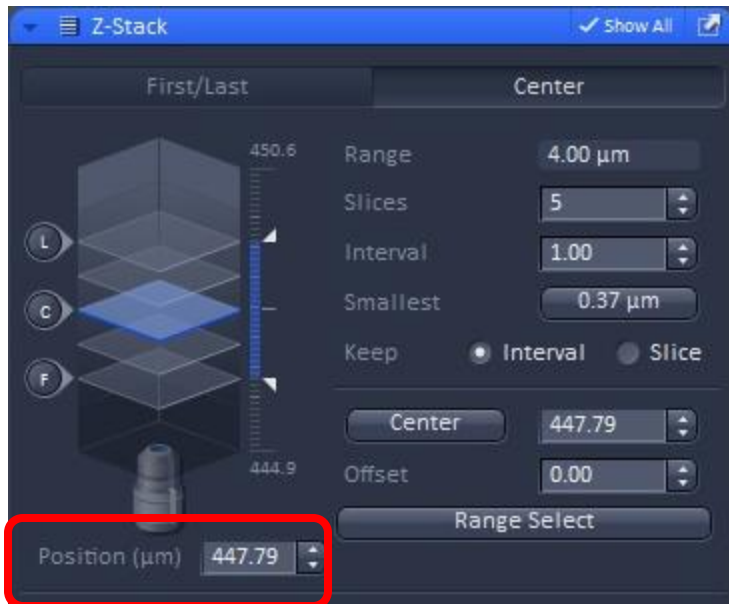


You will now need to set or “train” the autofocus:

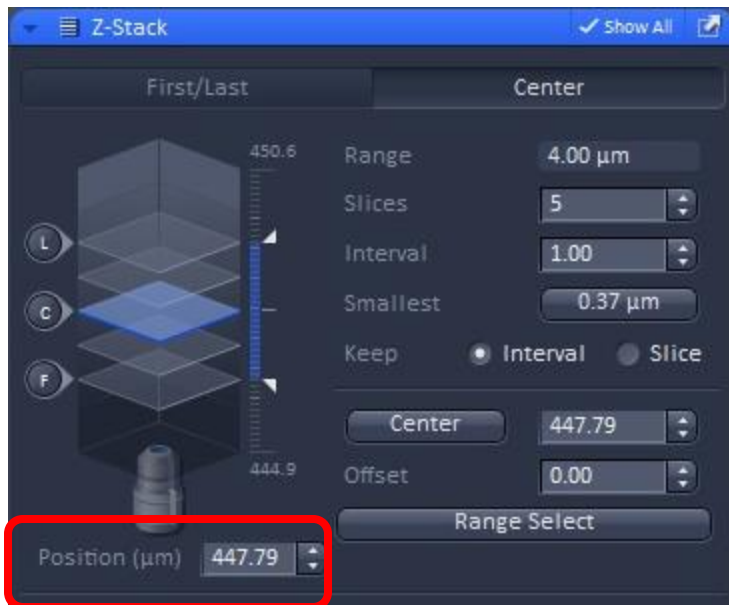
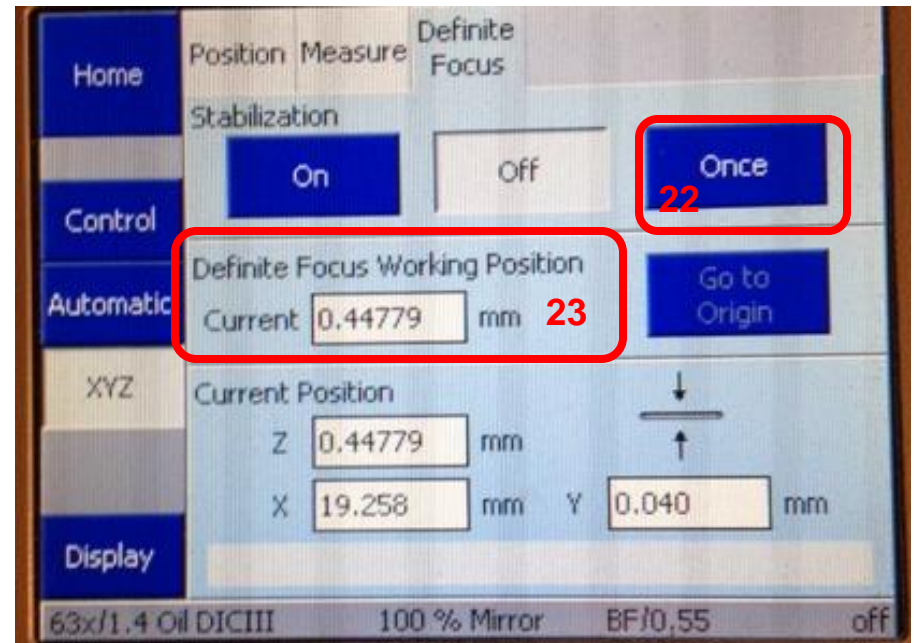
17. Click On.
18. Wait until you see numbers populate the Current Box under Definite Focus Working position.
19. Click Off.
20. You can double check the numbers in the Definite Focus Working Position with the Z Position in the Z-Stack window to make sure they are the same. Note mm vs μm .



The autofocus is now trained.

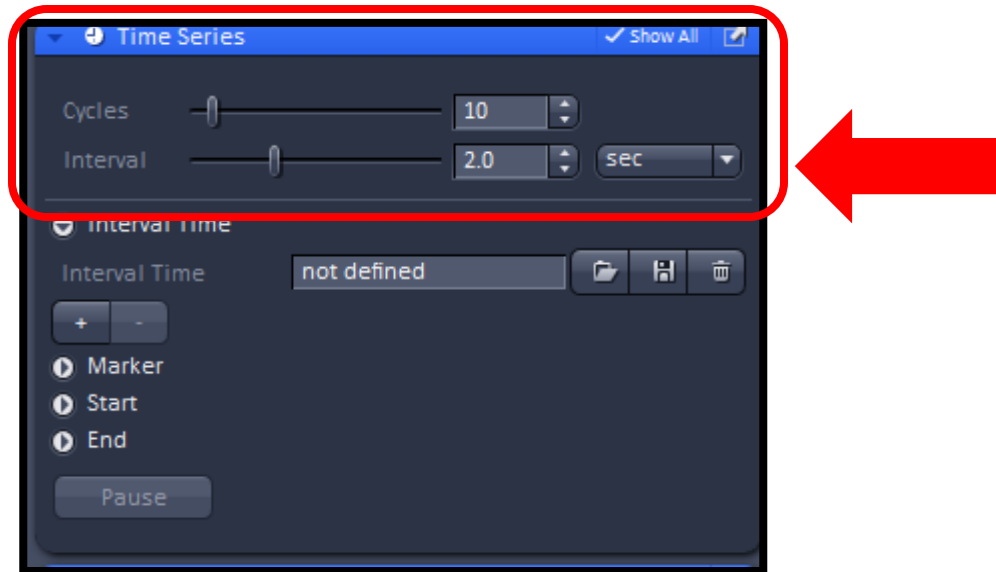


- If you want to test the Definite Focus:
21. Move slightly out of focus.
 22. Click Once.
 23. See that focus returns to proper position. If not repeat training of definite focus.

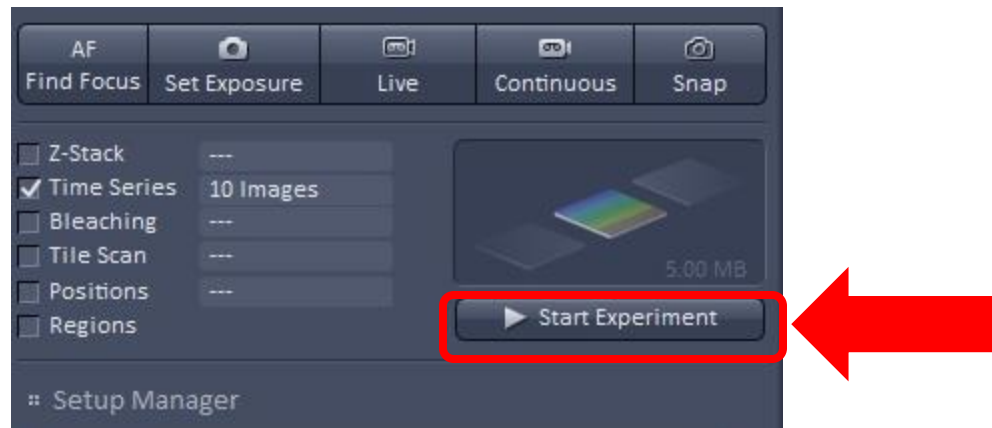


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24. In the “Time Series” box: Select the number of images you wish to take in “Cycles” and the time between each cycle in “Interval.” You can change the increment of interval in the pull down menu [red arrow]. Choices are msec, sec and min.

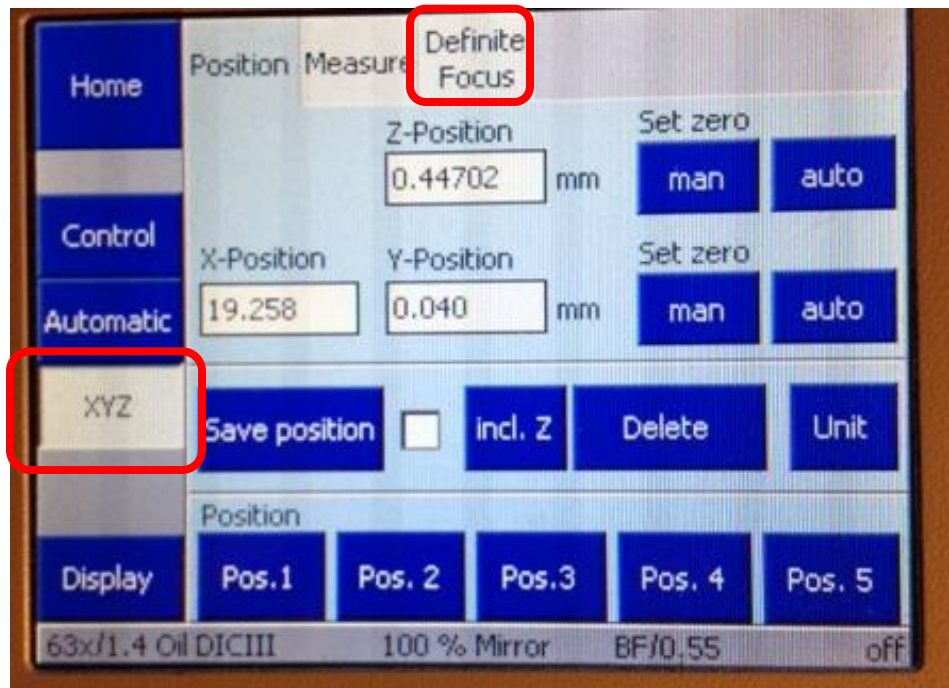


25. Click “Start Experiment” to begin imaging.



Troubleshooting Definite Focus:

If you go to the XYZ tab and do not see the Definite Focus tab then the Definite Focus Power Supply is turned off.



The power supply for the definite focus is located under the microscope table. From the front of the microscope table you can only see the side of the power supply but it is labeled with blue tape. The power switch is around the right side of the table and is seen in the right hand photo.

1. Turn on definite focus power supply.
2. Exit software.
3. Turn off computer.
4. Turn off microscope.
5. Restart microscope and computer.

