

Multi-Position Time Series on the LSM710

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IMPORTANT INFORMATION TO AVOID FOCUS DRIFT

In order to stabilize the focus one must:

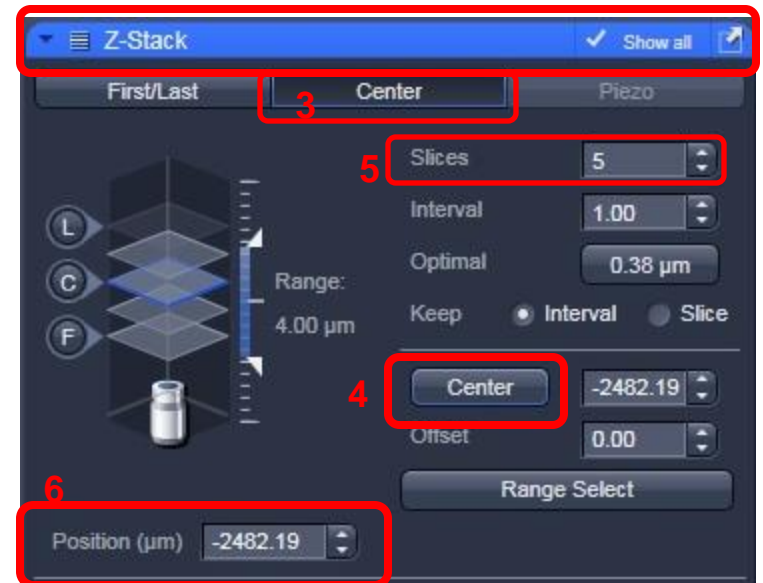
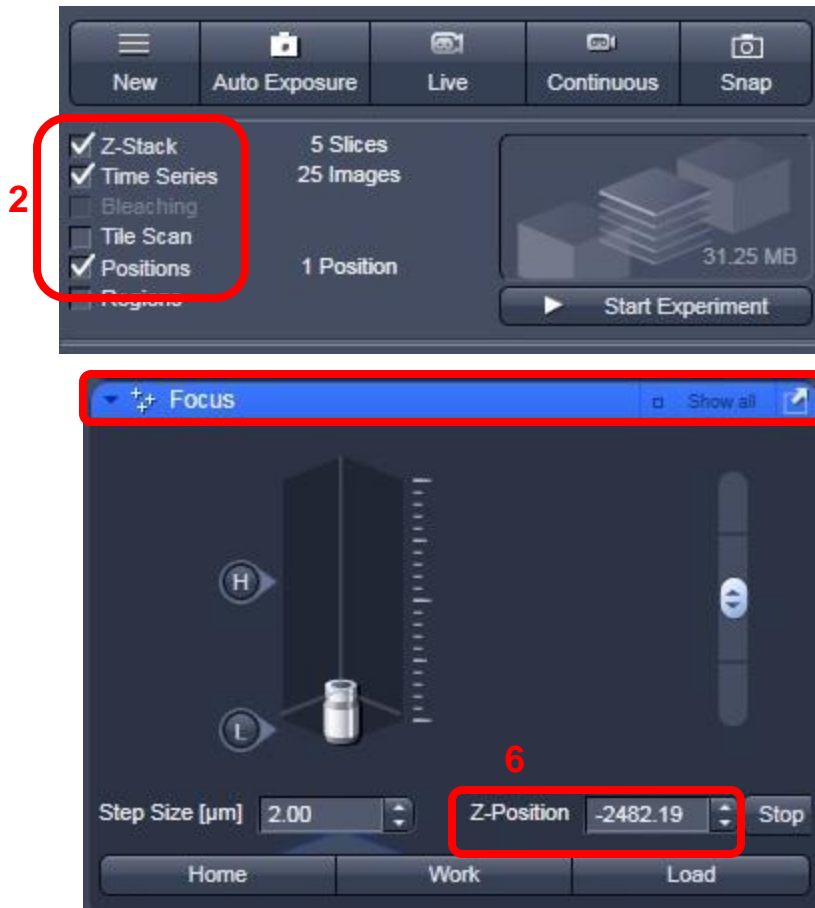
- 1. Have whole microscope on for 2 hours before starting any experiment.**
- 2. The sample should sit on the microscope for 15 minutes before you start imaging.**

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.

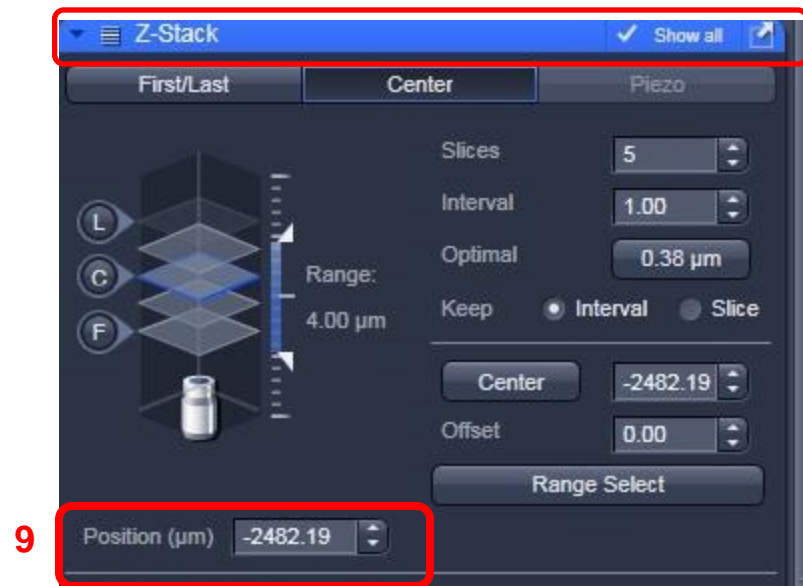
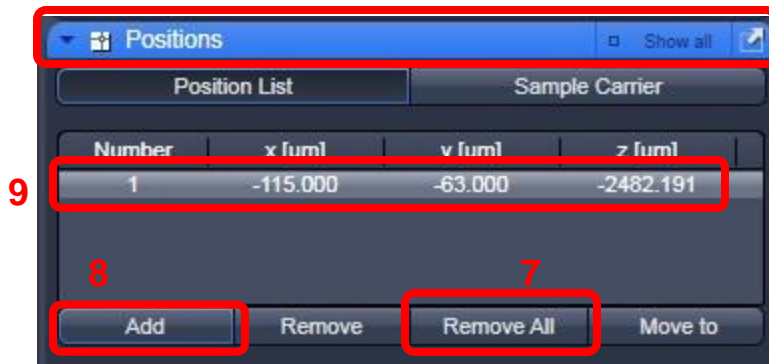
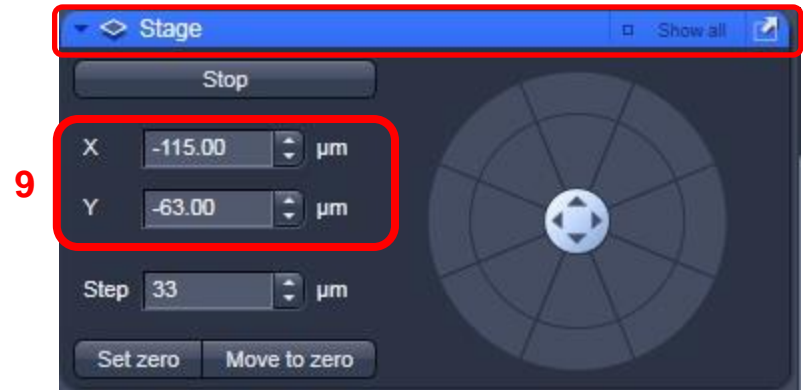
The following items that are critical to turn off in this scenario:

- 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 Positions Boxes.
3. In the Z-Stack box set the Z stack by selecting Center.
4. Click the Center button.
5. Select the number of slices you want. It must be an odd number.
6. Double Check the Z position in the Z-Stack and Focus Box to make sure they match.



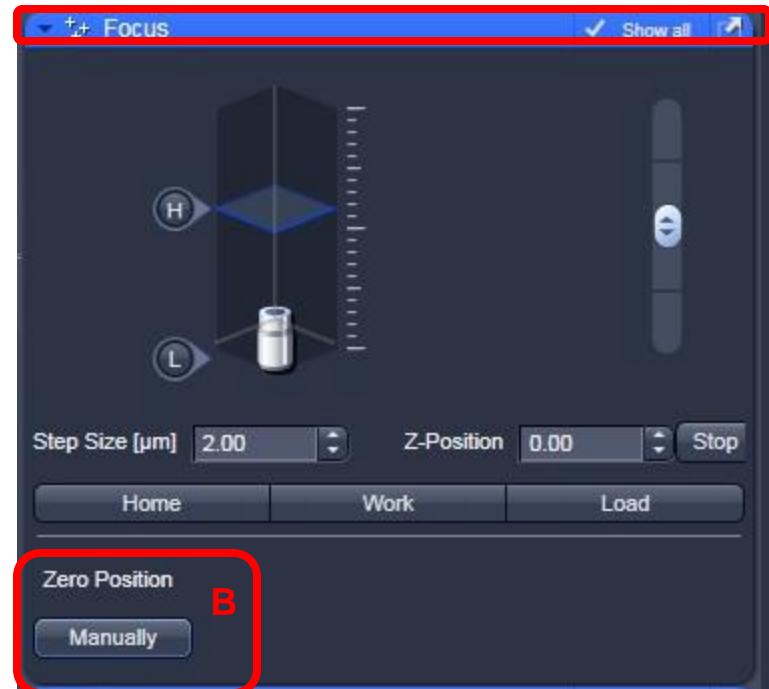
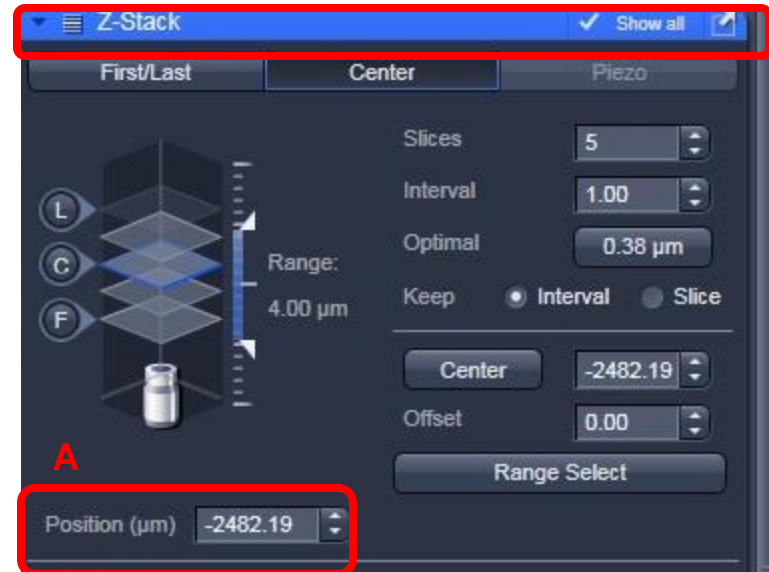
7. In the Positions window if there are any positions listed click Remove All.
8. Click Add to add your current position.
9. Double check x, y and z numbers in Positions window with x, y and z numbers in the Stage [x and y] and Z-Stack [z] windows. They should match.



10. Before adding other positions:
- A. In the Z-Stack window check the number in the Position box.
 - B. If it is a large number like you see here, in the Focus window under Zero Position click the Manually button.

This enables the Z offset to function properly when you add positions.

Z-Stack is ONLY set at Position 1.



11. Move to next position.
12. Focus the sample and get your settings.
13. **DO NOT do set the Z stack.**
14. In Positions box click Add.
15. Double check the X, Y and Z positions in the positions window matches the X,Y position in the Stage window and Z position in the Z-Stack window.
16. Repeat Steps 11-15 for all other positions.
17. When finished with all positions move through each one again to check that positions are correct. Highlight the position you wish to move to then click Move To in order to move to that position.

Number	x [µm]	y [µm]	z [µm]
1	115.000	63.000	0.000
2	258.750	-436.250	1.438
3	-966.750	2306.750	-6.096

When you have set and double checked all of your positions:

18. Move back to Position 1 by highlighting the position.
19. Click Move To.
20. Check Stage and Z-Stack windows to ensure you are on Position 1.

Important Reminders Before You Continue:

1. Z Stack is ONLY set for Position 1. Do not click anything in the Z-Stack window [Steps 3-5] for Position 2 and onward.
2. You MUST be in Position 1 when you perform ALL the steps that follow.

Number	x [µm]	y [µm]	z [µm]
1	-115.000	-63.000	0.000
2	258.750	-436.250	1.438
3	-966.750	2306.750	-6.096

X: -115.25 µm
Y: -62.75 µm
Step: 33 µm

Slices: 5
Interval: 1.00
Optimal: 0.38 µm
Keep: Interval (selected)
Center: 0.00
Offset: 0.00
Range Select
Position (µm): 0.00

21. 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.



22. Click “Start Experiment” to begin imaging.



Looking at the results:

23. Click Dimensions Tab.
24. In Gallery Tab Click P for Position.
25. Back in Dimensions tab you can use the slider to move through
 - A. Z Position.
 - B. Time.

