



ZEISS Lightsheet Z.1

Quick Guide: Imaging with Water or Clearing Immersion



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Background

Light Sheet Fluorescence Microscopy has advantages for imaging live as well as cleared samples. Lightsheet Z.1 equipped for Clearing [1] can be used for both applications equally well. A detailed description on how to use ZEN 2014 for Lightsheet Z.1 and the hardware components is available in the User Manual.

This document describes the necessary steps to change between water immersion and clearing immersion imaging options.

Imaging with Water Immersion

Water immersion are all media commonly used for live imaging (e.g. culture media, PBS, zebrafish embryo medium, artificial salt water), which have a refractive index of $n = 1.33$.

1. Select the wanted detection optics for water immersion: Lightsheet Z.1 Detection Optic 5x/0.16; 10x/0.5; 20x/1.0; or 40x/1.0
2. Assemble the sample chamber for water immersion, using the correct detection optic adapter for the used detection optic.
3. Place detection and illumination optics and the sample chamber in the Lightsheet Z.1 system.
4. Fill the sample chamber with medium and make sure that the sample chamber is not leaking.

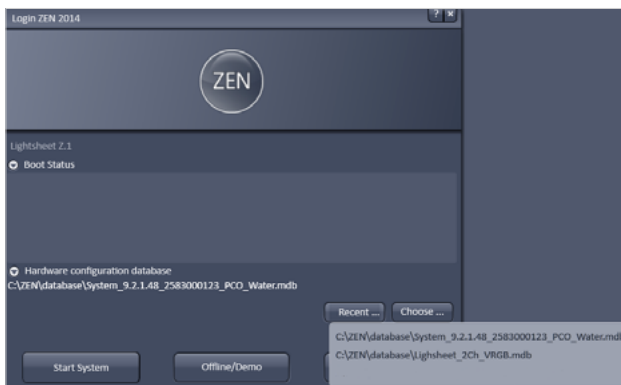


Figure 1 Start-up window, choosing database for water immersion

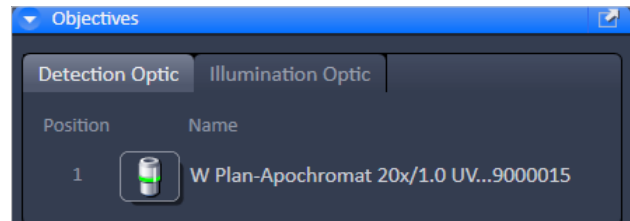


Figure 2 Maintain tab, Detection and Illumination Optic

5. Make sure to have the Reflector Turret for Laser Blocking Filters in the system [2].
6. Double-click on the ZEN icon.
7. Open the **Boot Status** and **Hardware configuration database** option in the start-up window, Fig.1.
8. Click **Recent...** or **Choose...** to open the correct database, ending with `_water` (e.g. `System_9.2.1.48_2583000123_PCO_Water.mdb`). It is possible that the database has a different name in your laboratory, please ask your advisor or lab administrator in this case.
9. Press **Start System**
A message "Attenuator calibration data is not for this system, use it?" will be shown, when the database is changed [3]. Press **yes** to proceed.
10. After ZEN has started, check in the **Objectives window** under the **Maintain Tab** if the correct detection and illumination optics are listed for imaging, Fig.2.
11. If this is not the case, choose the optics as necessary in the according menus.
12. If the optics configuration needed changing, restart ZEN software with the same database again to fully apply the changes.
13. Place the sample holder with the sample in the system and use the **Locate Tab** in ZEN for Lightsheet Z.1 to place the sample in front of the detection optic.
14. Proceed with imaging as needed.

Imaging with Clearing Immersion

Clearing media are used to enhance transparency of tissue. In Lightsheet Z.1, water-based clearing media can be used (e.g. FocusClear™, Scale, Cubic, LUMOS) with the refractive indices $n = 1.38$ or $n = 1.45$.

1. Select the desired detection optics for clearing immersion
 - Lightsheet Z.1 Detection Optic 5x/0.16 with adapter, $n_d = 1.45$
 - Objective Clr Plan-Neofluar 20x/1.0 Corr $n_d = 1.45$
 - Objective Clr Plan-Apochromat 20x/1.0 Corr $n_d = 1.38$
2. Assemble the specific sample chamber for the chosen detection optic. The available sample chambers are labelled with the corresponding magnification and the refractive index (e.g. 20x/1.45).
3. Insert detection and illumination optics in the Lightsheet Z.1 system. Do not insert the sample chamber at this time.
4. Make sure to use the Filter slider for laser blocking filter. It is not possible to use the Reflector Turret for Laser Blocking Filters with clearing immersion detection optics.
5. Double-click on the ZEN icon.
6. Open the **Boot Status** and **Hardware configuration database** option in the start-up window, Fig.3.
7. Click **Recent...** or **Choose...** to open the correct database ending with `_Clearing` (e.g. `System_9.2.1.48_2583000123_PCO_Clearing.mdb`). It is possible that the database has a different name in your laboratory, please ask your advisor or lab administrator in this case.
8. Press **Start System**.
9. A message "Attenuator calibration data is not for this system, use it?" will be shown, when the database is changed [3]. Press yes to proceed.
10. After ZEN has started, check in the **Objectives window** under the **Maintain Tab** if the correct detection and illumination optics are listed for imaging, Fig.2.
11. If this is not the case, choose the optics as necessary in the according menus.
12. If the optics configuration needed changing, restart ZEN software with the same database again to fully apply the changes.
13. Open the upper system cavity door and insert the sample holder, sample holder disc with inserted sample holder stem universal, into the system.
14. Open the front system cavity door.
15. Press the Safety switch at the lower left side of the front system cavity, and lower the stage with the **System Navigator tool window** or the Ergo Drive Operating Panel with the front cavity door open, until the end of the sample holder stem universal is visible and can be reached.
16. Hinge the sample adapter with the attached sample in the sample holder stem and fasten it loosely with the locking rod.
17. Press the Safety switch at the lower left side of the front system cavity, and lift the stage with the sample again using the **System Navigator tool window** or the Ergo Drive Operating Panel.

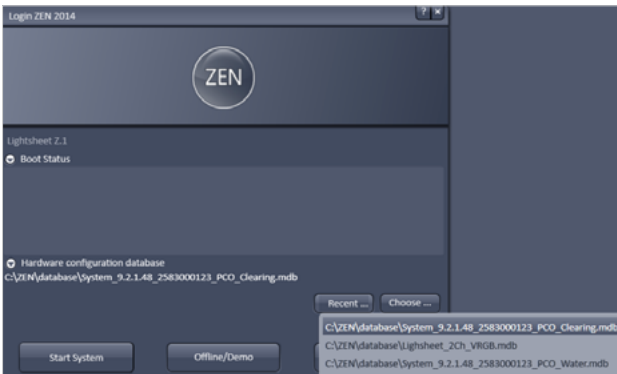


Figure 3 Start-up window, choosing database for Clearing

18. Open the upper system cavity door in necessary and fasten the locking rod tightly.
19. Close the upper system cavity door.
20. Insert the sample chamber into the system, make sure the sample is high enough to not be touched during this process.
21. Fill the sample chamber with medium. Do this slowly and carefully to prevent the media to form streaks or air-bubbles.
22. Press the Safety switch at the lower left side of the front system cavity and carefully lower the sample into the sample chamber, using the **System Navigator tool window** or the Ergo Drive Operating Panel.
23. Close the front system cavity door.
24. Use the **Locate Tab** in ZEN for Lightsheet Z.1 to place the sample in front of the detection optic.
25. Proceed with imaging as needed.

References:

- [1] When in doubt, if your system is equipped for Clearing, please contact your local ZEISS sales representative or specialist.
- [2] For water immersion it is possible to work with the Filter slider for laser blocking filter. This will result in error messages during start-up of ZEN for Lightsheet Z.1
- [3] The calibration file for the laser attenuation is shared between databases.



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