



ZEISS Lightsheet Z.1

Quick Guide: Sample Holder Universal for Capillaries,
Clearing and Large Samples



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Background Information

Depending on the size of the sample there are two different types of sample holders available:

- Sample holder for syringes (1 ml)
- Sample holder universal for capillaries and for sample adapters in both long and short versions.

If the sample is embedded in a hydro-gel matrix, use the smallest cylinder diameter of capillary or syringe that fits your specimen to avoid excessive amounts of embedding medium. The sample adapters in combination with the sample holder universal allow you to attach samples without the usage of an embedding medium. e.g. for cleared samples, plants, etc. This document gives an overview on the assembly and usage of the sample holder universal with capillaries, sample adapter short and sample adapter long. Furthermore the physical dimensions of these interfaces are given, so your own sample holder adapters can be designed independently from ZEISS and attached to the sample holder.

As a prerequisite you need to know how to assemble and place the sample chamber into the system as well as illumination and detection optics. It is preferable to already have experience with mounting samples in capillaries and reading Chapter 2: Sample Preparation of the ZEN for Lightsheet Z.1 manual. Furthermore knowledge of ZEN for Lightsheet Z.1 operation is necessary.

IMPORTANT: The sample adapter short and long are designed for imaging of cleared samples. If used with water immersion detection optics 20x, 40x or 63x, these sample adapters can touch the front lens of the detection optics. Please handle with caution to prevent damage to the lens.

IMPORTANT: The sample adapter long, cannot be used for Multiview experiments with different angles because rotation of the holder will probably touch and risk damage to the detection optics. This is the same for any sample or sample holder that is not limited to the rotation axis of the sample holder.

Assembly of the Sample Holder Universal with Capillaries

1. The sample holder and the capillary are assembled before being placed into the Lightsheet Z.1 system. The sample chamber can be already in the system and filled with the appropriate media.
2. Select the corresponding colored sleeves (Fig. 1/3) to match the color of the capillary of choice (Fig.1/6).
3. The capillary should hold the sample and the appropriate plunger (Fig. 1/5). The plungers that fit into capillary size 2–4 have to be used with the corresponding Teflon tips that are already assembled onto the plungers. Note that in addition 10x Teflon tips of each as well as matching Teflon tip tools are provided in the Chamber & Sample Holder Starter Kit Lightsheet Z.1 (Tab. 1). The Teflon tips can be assembled as a replacement onto the matching tip-less plungers.

Capillary Size (Inner Diameter)	Capillary Color Coding/ Reference Number	Plunger Reference Number	Teflon Tip	Teflon Tip Tool Color Coding
Size 1 (~0.68 mm)	Red/701902	#701930	NA	NA
Size 2 (~1 mm)	Black/701904	#701932	Size 2	Clear
Size 3 (~1.5 mm)	Green/701908	#701934	Size 3	Green
Size 4 (~2.15 mm)	Blue/701910	#701936	Size 4	Blue

Tab 1 Capillary components in the Chamber & Sample Holder Starter Kit Lightsheet Z.1

4. The sleeves are tube-shaped with four slits at one end. Insert the sleeves into the sample holder stem (Fig. 1/2,3) so that the two slit endings point outwards and the non-slit endings are next to each other in the center.
5. Take the clamp screw (Fig. 1/4) and position it onto the sample stem holder turning clockwise three times (360° each turn) which will not fix the screw.

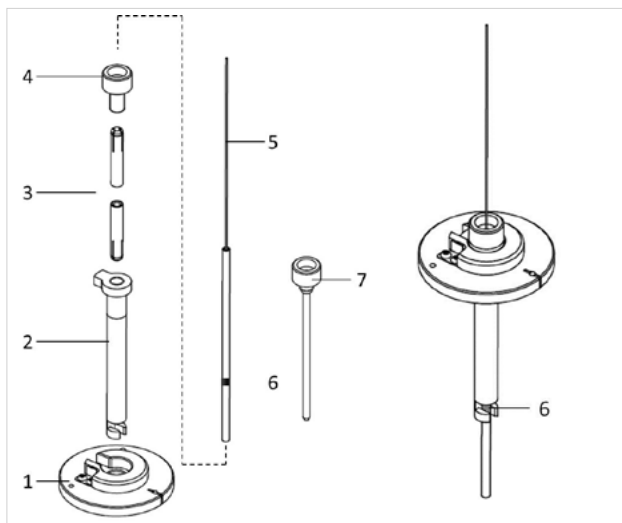


Figure 1 Assembly of the sample holder universal with capillaries

- | | |
|--------------------------------|--------------------------------------|
| 1 Sample holder disc | 5 Plunger |
| 2 Sample holder stem universal | 6 Capillary with color-coded marking |
| 3 Sleeves (color-coded) | 7 Locking rod with screw |
| 4 Clamp screw | |

6. Take the capillary by the glass portion (avoiding the plunger) with the agarose embedded specimen inside and carefully place it through the middle of the holder from the screw end. Do this holding the sample holder horizontally to prevent the plunger sliding all the way through.
Newly bought sleeves can be slightly tight at their slit opening ends so that it can be difficult to push the capillary within the holder. In this case take the capillary with matching plunger and insert it carefully into the corresponding sleeves at their non-slit endings. Make sure that the slits point outwards. Position the capillary surrounded by the sleeves into the sample holder stem and place the clamp screw. You can proceed with step 6.
7. Push the capillary within the holder stem through the holder until the color-coded capillary marking just becomes visible (Fig. 1/6 right hand side). It is better to pull the glass capillary as it becomes visible than push the plunger and risk moving the sample. Having the color marker at the edge of the metal is the recommended -position for later imaging. However, if there is more space needed in between the sample chamber and the sample holder positioning stage it can be useful to push the capillary through further. In these cases, carefully test if the capillary still can be placed tightly within the sample holder.

8. Tighten the clamp screw tight and check that the capillary is held by pulling it lightly before turning the holder back to the vertical position. Take the sample holder disc (Fig. 1 / 1) with the protruding side with positioning notch facing you. Carefully position the sample holder stem with specimen into the sample disc holder until the ball bearing click-position is felt.
9. Ensure that the system motors have been moved to the load position, then open the upper system cavity door.
10. Insert the sample holder gliding the sample holder disc along the guide rails. It is placed correctly into a click position if the sample holder disc ball bearings lock into the three holes. It helps but is not essential to align the white marking of the holder to the white marking on the motor.

Removal of the sample after imaging:

11. Move the motors back to the load position and remove the holder through the upper door.
12. For removal of the capillary, first turn the holder back to the horizontal position to prevent the capillary dropping, before loosen the clamp screw and carefully pull the capillary through the stem sleeve by using the glass end nearest the color-coded marking.
13. Unscrew the clamp screw completely and remove the plastic color coded sleeves by using the locking rod as an ejection tool by poking it through the non-screw end of the holder (Fig. 1 / 7).

The sleeves as well as the glass portion of the capillary can be cleaned with a lint-free cloth moistened with water.

Sample Adapter Short and Sample Adapter Long

With the sample adapters, provided by ZEISS or self-made, it is possible to attach samples that are large and not necessarily embedded in agarose to the sample holder.

The sample adapters do not fit through the opening of the stage, in which the sample holder stem is inserted through the upper system cavity door. Hence the sample adapter is attached onto the sample holder universal through the front cavity door of the system when the holder is already in the system.

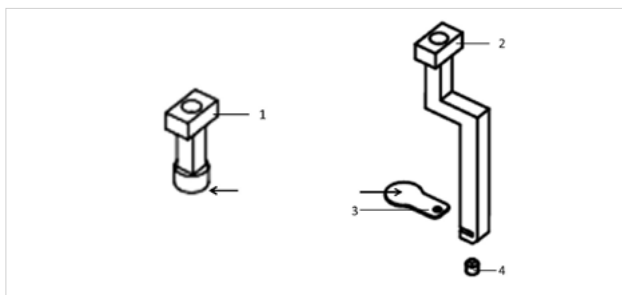


Figure 2 Sample adapter short and sample adapter long. Sample placement marked with an arrow.

- | | |
|------------------------|---------------------------|
| 1 Sample adapter short | 3 Sample plate |
| 2 Sample adapter long | 4 Screw (ISO 4026 M2 x 2) |

Sample Adapter Short

IMPORTANT: This sample adapter can touch the front lens of the water immersion detection optics 20x, 40x and 63x.

The Sample Adapter Short can be used to either attach the sample directly or necessary components for sample attachment (metal plates, glass components etc.) (Fig. 2 / 1, arrow). This can be done with a variety of glues:

- super glue/instant glue (based on cyanacrylat, exothermic reaction)
- two component adhesives (epoxy resin)
- all-purpose adhesives

Depending on the sample and the media it is kept in, different glues work with diverse results. Some experimenting might be necessary. For tissues, super glue has been proven useful and is recommended here. Super glue can flow quite thin and the following tips might help:

- during the hardening of the glue, hold the sample adapter and the sample in a way, that the glue will flow away from the sample, otherwise the sample can be covered in more glue than necessary
- use a third hand tool (adjustable clamp) to hold the sample adapter
- use modelling clay (modelling dough) to support sample adapter or sample as necessary
- use a base of hardened agarose to work on, to prevent the sample adapter or the sample to be glued to the base (e.g. a petri dish)

Glues can be removed from the sample adapter by placing it into solvents (e.g. EtOH) and scraping off the glue with a razor blade or a scalpel.

Sample Adapter Long and Sample plate

IMPORTANT: This sample adapter **cannot** be used for Multiview experiments with different viewing angles! Due to its design, parts of the sample adapter are not in the rotation axis and will move through a large circle when changing the angle. This will lead to collisions with the front lens of the detection optic.

The sample adapter long can be used to either attach the sample plate provided by ZEISS or to attach third party/own constructed sample plates/adapters/holders onto the sample holder.

The sample adapter long and the sample plate are normally assembled before the sample is attached. The sample plate has a round side on which the sample is placed, and a square side with a hole to fix it to the sample adapter. To do so, slide the sample plate with the hole into the slit on the lower part of the sample adapter long and fixate it with a screw (ISO 4026 M2 x 2, Screwdriver: 0.9x40 mm allen key; Hex Inch 0.035 x 40) from below.

Any other device, that fits the given physical dimensions of the attachment side (see section Hardware Physical dimensions) can be attached to the sample adapter long in the same way.

To fix the sample onto the sample plate, use either glue:

- super glue/instant glue (based on cyanacrylat, exothermic reaction)
- two component adhesives (epoxy resin)
- all-purpose adhesives

Or use other adhesives (e.g. agarose, double-face tapes) or mechanical locking devices to hold the sample in place.

Assembly of the Sample Holder Universal with Sample Adapter Short and Long

When the sample is fixed to the sample adapter short or long, all can be attached to the sample holder universal. A sample adapter of the correct physical dimensions can as well be attached to the sample holder universal (see section Hardware Physical dimensions) in the same way.

The sample holder and the sample adapter with the sample often need to be placed into the system, before the sample chamber is introduced.

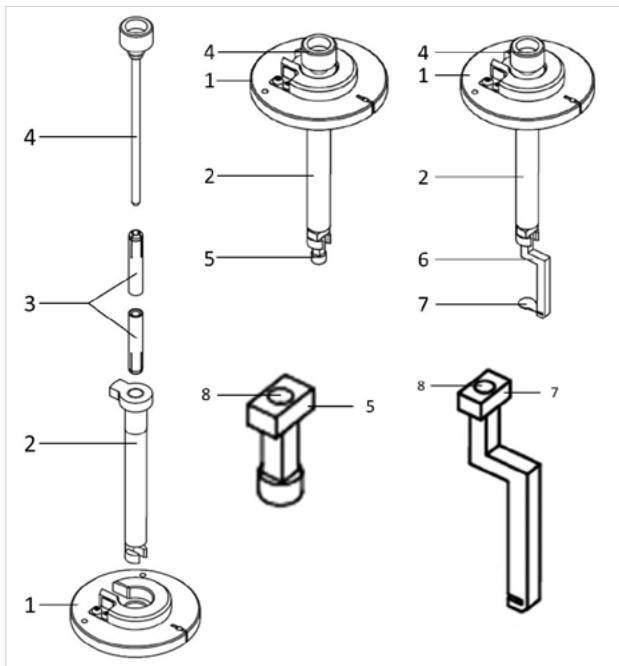


Figure 3 Sample adapter short and sample adapter long

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|--------------------------------|---------------------------|
| 1 Sample holder disc | 5 Sample adapter short |
| 2 Sample holder stem universal | 6 Sample adapter long |
| 3 Sleeves (blue) | 7 Sample plate |
| 4 Locking rod with screw | 8 Opening for locking rod |

1. The sample adapter with the sample will be attached, after the sample holder stem universal is placed into the Lightsheet Z.1 system.
2. Place two blue sleeves (as for the blue capillaries) into the sample holder stem universal. Make sure that the slits point outwards (Fig.3/3).
3. Take the locking rod and push it into the sample holder stem, through the blue sleeves (Fig. 3/3,4).
4. Take the sample holder disc (Fig.3/1) with the protruding side with positioning notch facing you. Position the sample holder universal into the sample disc holder until the ball bearing click-position is felt.

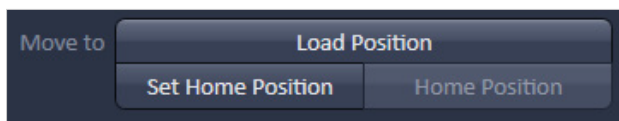


Figure 4 Navigation tool window, Load Position button

5. Move the stage of Lightsheet Z.1 in the Load Position (Fig. 4).
6. Open the upper system cavity door.

7. Insert the sample holder gliding the sample holder disc along the guide rails. It is placed correctly into a click position if the sample holder disc ball bearings lock into the three holes.
8. Open the front system cavity door as well.
9. Press the Safety switch at the lower left side of the front system cavity, to allow movement of the stage, with the door open.
10. Use the Ergo Drive Panel or the Navigation Tool in ZEN to lower the stage until the end of the sample holder stem universal is comfortably reachable. Rotate the stage until the open part of the stem is facing towards you (Fig. 3/2).
11. Lift the locking rod up with one hand and place the sample adapter in the opening of the sample holder stem with the other hand (Fig. 3, middle and right hand images).

For the sample adapter long, make sure that the vertical bar is facing towards you and the sample away from you (see Fig. 3, right hand side).

Depending on the detection optics in the system, the vertical bar can otherwise touch the front lens of the detection optics.
12. Turn the clamp screw at the end of the locking rod a couple of times. The locking rod will hold the sample adapter by fixing its opening (Fig. 3/8)

Do not apply pressure on the stage while turning the clamp screw.
13. Raise the stage, again pressing the Safety switch on the system, until the sample adapter and sample are not visible when looking directly into the front cavity anymore. Turn the clamp screw of the locking rod while holding on to the sample holder disc, until tight. The locking rod will be fixed in the opening of the sample adapter (Fig. 3/8) during this process.
14. While the sample is in this upper position it is possible to slide the sample chamber into the system and tighten it against the detection optic.
15. Fill the chamber with the appropriate medium.
16. Slowly lower the sample into the chamber and place it in front of the detection optics. Either do that with the front system cavity door open (pressing the safety switch) or with the Locate Sample option in ZEN.
17. The sample is ready for imaging in the Lightsheet system.

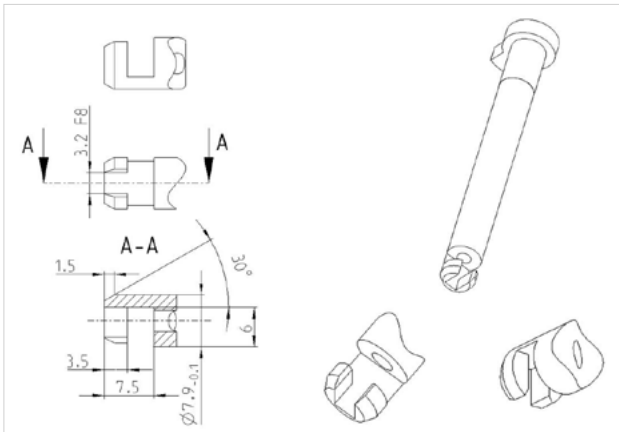


Figure 5 Sample holder stem universal

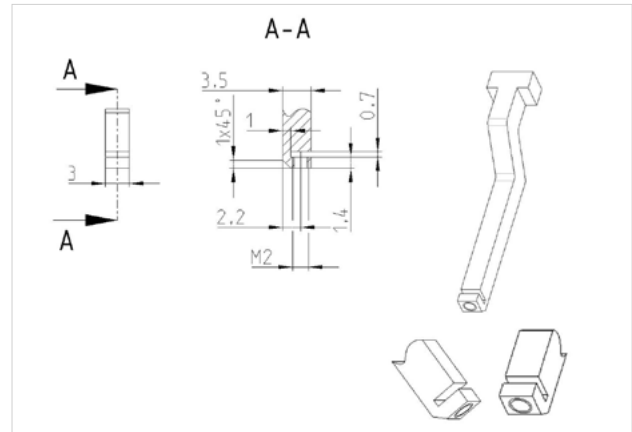


Figure 7 Sample adapter long interface, slit for sample plate

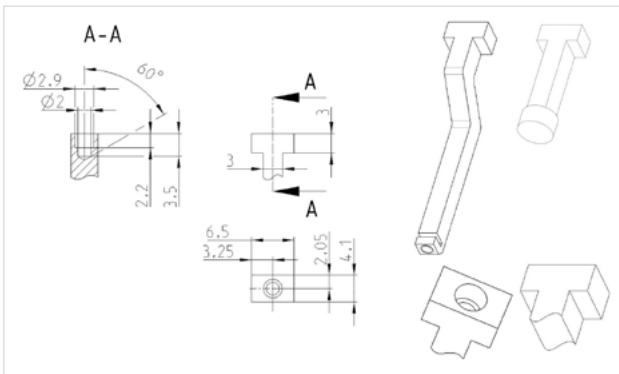


Figure 6 Sample adapter, interface with sample holder stem universal

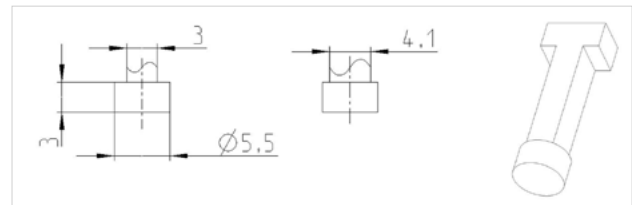


Figure 8 Sample adapter short, physical dimensions of lower end

Hardware Physical dimensions/Interfaces

The sample holder stem universal as well as the sample adapters are designed with several interfaces, to attach self-made or third party solutions to hold and mount your samples.

The sample holder stem universal as an easy mechanism to hold sample adapters. This mechanism relies on the locking rod, which is entering a depression in the sample adapter.

The locking rod is as well fixed with the blue sleeves and the screw.

Hence any adapter which is fitting into the holding opening of the sample holder stem universal and offers a depression for the locking rod to fix into, can be attached to the sample holder stem.

When designing your sample adapter also consider that the available space for the sample depends on the sample chamber and the detection optic you are using.

IMPORTANT: Every sample or sample holder that is not limited to the rotation axis of the sample holder, cannot be used for Multiview experiments with different angles! Such samples or sample holders can touch the detection optics during rotation of the sample.

The sample adapter long allows to attach other parts than the sample plate offered by ZEISS. The customized part needs to fit into the slit of the sample adapter long and must provide an opening for the screw to fit through.

Please use the physical dimensions of the drawings to design your sample plate etc. to fit to the sample adapter long.

The needed screws are ISO 4026 M2 x 2, which are available in different materials. We recommend steel, if this is compatible with your sample and material.



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