



ÉCOLE POLYTECHNIQUE
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A Generic Tool for Lightsheet Sample Holders

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Introduction

When imaging using conventional microscope configurations (Inverted or Upright), most samples have a reasonable thickness ranging from 4 to 20 microns and in rare cases a hundred microns. To increase the penetration of light into thick tissues, tissue clearing protocols have been developed that allow for imaging of samples many millimeters, even centimeters thick.

These new "unusual" samples are now becoming the norm, and present new constraints linked to their size and bulk, which were not necessarily considered when the first Zeiss LZ1 was designed.

For example, samples are usually glued to a stem and then suspended into the chamber. Mounting large samples involves a delicate process involving many steps that risk damaging both the sample and the system. Delicate samples, such as thymus, are not coalescent enough to be suspended directly onto the stem and detach. Long samples, such as mouse spine tend to buck in the thick imaging medium during Z movement, reducing the quality of acquisitions.

We designed a series of reusable holders to 1. Help the mounting of large samples onto the LZ1 and equivalents. 2. Cover a range of sample geometries and consistencies and 3. Try to make use of the most of space in the LZ1 chamber to image as deep as possible while keeping the optics safe.

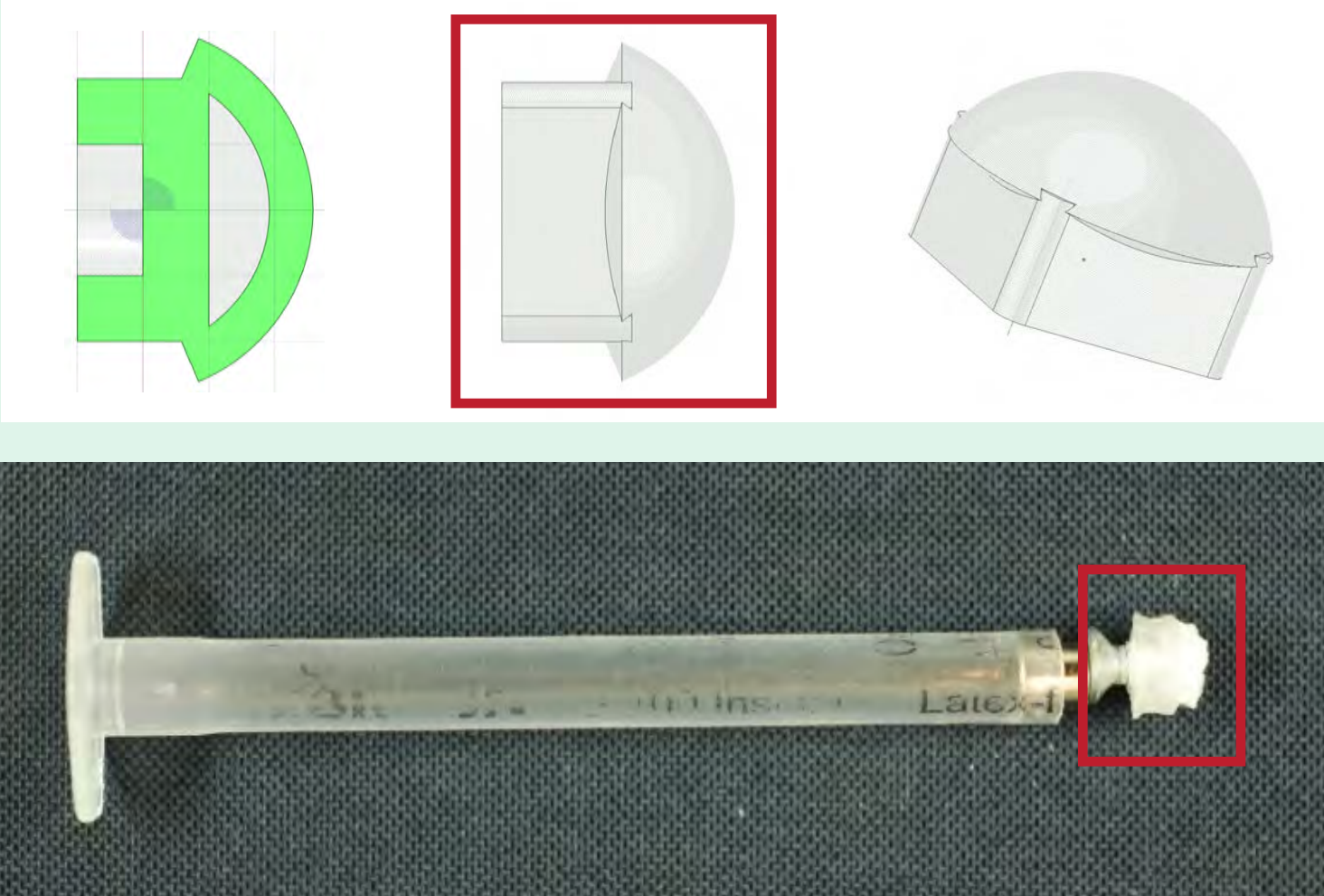
Here we present these holders, their general design concept and the universal magnetic holder socket along with example cases where these devices were used.

Patch Holder

Using a plastic filament 3D printer, we printed a multi-use PLA cube with a domed top to which we attach an iron flat head M2 screw. After removing the tip of the syringe, a 4.5mm magnet is attached.

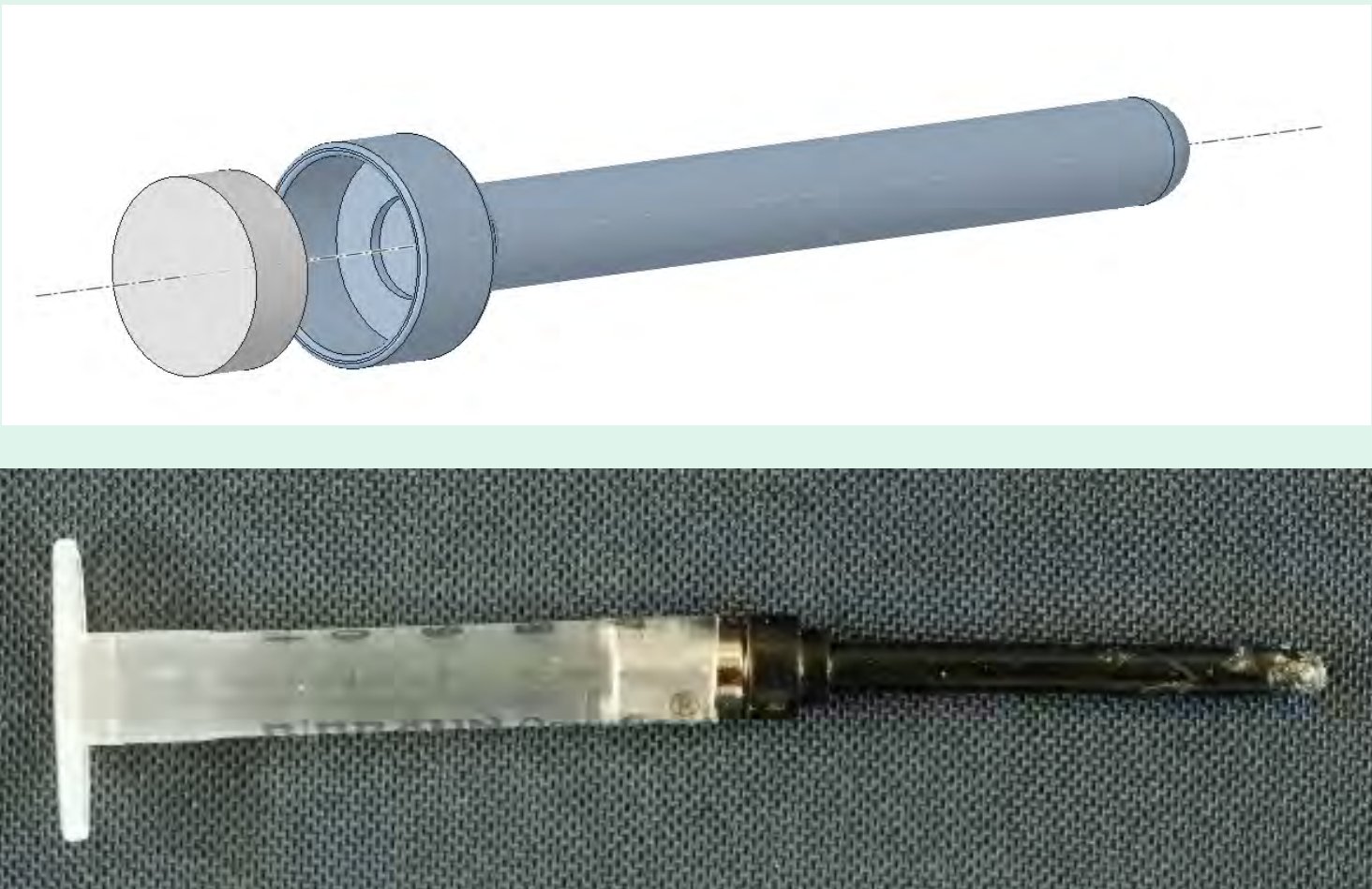
The sample is glued to the PLA cube using CYANOLIT and then placed as per the protocol described.

For varying sample lengths, the length of the screw can be adjusted. A typical sample using this holder is cleared mouse brain.



LEGO Antenna Holder

Lego pieces are excellent for use as holders in Lightsheet microscopy. They are autoclavable, mechanically resistant, chemically inert and have low temperature expansion. As per above, after cutting the syringe in half, a 4.5mm magnet is inserted. A 4mm iron washer is glued inside the Lego piece, and the sample is then glued to the antenna using CYANOLIT. This holder was used with sectioned mouse brains (lobe, cerebellum)



Sliding Slide Holder

By attaching a magnet to the plunger of a syringe cut in half, we then manufactured a metal rod with a flat end that allows for the positioning of a 5x25mm coverslip held in place by a magnet.

Samples that are not strong enough to be held by a single attachment point or that are very long can be glued to the coverslip using CYANOLIT. These include lymph nodes, mouse intestine and thymus.



Sample Characteristics For Each Holder

Holder	Max Sample Weight	Max Sample Width	Multiview Acquisition	Tiling	Typical Tissue
Patch	2 grams	25mm	Yes	Yes	Brain Lobes
LEGO Antenna	<1 grams	25mm	Yes	Yes	Brain Lobes
Sliding Slide Holder	>2 grams	5mm	No	Yes	Thymus, Mouse Spinal Cord,
New Generic Slide Holder	>2 grams	5mm	No	Yes	Lymph Node, Mouse Intestine

Important Considerations

Due to the fact that cyanolit glue is light scattering and autofluorescent, care should be taken to properly select the region of the sample that will be attached.

While samples larger than 5mm run the risk of being in contact with the objective, this is hardly a problem as samples are very soft and cannot damage the objective.

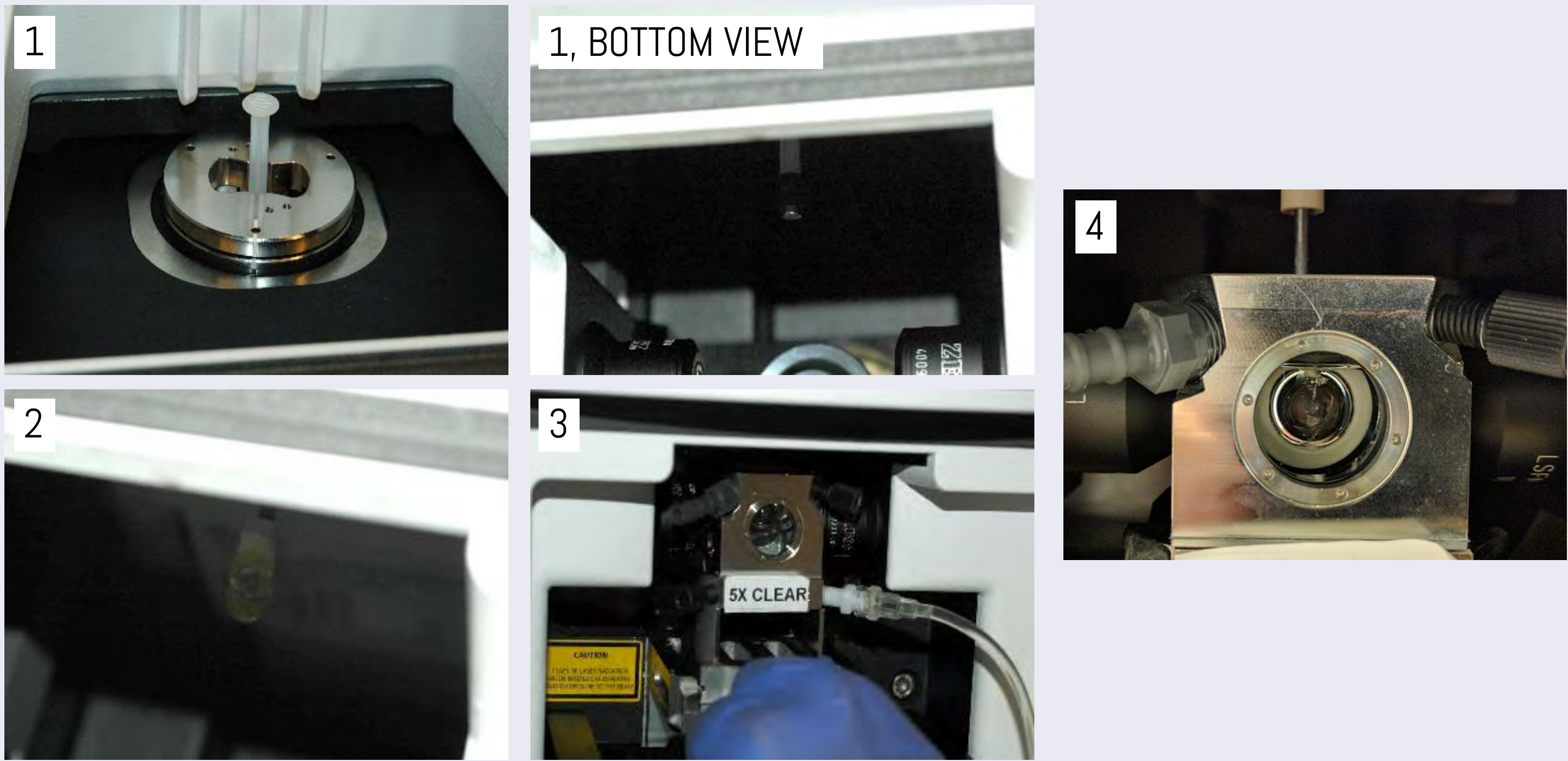
As for all cleared samples, care should be given not to dip the syringe part of the holder, to avoid the risk of having medium in contact with the morotized stage during removal.

Sample Insertion Protocol

Zeiss allows for the use of a 1mL syringe to mount the largest possible samples, so we have transformed it by creating different holders on which we can attach the specimen.

Due to the size of the samples, they cannot be inserted from the top of the LZ1, as the opening matches the 4.5mm limit. For this reason, we use the follwing protocol:

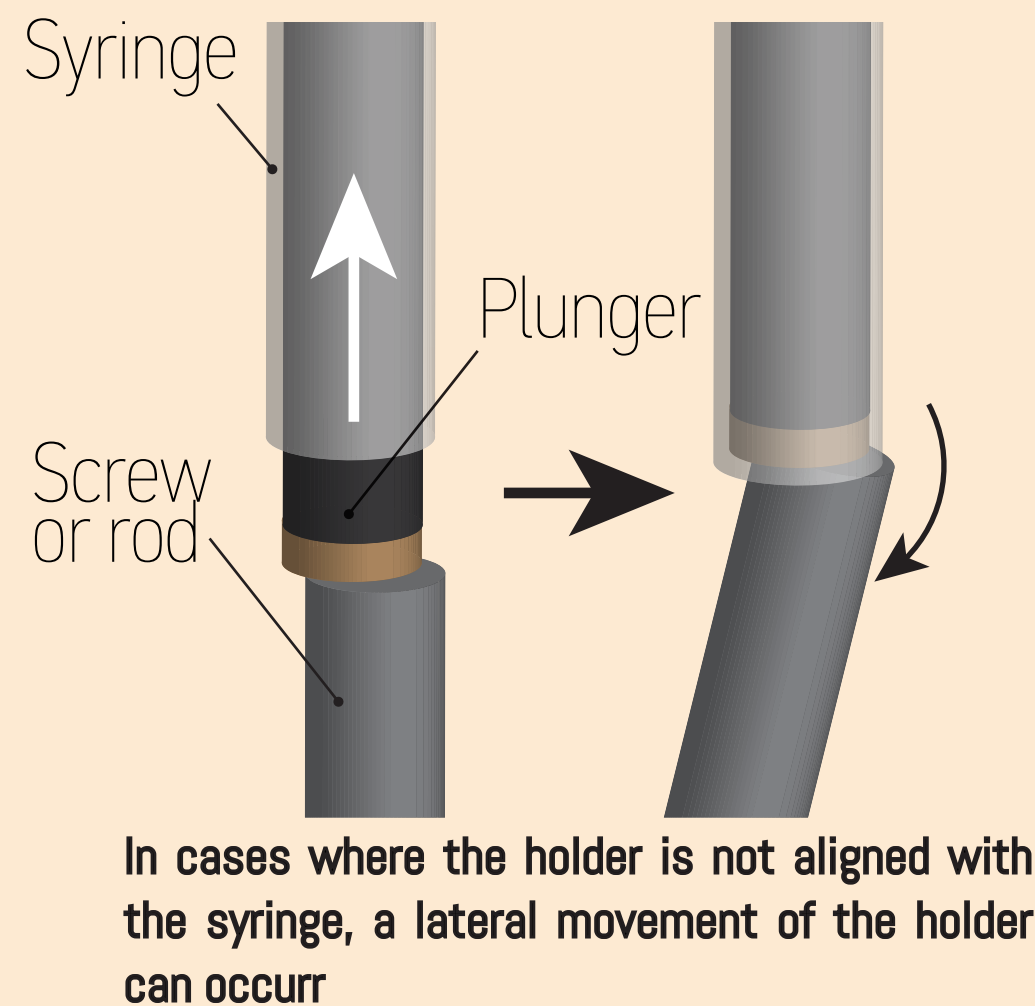
1. Insert the modified syringe onto the LZ1 stage
2. Through the front opening, attach the sample to the syringe and raise it
3. Introduce the chamber, fill with medium
4. Lower the sample onto the chamber.



Towards a Generic Holder

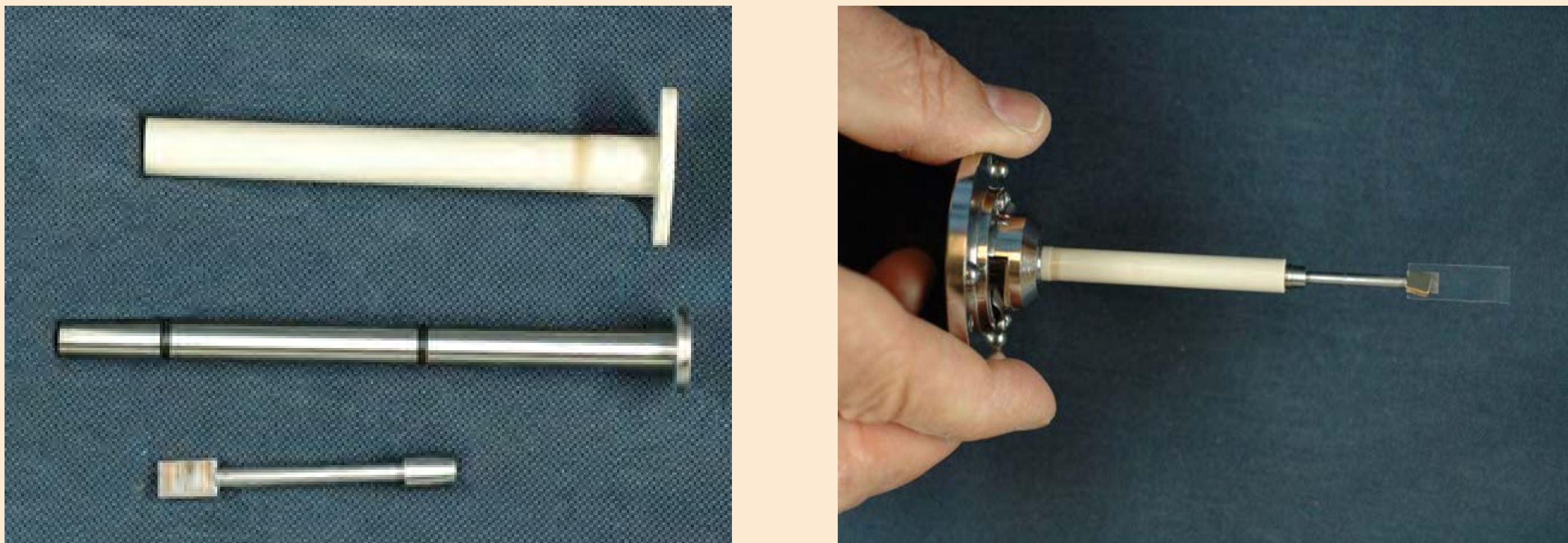
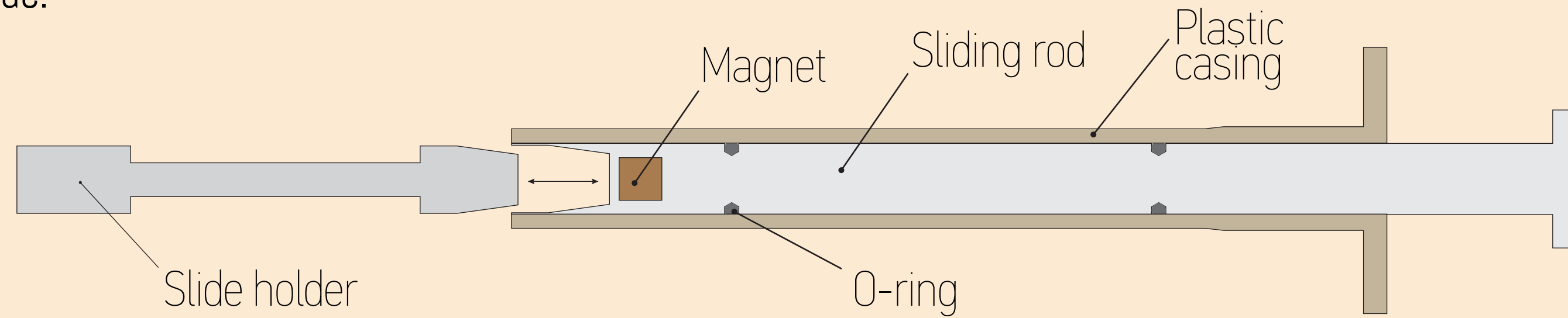
While simple to implement, using the holder with magnets has two main disadvantages

- Samples can be jerked around a lot when the metal piece snaps to the holder magnet, risking detachment and damage of the sample.
- The positioning of the metal screw or rod on the magnet can lead to a misalignment of the two pieces, resulting in a tilting of the holder which could damage the sample.



To overcome these limitations we have engineered a holder where the magnet is housed in a cavity that is shaped to guide the sampled holder onto the right position.

Moreover the magnet is positioned farther from the holder which helps to avoid the 'snapping' issue.



Outlook And Future Work

The counter piece to the sliding rod can be manufactured separately and could then be attached to any other kind of holder using either screws or magnets. Considering the sturdy construction, this can lead to a simple customizable universal adapter that can use all the holder types described here.

