P3 – Miniscope assembly protocol

An overview of the MINI2P miniscope optical components and objectives can be found in Figs.S1-S2 in *Zong et al., 2022*, whereas the materials for assembly are shown in Fig.S10, also in Zong et al., 2022. This section describes the main steps to assemble the microscope´s optical and mechanical parts, steps that are illustrated in Fig. (2) schematics. Other figures are provided along the way, to show real examples of the Mini2P scope assembling in our workshop.

Reagents:

- Super glue (Loctive, 415)
- Epoxy adhesive (3M Scotch-Weld, item p)
- Silicone Adhesive (Winjee, item o)
- Absolute ethanol (100%)
- Air duster Green (PRF 4-44, item w)
- UV curing optical Adhesive (Thorlabs, item l)

Equipment: to prepare MINI2P (see Fig.S1-2 in Zong et al., 2022)

- 1-2 Tweezers
- Screwdrivers (Philips's cross)
- 1 Handle with scalpel blades
- Splice protector sleeve (Item m) – multiple thicknesses
- Lens cleaning tissue (Thorlabs, item j)
- Cotton buds & swabs for electronics (Chemtronics, item k)
- Tack-it (Faber Castell)
- Medical tape
- Laboratory gloves
- Stereo-microscope (Zeiss Trino, WL37166)
- UV curing light (Thorlabs, Item e)
- Handheld red laser source (Thorlabs, item f)
- NIR detector card (Thorlabs, item i)
- Ultrasonic cleaner
- Calibration target (e.g. wall or cardboard)
- Components of the Mini2P Scope (Table1)

Table 1: Components of the Mini2P miniscope

Component Name	**Amount**	**Item Number (see S1, shopping list)**
Scopebody P1	1	102
Scopebody P2	1	103
Scopebody P3	1	104
μTLens stacked	4	114
μTLens connector to MEMS	1	114
MEMS	1	115/116
MEMS PCB	1	117
MEMS connector to μTLens	1	117
Scan lens	1	109

6-wires for MEMS	1	50
Objective	1	111/112/113
Dichroic Mirror	1	110
M1.2 cap screws M1.4 cap screws + M1.6 setscrews	2 2 >6	108
Stitching Adapter	1	105
Baseplate	1	106
Prepared HC-920 assembly	1	Protocol 2
Prepared TFB	1	Protocol 2

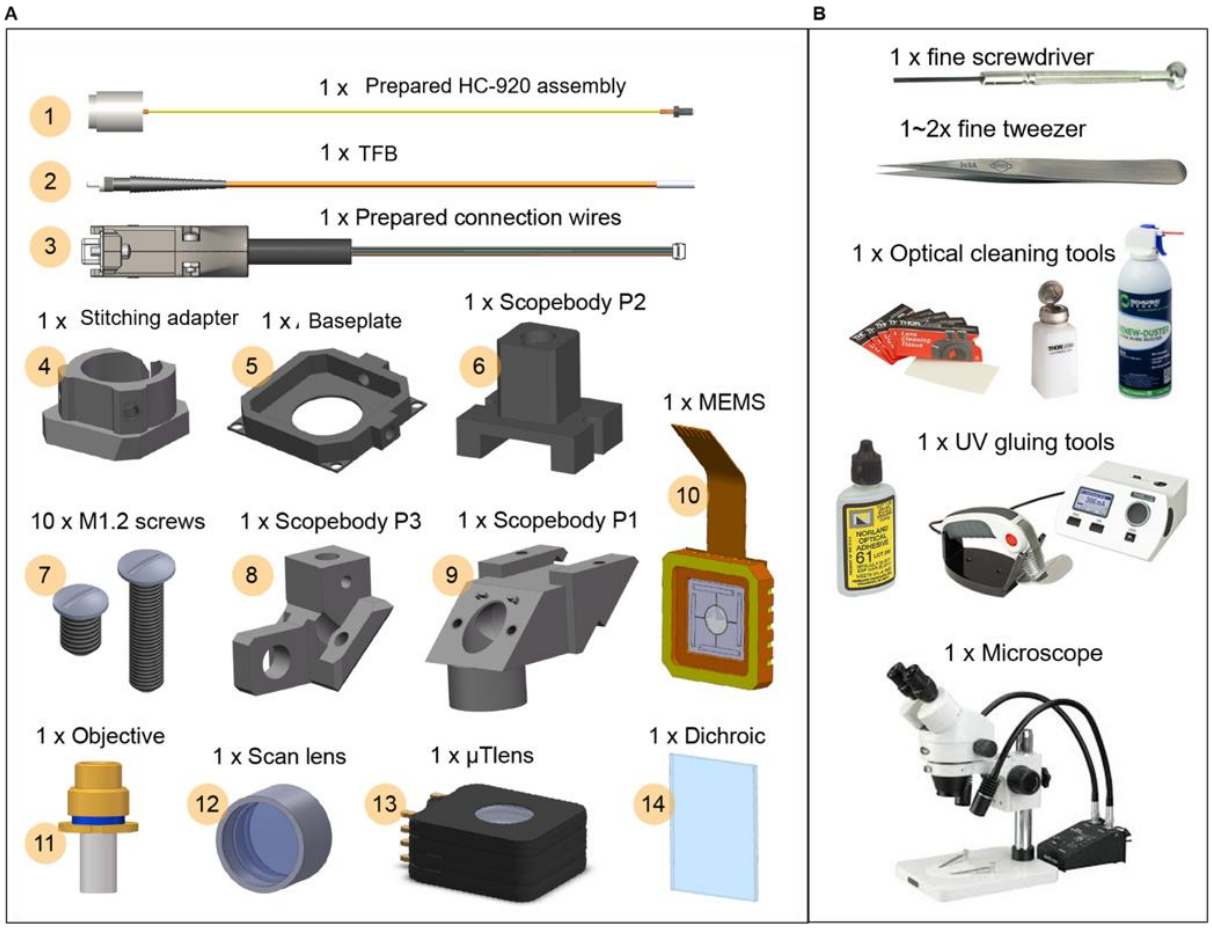

Fig. 1: Taken from Fig.S10 in Zong et al, 2022: Schematic of (A) Optical and mechanical parts; (B) Reagents and tools for Mini2P assembling.

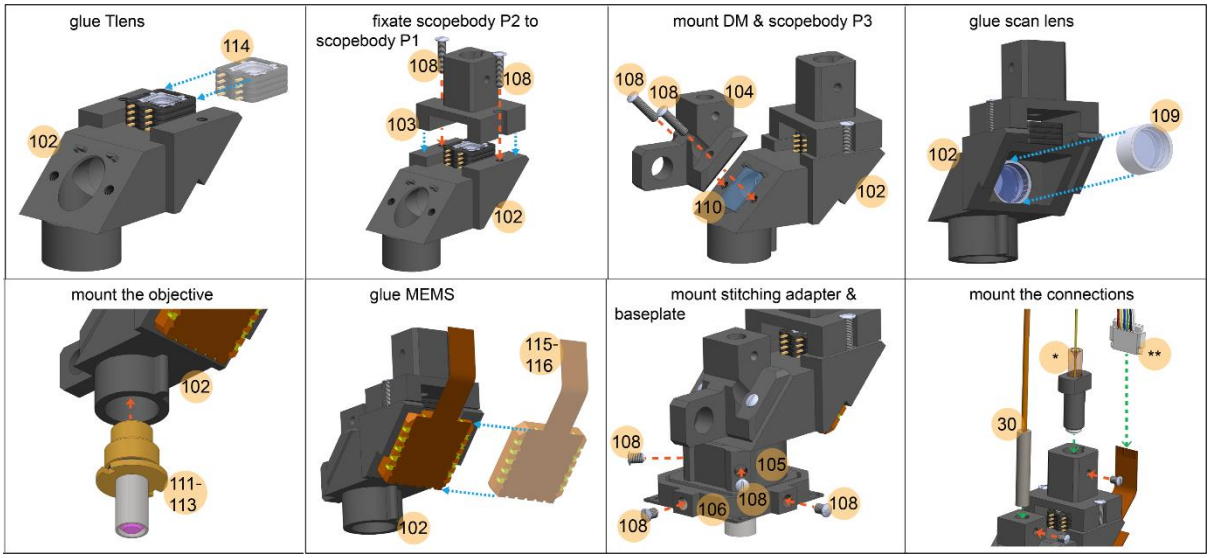

Fig. 2: Summary of miniscope assembly key steps

Miniscope assembly tutorial video can be found on the link:
https://www.youtube.com/watch?v=2B0UnX2e5S8&list=PLonWNO9SywvJXpIgQEp6jQMSh_jrn_jPU&index=4

See also MINI2P – Video tutorial package:
https://www.youtube.com/playlist?list=PLonWNO9SywvJXpIgQEp6jQMSh_jrn_jPU

Step1. Pre-assembly step: Cleaning of scopebody parts (P1, P2 & P3)

Prepare to mount the miniscope, but first start by cleaning all three scopebody parts, P1 to P3. This step must be done under a stereo-microscope and in a clean room.

1.1 Remove any residues from the scopebody parts with a scalpel. Surfaces where optical components will be mounted should be cut as flat as possible, and not have "bumps or chamfers/rough parts" standing out.

Note! This is especially important and more frequent in scopebody P1, where the dichroic mirror, MEMS scanner and scan lens are mounted into (see example Fig.3).

Fig. 3 Preparation of microscope surface to glue the MEMs onto. Cut excessive material with a blade all the way from the top.

1.2 After cutting, press a piece of tack-it against the scopebody P1 surface to remove any small particles and residues left from the cleaning.
1.3 Clean all parts with a cotton swab dipped in 100% ethanol.
1.4 Rinse all three scopebody parts in 100% ethanol and clean them in an ultrasonic bath. 10min should be enough.
1.5 Once the scopebody parts are clean, they can be sent to PoLight, the company that glues the four stacked uTlens onto the scopebody P1 (see Fig.4).

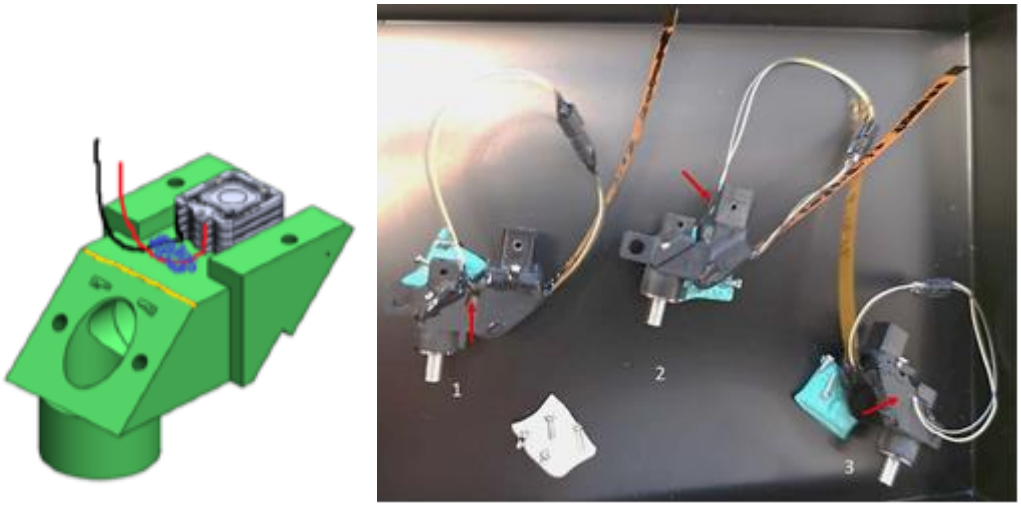

Fig. 4: (left): Schematic showing area (blue) where to glue the µTLens wires on Scopebody P1; (right) Photography of three assembled miniscopes with a red arrow pointing where superglue and epoxy were applied to garantee sturdiness of uTLens wires.. PLEASE AVOID REGIONS NEAR THE uTLENS, so preferably add glue only in regions pointed with a red arrow.

Step2. Testing the MEMS scanner

The aim of this step is to confirm that the MEMS scanner is working. By letting the MEMS scan while directing the handheld red laser source at it, the scanning pattern is reflected in a calibration target (see Fig.5). This gives an estimation of the in-vivo FOV.

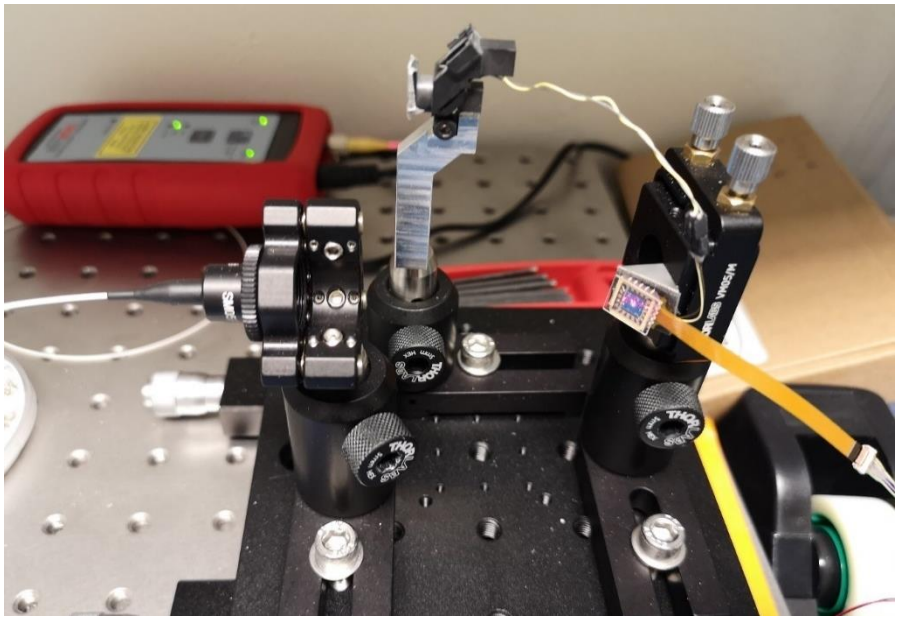

Fig. 5: MEMS testing setup: red laser (item f) directed to MEMS mirror to confirm MEMS scanner works.

2.1 Attach the MEMS scanner to the holder using tack-it. Then connect its wires.
CAUTION! Make sure the BNC +5V from the vDAQ to the controlbox is not connected.
2.2 Connect BNC +5V that goes from the vDAQ to controlbox.
2.3 Turn on the handheld red laser and adjust its holder until the light spot hits the center of the MEMS mirror.

Note! A good indication that the red laser source hits the center of the MEMS mirror is that there are two visible beams on the calibration target (one from the glass that protects the MEMS mirror).

2.4 Move the reflected beam to the center of the calibration target axis (see Fig.6-left). Both the laser and the MEMS holder can be adjusted to achieve this.
2.5 To initiate the MEMS scanning, open the controlling software ScanImage. Select the scanning speed which is determined by pixels/line (e.g. 128 or 256) and click ”FOCUS”.
2.6 The MEMS pattern should now appear on the calibration target (see Fig.6-right).
Note! The pattern will not be square in this case because the laser is hitting the MEMS with an angle different than 45°
2.7 The expected in vivo FOV is estimated by the intersection with the smallest axis.
Note! In Fig.6-right) can be expected a FOV of around 400 um x 400 um

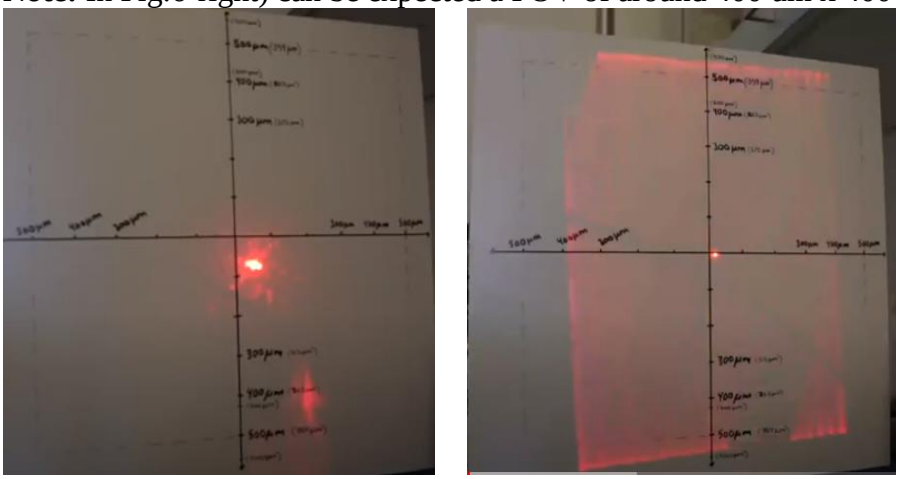

Fig. 6: (left) Red laser reflected from the MEMS mirror into a target; (right)MEMS scanning pattern.

Step3. Aligning and attaching scopebody P1 & P2

This step must be done right next to a working MINI2P platform because the miniscope must be aligned to the HC-920 fiber assembly prepared in Protocol P2.

3.1 Attach scopebody P2 to scopebody P1 using M1.4 (L=4 mm) capscrew. Do not screw too tightly because there should be some room for adjustment.
Note! Confirm you can move scopebody P2 before the adjustment step.
3.2 Attach scopebody P3 to scopebody P1 using M1.2 (L=4 mm) capscrew. Scopebody P3 is needed to attach the miniscope to the alignment holder (modified version of 75-MINI2Pholder P4)
Note! Screw scopebody P3 tightly to P1 to guarantee both parts do not move during alignment.
3.3 Place a M 2.5 screw (L= 4mm) in the hole of scopebody P3 and screw the miniscope onto the MINI2P alignment holder.

3.4 Place HC-920 fiber assembly in the scopebody P2 inserting the collimator holder all the way in.

3.5 Connect the uTLens to a MEMS scanner which is connected via 6-wires cable onto the MINI2P platform (controlbox output).

3.6 Adjust the position of the scopebody P2 relative to P1 until the light from the fiber is hitting the center of the uTLens. While you adjust P2, visualize the light spot after passing through the uTLens on a NIR laser detector card placed in front of the miniscope. Continue adjusting P2 until the spot is no longer cut off.

3.7 Optimise settings in ScanImage to alternate the uTLens between two different focal lengths (see Fig.7). This approach allows to re-adjust scopebody P2 until the two spots at two different optical powers are centered.

a. Increase the laser power to 40%
b. In bounded stack, prepare to image two slices and a very large number of volumes (e.g. 100 000)
    i. Change the uTLens focal length to -20 in the motor controls menu.
    ii. In bounded stack, set START to -20.
    iii. Change uTLens focal length to -40, and set end.
c. Set the number of slices to 2 and number of volumes to a large number (e.g. 100 000)
d. Press Grab to start alternating between the two focal lengths

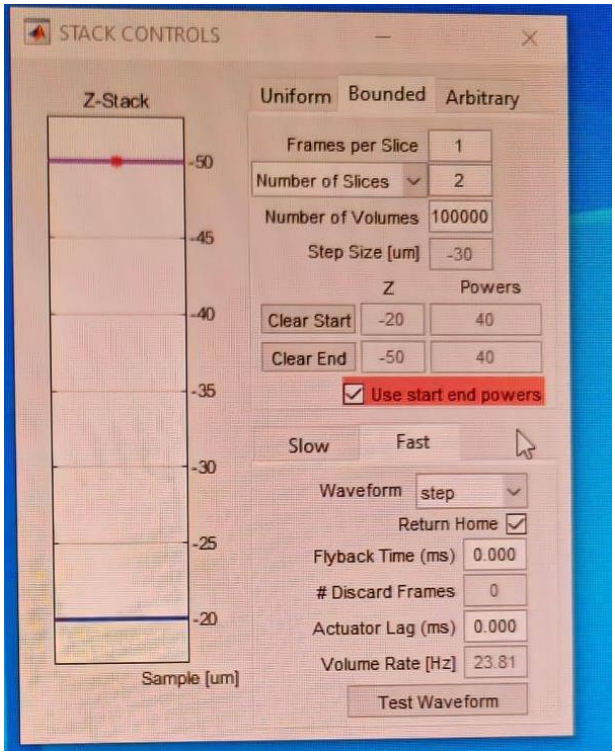

Fig. 7: ScanImage setting to alternate between two spots at different optical powers. Stack must be ENABLED.

3.8 Adjust the tightness of the screws and the position of scopebody P2 until the spots coincide.

3.9 The resulting output should be something as illustrated in MINI2P video tutorial, 4:29.

3.10 Once the alignment is good, P1 and P2 can be glued together. Add tiny amounts of glue along the edges between P1 and P2. Also add glue to the M1.4 screws.
Caution! Do not add too much glue as this can go into the uTLens.

3.10 Leave the glue to dry for approximately 1 h

3.11 Once the glue is dry, protect and shield off the uTLens.

a. Add a piece of tack-it to cover the gap to the uTlens on the top side.
b. Flatten it and attach well with the aid of a pair of tweezers.
c. Repeat a) and b) for the gap on the other side of the miniscope.

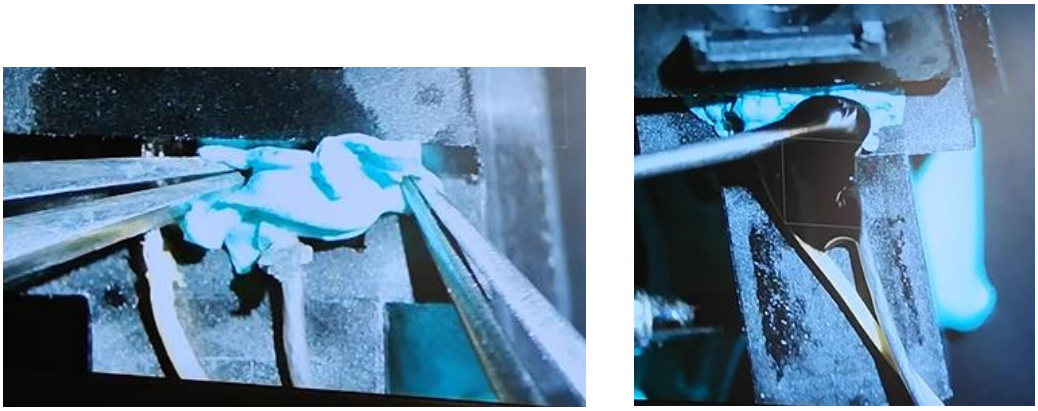

Fig. 8:(left) Illustration showing the µTLens slit covered with Tack-IT; (right) deposition of black epoxy to cover all Tack-It and µTLens wires.

3.12 Cover the tack-it with epoxy to seal the uTlens off (see Fig.8-right).

a. Prepare epoxy by stirring both parts with the back side of a cotton swab
b. The tack-it, screws and gap between P1 and P2 should be covered with a thin layer of epoxy

Caution! Do not cover at the back side with tack-it all the way, as the MEMS will be placed there later on and epoxy might block it from being positioned correctly.

3.13 Protect the inside from dust using tape and leave to dry over night

Step4. Mount dichroic mirror & scopebody P3

4.1 Attach the miniscope using tack-it
4.2 The surface for the dichroic mirror should be facing upwards, positioned as flat as possible.
4.3 Prepare UV glue.
4.4 Carefully get the dichroic mirror from its packaging
4.5 Before gluing, clean the mirror with a cotton swab covered with lens tissue. Spray the tissue with ethanol and clean both sides of the mirror.
4.6 Place mirror at its intended position on scopebody P1. Make sure the side with dichroic coating is facing downwards.
4.7 Add UV glue at all four corners of the mirror with tip tool of splice protector sleeve. **Note!** The glue will flow in between the mirror and the scopebody
4.8 Stroke along edges
4.9 Push the mirror down while curing it with UV light. The reason for this is to not get any gap between the scopebody P1 and mirror.
See example that looks good!
4.10 Cure the adhesive glue with UV light for around 60s
4.11 With the mirror in place, seal it off with scopebody P3. Do this using two M1.2 setscrews.

a) *b)*

Fig. 9: (a) A drop of UV glue is added to the surface adjacent to the dichroic mirror, on the tip of a splice sleeve. It is also visible some glue is flowing beneath the DM, on the right side next to the slit. (b) Cotton Swab pressed against the DM while UV light is shined perpendicularly over it.

Step5. Glue the scan lens

5.1 Grab the uTLens around ¾ from the top with a tweezer
5.2 Place the scan lens in scopebody P1
5.3 Push lens down with cotton swab to really flush it and get it into position
Tips! The lens should be pushed lower than the circular edge!
5.4 Add drops of UV glue in the gap between the lens and scopebody
Caution! Be careful, do not add glue on the actual lens.
5.5 Cure the adhesive glue with UV light for around 60s

Fig. 10: Splice protector sleeve with a drop of UV glue being released on the gap between the miniscope and the scan lens.

Step6. Screw in the objective.

6.1 Screw the objective until it is almost does not move.
6.2 Then use a tweezer to tighten it further until there is no apparent gap between the objective and the scopebody.

Step7. Glue the MEMS scanner.

All the following steps regarding the gluing of the MEMS scanner should be performed in an as clean as possible environment. It is recommended to use a portable fume hood bench.

7.1 Place the assembled MINI2P miniscope back to the alignment holder.
7.2 Carefully loosen the MEMS scanner from its cover glass
7.3 Peel off any glue residues remaining on the edges
7.4 Place the MEMS at its intended position on the miniscope
7.5 The MEMS should be positioned such that the outgoing laser spot is completely circular
7.6 Prepare superglue and apply a thin layer of glue along the edges of the MEMS scanner along between the MEMS and miniscope
7.7 Leave the glue to dry for at least 1h.
7.8 Prepare some epoxy.
7.9 Apply a layer of epoxy over the entire visible part of the MEMS. Do not forget the top gap between the MEMS PCB and the miniscope surface.
Note! Applying epoxy will protect and fixate the MEMS additionally but also prohibit light contamination.
7.10 Leave it to dry overnight.

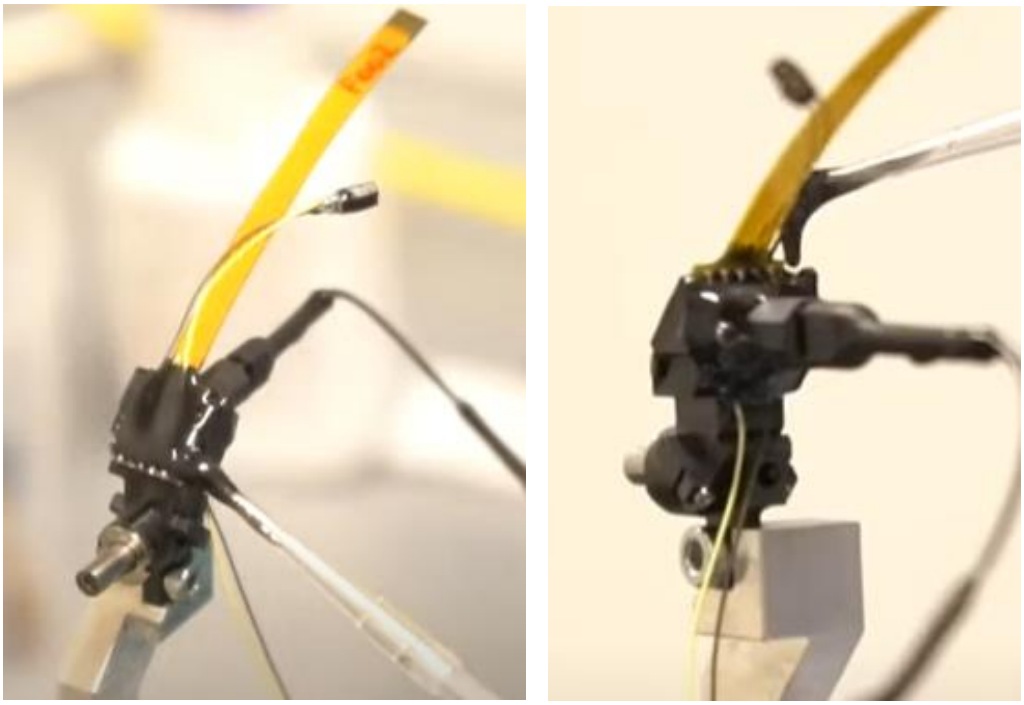

Fig. 11.: Finished miniscope after mounting MEMS into Scopebody P1 and glued with superglue (Step 7.6) and epoxy (7.9).

Step8. Mount stitching adapter and baseplate

8.1 Insert the stitching adapter onto the miniscope and tighten it with two M1.6 setscrews
8.2 Attach the baseplate again using two M1.6 setscrews.