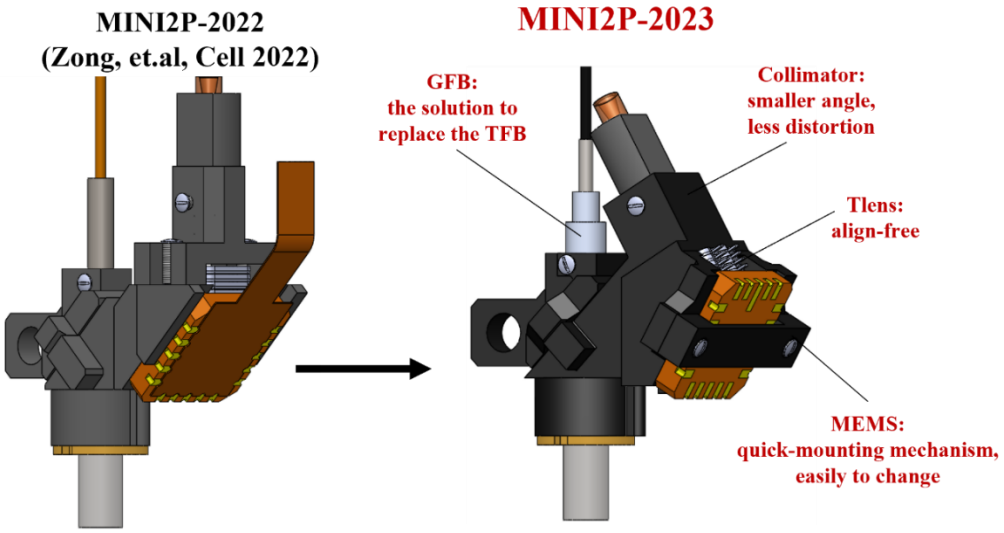
MINI2P 2023 – Miniscope assembly protocol

This protocol describes the assembly of the newest miniscope version, MINI2P 2023, illustrating its new features compared to MINI2P 2022. A table with useful equipment is also included. Preliminary cleaning and testing of parts, combined with the four main steps take approximately 2h to complete.

New features in MINI2P-2023



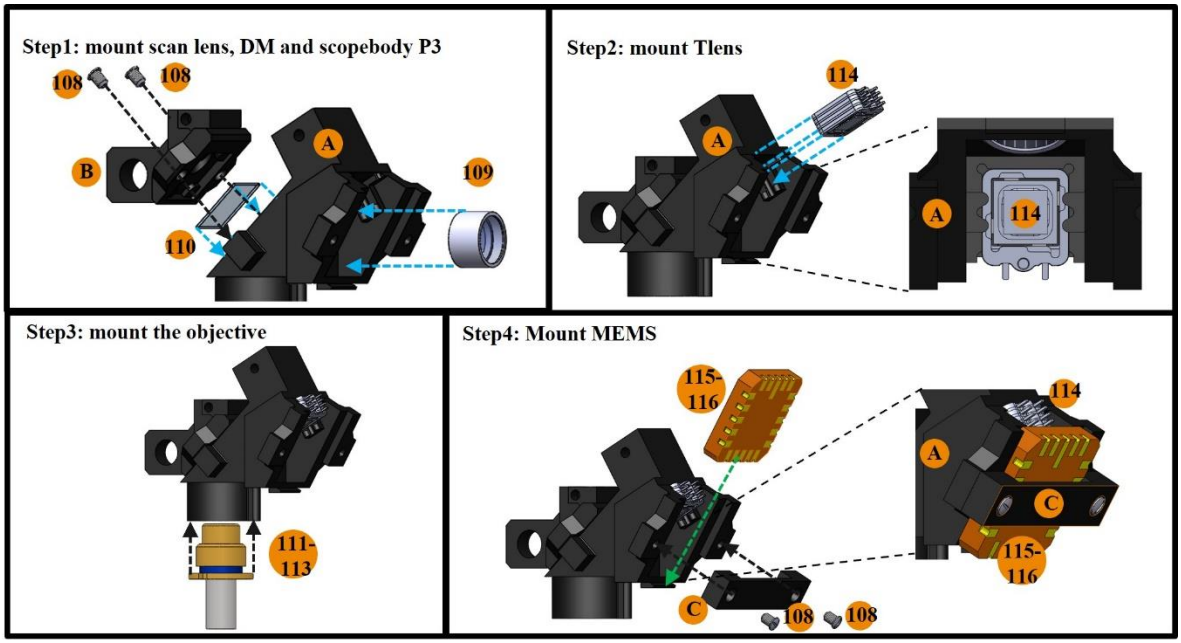
- A GRIN-connected fiber bundle (GFB) to replace the not-available TFB;
- Small incident angle of the collimator decreases the scanning distortion;
- Tlens can be mounted in-house and it is “align-free”;
- A quick-mounting mechanism for mounting the MEMS on the scopebody; MEMS can be easily changed if broken.
- Can be assembled in 2 hours;
- 8% less weight (2.23 g now).

MINI2P 2023 assembly tutorial video can be found on the link:
https://youtu.be/wqhDkIUZHgM

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

GitHub 2D/3D models under MINI2P_toolbox/Hardware/
New P1 – Shopping Machining List 2023 under MINI2P_toolbox/Protocols/

MINI2P 2023 key steps



Reagents and other useful equipment

Component Name	**Supplier**	**#Item**
Scopebody P1	3A prototype	**#A102_2023**
~~Scopebody P2~~	~~3A prototype~~	**Does not exist**
Scopebody P3	3A prototype	**#B104_2023**
MEMS locker	3A prototype	**#C118 (new!)**
TLens testing holder	3A prototype	**#D107 (new!)**
MINI2P holder P4 version 2	Thorlabs	**75 version 2**
Optical post	Thorlabs	TR75/M*
Post holders	Thorlabs	UPH75/M*
Locking ball	Thorlabs	TRB1/M*
TLens (4 stacked)	PoLight	114
TLens connector to MEMS	Polight/LabMaker	114
MEMS mirror (L or F)	Mirrorcle	115/116
MEMS PCB	LabMaker	117
MEMS connector to TLens	LabMaker/Polight	117
Scan lens	Domlight	109
Dichroic Mirror (DM)	Fuzhou Sunlight	110
Objective	Domilight	111/113
M1.2 cap screws (2 pcs) M1.4 cap screws (2 pcs)	-	108
Cotton Swabs	Chemtronics	k
Tack-It	Faber Castle	
Lens cleaning tissue	Thorlabs	j
Air duster	PRF 4-44	w
UV Adhesive glue	Thorlabs	l
UV curing gun	Thorlabs	e
Super Glue 415	Loctive	p
Silicone Adhesive	Winjee	o

Allen keys	-	
Splice protector sleeve	Thorlabs	m
Tweezers & scalpels	-	-
Ultrasonic bath	-	
Medical tape	-	
Handheld red laser	Thorlabs	f
Absolute ethanol	-	

Unless otherwise indicated, only 1 pc needed. *Variations of these items work too.

Preliminary steps before assembly

Prepare to mount the miniscope, but first it is recommended to both clean scopebody parts and test MEMS mirror and TLens. These steps must be done under a stereo-microscope and in a clean room.

i. Cleaning scopebody parts P1 (A102_2023) and P3 (B104_2023)

Cleaning scopebody P1 (item #A102_2023)

- ✓ With a scalpel, remove any chamfers sticking out.
- ✓ Cut surfaces where optical components will be mounted as flat as possible (Fig.1)

a) *b)*

Fig.1: Preparation of scopebody P1 surface to glue the MEMs onto. Cut excessive material with a blade in a single movement from the top (a) to the bottom(b). Photos of previous miniscope version, MINI2P 2022.

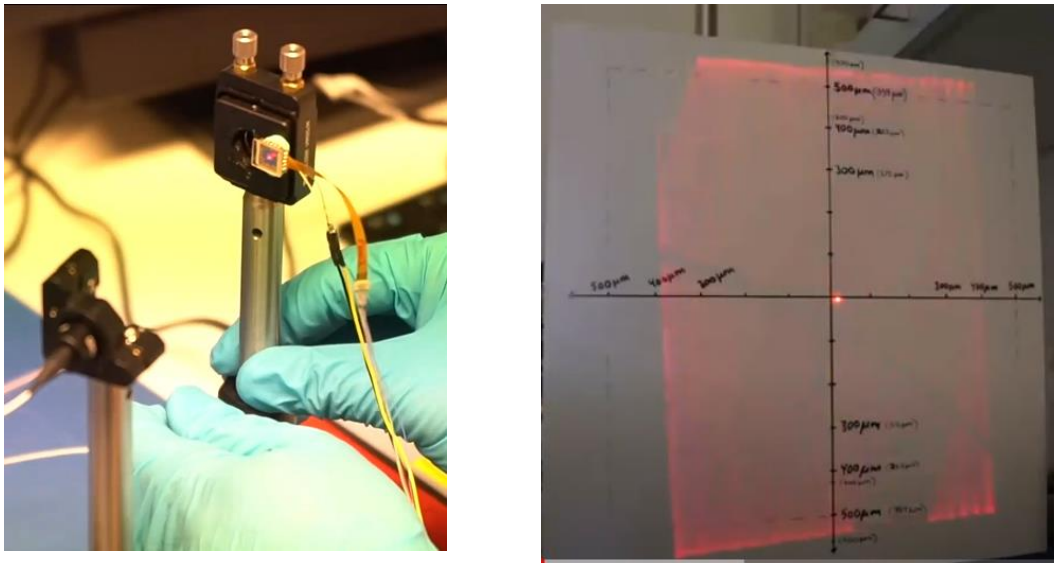
- ✓ Press with tack-it to remove any other residues, and clean scopebody P1 with a cotton swab dipped in 100% ethanol.
- ✓ Rinse all parts in 100% ethanol and clean them in an ultrasonic bath for 10min

ii. 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.2). This gives an estimation of the in-vivo FOV.

- ✓ Attach the MEMS scanner to the holder using tack-it. Then connect its wires. **CAUTION!** Make sure the power to the MEMS driver (BNC +5V) from the vDAQ to the controlbox is not connected.
- ✓ Connect BNC +5V
- ✓ Turn on the handheld red laser and adjust its holder until the light spot hits the center of the MEMS mirror (Fig.2a)
- ✓ Adjust the MEMS holder position until the reflected light spot hits the center of the calibration target axis (Fig.2b)

- ✓ Start the MEMS scanning using ScanImage. Select the scanning speed which is determined by pixels/line (e.g. 128 or 256) and click ”FOCUS.
  CAUTION! Always start with higher zoom (e.g., 2.0) and **NEVER change the zoom while scanning**, i.e. click Abort, change zoom and only after click ‘FOCUS’.



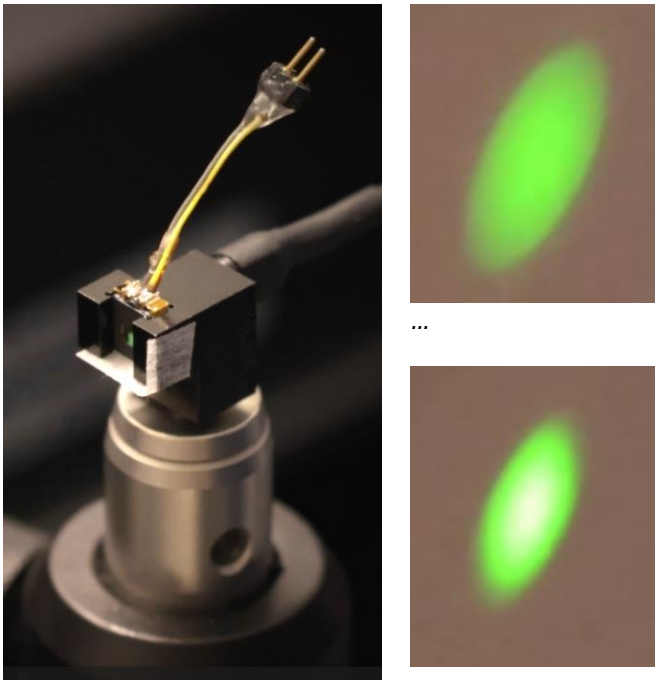
a) *b)*

Fig. 2: MEMS testing setup: red laser directed to MEMS mirror (a) and MEMS scanning pattern (b).

- ✓ The MEMS pattern should now appear on the calibration target (see Fig.2b).
  Note! The pattern will not be square in this case because the laser is hitting the MEMS with an angle different than 45°
- ✓ The expected in vivo FOV is estimated by the intersection with the smallest axis.
  Note! In Fig.2b) can be expected a FOV of around 400 um x 400 um for Fast MEMS

iii. Testing Tlens

- ✓ Attach TLens to Tlens testing holder sitting on a post (Fig.3a)
- ✓ Insert HC-920 assembly into TLens holder`s slit
- ✓ Connect cable to TLens driver (piezocontroller)
  CAUTION! TLens driver must be switched off before connecting.
- ✓ Turn on TLens driver and scrow the knobs to enable HV.
  CAUTION! Confirm 75V is the maximum voltage range selected, otherwise it can permanently damage the TLens.
- ✓ Change the focal plane via ScanImage by selecting the arrows in one direction in the right side of “Motors Control Menu” (from 0 to - 200). Observe the change of beam profile on a NIR detection card (Fig.3b). This means the TLens works!



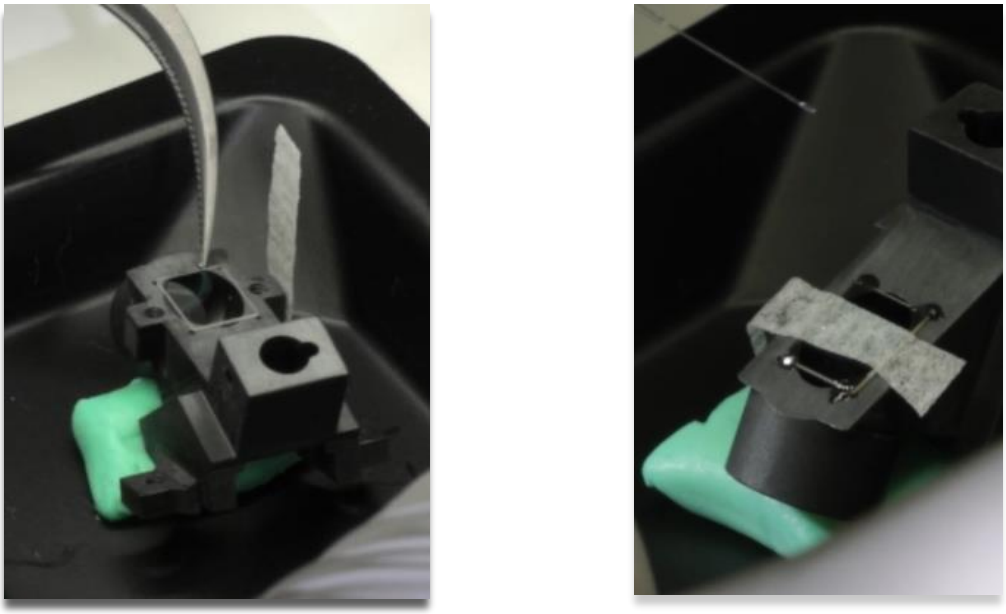
a) *b)*

Fig.3: Setup to test Tlens with HC-920 collimator holder on opposite side (a); Change of beam profile for two focal planes(b).

Step1. Mount scan lens, dichroic mirror and scopebody P3

1.1 Mount the dichroic mirror (DM)

- ✓ Clean DM coated surface (facing down towards white paper) with lens cleaning tissue soaked in ethanol and wrapped in a cotton swab.
- ✓ Cut some medical tape and fixate it to the side of P1 as shown in Fig.(4a)
- ✓ Place the DM with coated surface facing down, slightly above the holes and leaving space for the objective on the bottom side near the collimator slit.
- ✓ Apply small drops of UV glue to the four corners of DM using the splice sleever (Fig.4b)
- ✓ Cure it with UV source while pressing the DM with the cotton swab for ≈60s
- ✓ Remove the tape and clean surface with a cotton swab wrapped in lens tissue soaked in 100% ethanol.



a) *b)*

Fig.4: Mounting DM with coated surface facing down: correct position (a) and fixating applying small drops of UV adhesive glue on all four corners while tape holds DM in place (b).

1.2 Mount scopebody P3

- ✓ Attach scopebody P3 to P1 to cover the DM using two M1.2 screws
  Tips! Screw until tight but do not force it to avoid breaking the DM.

1.3 Mount scan lens

CAUTION! This step must be done before mounting TLens otherwise there is no space.

- ✓ Grab the Scan lens around ¾ from the top with a tweezer and insert scan lens into slit.
- ✓ Apply small drops of UV glue in the gap around the lens with a splice sleever (Fig.5)
- ✓ Cure it with an UV gun for about 60s

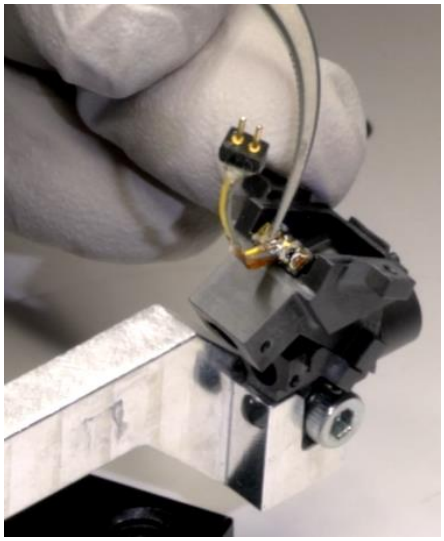
Tips! If there are any UV drops, first cure the UV glue and only then remove it with a tweezer in a parallel direction to the lens surface to avoid scratching it.

Fig. 5: Splice protector sleeve with a drop of UV glue being released on the gap between the miniscope and the scan lens.

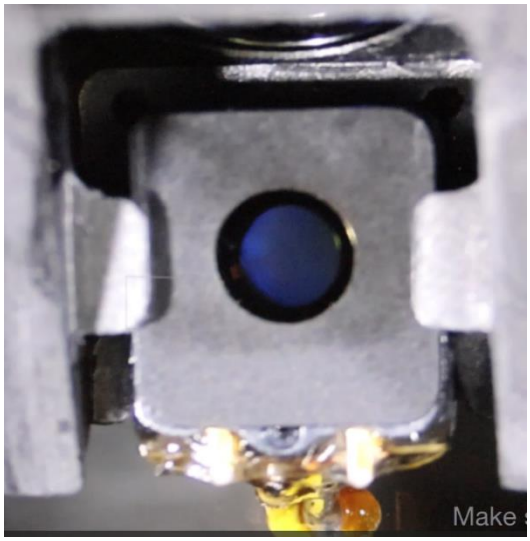
Step2. Mount Tlens

Steps 1.1 to 1.3 were similar to MINI2P 2022. However, Step 2: mounting TLens is novel and roughly “align-free”, done in-situ by the user instead of being sent to PoLight.

2.1 Gently remove protecting tapes on both sides of the stacked uTLens
2.2 Slide TLens into scopebody with coated surface facing down towards the HC-920 fiber and wires sticking outwards as in Fig.(6a). Ensure to push it all the way in as in Fig.(6b) as close as possible to the scan lens.



a)



b)

Fig.6: Mounting TLens: slide TLens with coated side facing HC-920 fiber collimator (a); top view with stacked TLens pushed all the way in, as close as possible to scan lens (b).

2.3 Add small amounts of UV adhesive glue around the edges of the uTLens.
2.4 Cure with UV gun for 60s
2.5 Add small amounts of super glue near the pins to fixate it better.
2.6 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.

Step3. Mount objective

✓ Screw the objective until it almost does not move.
✓ Then use a tweezer to tighten it further until there is no apparent gap between the objective and the scopebody P1.

Step4. Mount MEMS mirror

4.1 Attach the MEMS locker with two screws without fastening them
4.2 Place the MEMS on a sticky surface with the protective glass facing down.

4.3 In a direction perpendicular to the glue, detach one side of the MEMS from the protective glass glue, then the other and lift it up.
CAUTION! This step should be done very quickly (< 15s) or under a fume hood

4.4 Slide MEMS in between scopebody P1 and locker and fix the screws.

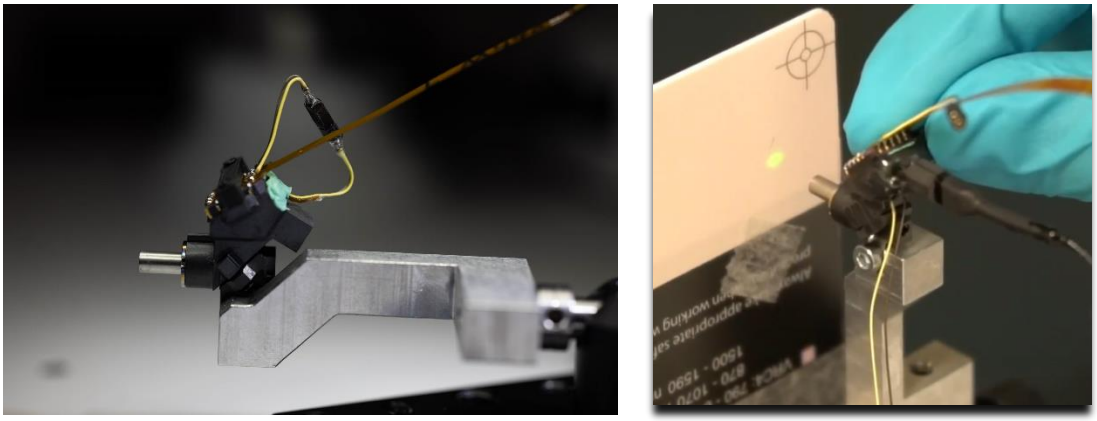
4.5 Cover the upper space between the MEMS and scopebody, sealing the TLens gap (Fig.6a) with tack-it

4.6 Attach the Tlens wires to the MEMS, matching wires colour (black to black and yellow to yellow), and connect the MEMS flex cable (PCB) to the 6-wires (black end facing clip)
CAUTION! Ensure TLens driver is switched off and +5V is not connected.
CAUTION! Ensure +5V BNC is not connected.

4.7 Power control box (+5V BNC vDAQ) and piezocontroller (activating HV)

4.8 Insert the HC-920 collimator into the scope. In ScaImage, open the laser shutter and increase power to 20%

4.9 Slightly loosen the screws of the MEMS brackets and adjust the MEMS position until the outgoing laser spot is completely circular and not trimmed (Fig. 7b).



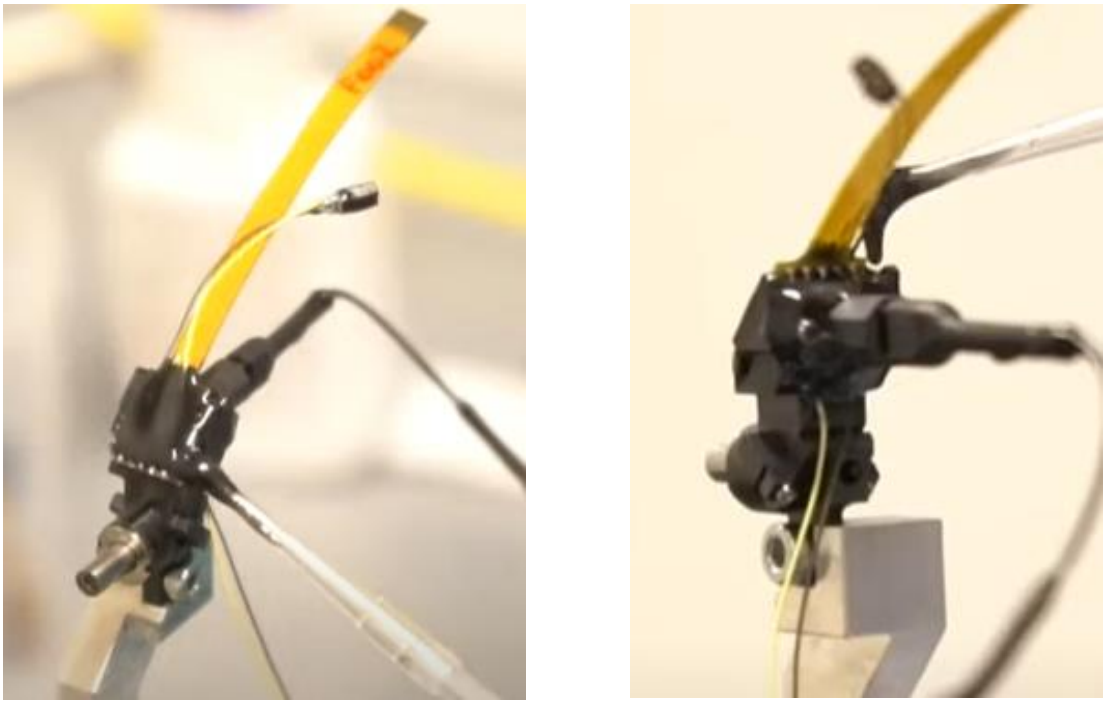
a) *b)*

Fig.7: Illustration showing the µTLens slit covered with Tack-it (a); finding optimal MEMS position with a Gaussian output, not cut (b).

4.10 Apply a thin layer of super glue along the edges of the MEMS scanner along between the MEMS and miniscope

4.11 Leave it to dry for 5min

4.12 Add silicone covering the entire visible parts of the MEMS in front (Fig.8a) and back (Fig.8b)



a) b)

Fig. 8.: Finished miniscope after mounting MEMS into Scopebody P1 and covering it with silicone: front (a) and back views (b).

Tips to replace MEMS mirror when necessary to swap by another type and/or it is damaged:

- ✓ Start by removing the whole coverage of silicone glue with a tweezer and scalpel
- ✓ Insert the scalpel blade in the bottom surface to open a gap between the MEMS mirror and the scopebody P1. Force it to slowly detach each side
- ✓ Then push the MEMS holding simultaneously the main body and PCB in one movement.
- ✓ Clear all remaining glues after removed MEMS.