# P2– System building protocol

## Part 1- Core Optics Assembly

**Reagents:**

- Super glue (Loctite, 415)
- Epoxy adhesive (item p)
- 100% alcohol
- Air duster Green (item w)
- UV curing optical Adhesive (item l)
- Rubber spray (black, item x)

**Equipment:**

- 2 Tweezers (preferably one flat & one curved)
- Splice Protector Sleeve (item m)
- Lens cleaning tissue (item j)
- Cotton buds & swabs for electronics (item k)
- Heat shrinkage tubings (black, item 7)
- Heat gun (item q)
- Hollow-core 920 nm fiber (HC-920) ≈ $2.5m$ (item 31)
- Screwdrivers
- Stereomicroscope (Zeiss Trino, WL37166)
- UV curing LED system (item e)
- Components of the coupling module (Table1)
- Components of the fiber coupling setup (Table 2)
- Components of the fiber collimating setup (Table 3)
- Components of the laser alignment (Table 4)
- Components of the detection module (Table 5)
- Components of the controlling module (Table 6)

**Table 1: Components of the coupling module**

| **Component Name** | **Amount** | **Item Number (see S1, shopping list)** |
|---|---|---|
| Breadboard | 1 | 1 |
| Sorbothane Feet | 1 | 2 |
| Seed laser | 1 | 29 |
| Coupling box | 1 | 21 |
| Coupling box cap | 1 | 22 |
| Glass rods | 3 | 32 |
| Prisms | 8 | 34 |
| Rotation mount | 1 | 5 |
| Half-wave plate (HWP) | 1 | 41 |
| Adjustable mirror mounts | 2 | 4 |
| Protected silver mirrors | 2 | 42 |
| Cap screws | 4 | 6 |

| | | |
|---|---|---|
| Coupling protection | 1 | 24 |

**Table 2: Components of the fiber coupling setup**

| | | |
|---|---|---|
| Coupling holder | 1 | 23 |
| Coupling lenses | 1 | 40 |
| Glass Flange | 1 | 33 |
| Spanner Wrench 1 | 1 | a |
| XYZ translation stage & right-angle bracket | 1 | 9 |
| Fiber clamp | 1 | 11 |
| Pulse check Autocorrelator | 1 | r |
| Base to fixate Autocorrelator to Breadboard | 1 | s |

**Table 3: Components of the fiber collimating setup**

| | | |
|---|---|---|
| 3 axis microblock stage | 1 | 8 |
| Fiber Clamp | 1 | 11 |
| Glass Flange | 1 | 33 |
| Fiber stripping tool | 1 | b |
| Collimator Holder | 1 | 26 |
| Collimating Lenses | 1 | 43 |
| Collimator assemble tool | 1 | 25 |
| Fiber Cleaver | 1 | c |

**Table 4: Components of the laser alignment**

| | | |
|---|---|---|
| Handheld laser source (635nm) | 1 | f |
| SM05 threaded adapter to laser source | 1 | 13 |
| Power and energy meter | 1 | g |

**Table 5: Components of the detection module**

| | | |
|---|---|---|
| Kinematic cage cube | 1 | 14 |
| Kinematic cage cube base | 1 | 15 |
| Emission filter 525 nm green channel | 1 | 35 |
| Emission filter 630 nm red channel | 1 | 36 |
| Shortpass filter | 1 | 37 |
| Dichroic Mirror | 1 | 38 |
| Lens Tubes | 4 | 16 |
| End cap for machining | 1 | 17 |
| Spanner Wrench 2 | 1 | d |
| Coupler | 3 | 18 |
| Optical beam shutter with controller for PMT | 1 | 19 |
| Post mounting adapter | 1 | 20 |
| Green LED | 1 | h |
| Aspheric Condenser Lens | 3 | 39 |
| µTlens driver | 1 | 51 |
| PMTs | 2 | 52 |
| Controller for shutter | 1 | 53 |

**Table 6: Components of the controlling module**

| | | |
|---|---|---|
| Control box shell | 1 | 27 |
| Control box cap | 1 | 28 |
| MEMS driver (controller) BDQ PicoAmp 5.4 T180 | 1 | 44 |
| Connectors J1,J2 | 2 | 44 |
| BNC male to BNC male | 8 | 95 |
| **DSUB15 to 8xBNC cables** | | |
| DSUB15 connector plug (male) | 2 | 46 |
| back shell | 2 | 47 |
| DSUB15 connector socket (female) | 2 | 48 |
| **DSUB15 to 6-pin (pcb/mems) cable** | | |
| 6-pin connector for MEMS | 1 | 49 |
| Single wire cables | 6 | 50 |

**Cleaning and preparing all components.**

**Step1.** All the custom machined components are cleaned with 100% alcohol. Some components are also cleaned in an ultrasonic bath for 10min including the coupling holder (item 23), collimator holder (item 26), and the glass flanges (item 33).

**Step2.** Spray all the cleaned components in Step 1 with Air duster (item w) and put them aside in a super clean bench.

**Assembling the coupling module.**

**Step3.** Put one half-wave plate (HWP, item 41) into the rotation mount (item 4) and screw the mount from the bottom of the coupling box using a M3 cap screw.

**Step4.** Fix the seed laser source (item 29) and the coupling box (item 21) on the breadboard (item 1) with four sorbothane feet mounted underneath (item 2). Make sure the entrance of the coupling box is centered to the output of the laser source

**Note!** To test the laser before using it, align its beam path to the Pulse Check autocorrelator (item r) to optimize the settings to remove the profile side lobes and minimize the pulse fit ($\approx 100fs$) and measure the maximum average power ($\approx 1.2W$@ 5V).

**Step5.** Place right-angle prisms (6 prisms, items 34) on the intended positions inside the coupling box (Fig. S7-B in *Zong, et al.,2022,* and Fig.1 in this document). Attach the surfaces of the prisms to the inner wall of the coupling box as close as possible. Then add drops of super glue in the contact points. Hold the prisms for a few seconds until they are fixed.

**Note!** Switch on the laser, place NIR Detection Card after the glued prisms to confirm the prisms were glued correctly, leading to an aligned laser beam path as designed.

**Step6.** Glue three glass rods (items 32) on the designed positions inside the coupling box (Fig. S7-B in *Zong, et al.,2022,* and Fig. 1 in this document) Check the laser spot after the glass rods. If centered, drop super glue on contact points of the box's base and the glass rod and press the latter.

**Step7.** Make two prism assemblies by gluing a prism (item 34) on top of a thick circular silver mirror (item 42). The thick circular silver mirror acts as an adapter to hold the prism on the mirror mount (see Fig. 2).

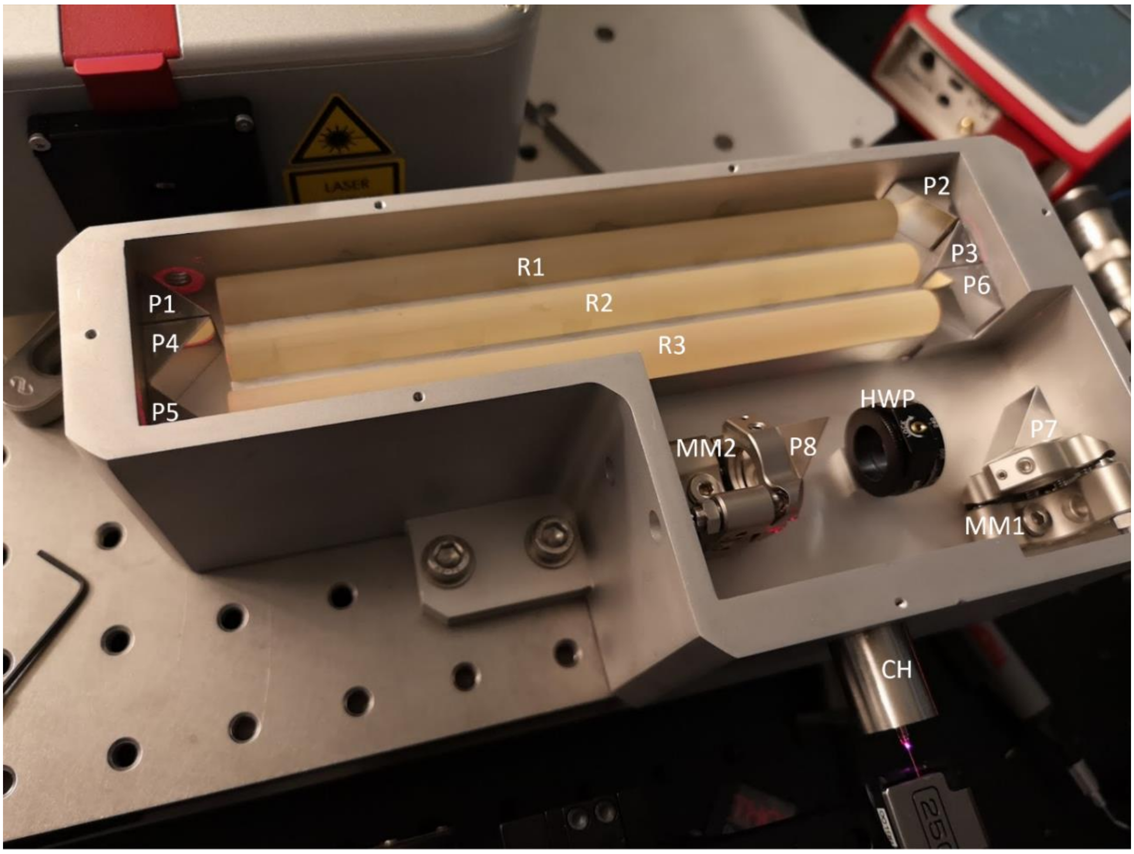


*Figure 1: Placement of coupling box in front of the seed laser, illustrating the components assembled inside as described in this protocol. The right-angle prims are labeled from P1 to P8, glass rods from R1 to R3 glass, Half-wave plate as HWP, mirror mounts as MM1 and MM2 and, finally, coupling holder as CH.*

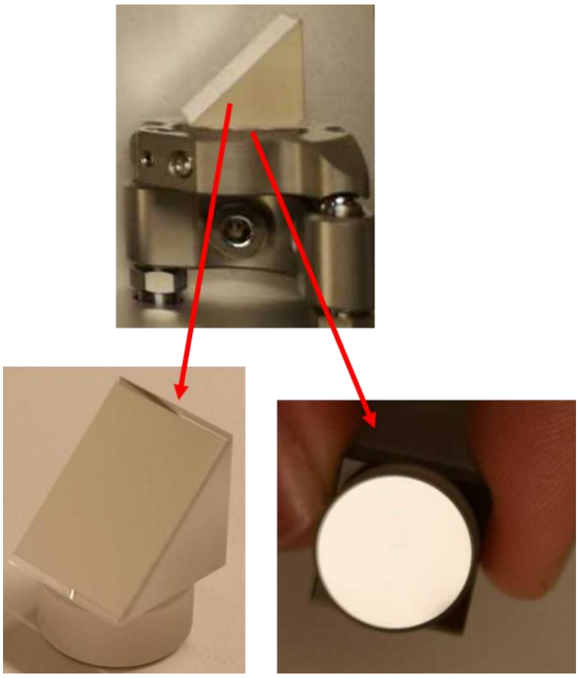


*Figure 2: Mirror mount fixated within the coupling box (top) with a schematic of the gluing of the prism on top of a silver mirror (bottom left) with prism centered on the mirror (bottom right).*

**Step8.** Fix the two mirror mounts (item 4) to the coupling box's base by tightening two M4-screws. The two Hex Adjusters of each mirror mount should face to the two diagonal holes on the vertical walls of the coupling box, such that adjustment of the mirrors' tilt/pitch can be done from outside of the box.

**Step9.** Mount the two prism assemblies made in Step 7 on the mirror mounts. Switch on the laser, place a NIR Detector Card before and after P7 and P8 (see Fig.1) to check the alignment of the light path. The laser should pass through the exit hole of the coupling box. If not, adjust mirror mounts until you see the laser from the output hole.

**Step10.** Mount the coupling lens (item 40) to the coupling holder (item 23) (see Fig. S7-B in *Zong, et al.,2022*) and fix the coupling holder onto the coupling box by screwing it from the outside.

**Preparing the HC-920 fiber assembly**

**The HC-920 assembly building video tutorial can be found on the link:**
https://www.youtube.com/watch?v=HjAtoPbDu8E

**Step11.** Cut about 2.5-meter HC-920 (item 31).

**Caution!** Throughout this and next steps, keep the fiber straight, always without nods, to not break or damage it.

**Caution!** Extra length at both ends of the fiber are added because multiple cuts are usually needed before obtaining a very flat and clean tip.

**Step12.** Prepare ~ 1-meter heat shrinkage tube1 (item 7). Slide the HC-920 fiber prepared in step11 inside the tube1. Attach tube1 with medical tape ≈10cm away from one of the ends to hold it in place for a temporary fixation.

**Caution!** Leave enough space (approx. 10 cm) between the top edges and the tube1 to avoid cutting the jacket if a flat tip was not obtained.

**Step13.** Slide the prepared HC-920 into the Coupling protector (item 24). Add two pieces of short heat shrinkage tubes with length of 10 cm for tube1 and 2 cm for tube2 (items 7) - see Fig. 3.

**Caution!** Must put the coupler protector before put shrinking tubes on.

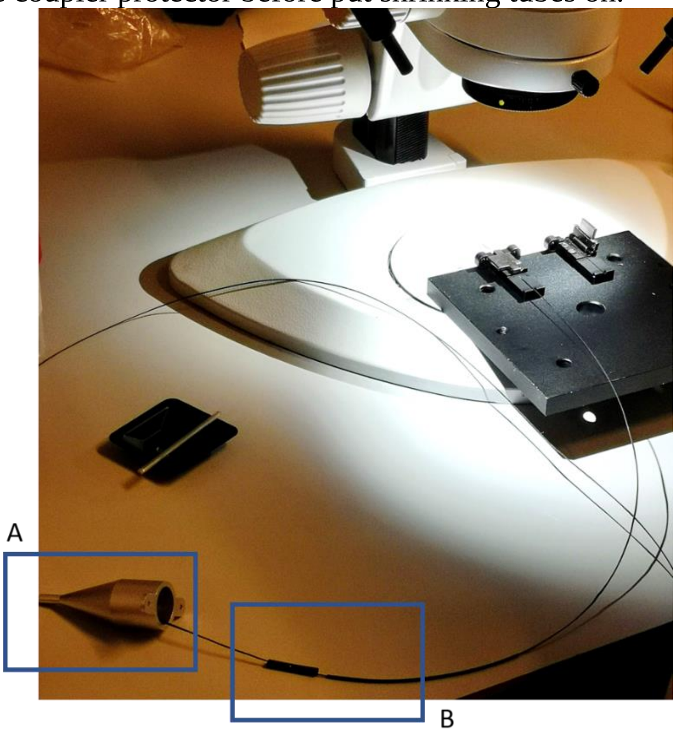


*Figure 3: Preparation of HC-920 fiber assembly: before cutting the fiber tips (steps 15-16), slide (A) the coupling protection; (B) heat shrinkage tubes through the HC-920 fiber.*

**Step14.** Prepare the other end to the collimator with three pieces of shrinking tube: 5cm of tube1, 2cm of tube2 and 1.5cm of tube3 (see items 7). Slide them over the HC-920.

**Step15.** Heat-shrink the longest jacket, tube 1 using heat gun at around 250°C. Start from one end and hold the gun for 1-2 seconds while sliding your fingers to both ends.

**Caution!** Handle the heat carefully – it is hot ($T_{\text{heat gun}} > 200°C$)!

**Caution!** Avoid radiating the heat gun into fiber for too long because it can damage it.

**Step16.** Remove about 2cm protecting claddings of the HC-920 on both ends by using a fiber stripping tool (item b). Clean with a paper tissue dipped in 100% ethanol.

**Step17.** Cut both ends of the fiber with the fiber cleaver (item c) based on the operating manual provided by Thorlabs.

**Note!** About 5 mm uncladded fiber should be kept on both ends (Fig. S8A-C). Check the ending tip quality under the stereoscope (see Fig. 4). If the ending tip is not flat and clean, re-do steps 15-16.

**Note!** You may also need to shorten the jacket to exposure enough length of fiber on the ends.

**Step18.** Slide a cleaned glass flange (item 33) on one of the prepared ends of the HC-920 with a curved tweezer (see Fig.5-A). Stop sliding the glass flange as soon as the end of the fiber tip is slightly sticking out at the other end of the flange (see Fig.4-A).

**Caution!** Do not touch the tip of the fiber, as this may damage it.

**Step19.** Add UV-glue using a small fiber tip (see Fig.5-B) to the funnel at the root of the Glass Flange. Wait for the glue to flow up. Repeat until the gap between the HC-920 and the glass flange is filled with glue, then curing the UV glue with UV light.

**Step20.** Prepare the other end of the fiber as in steps 17-18.

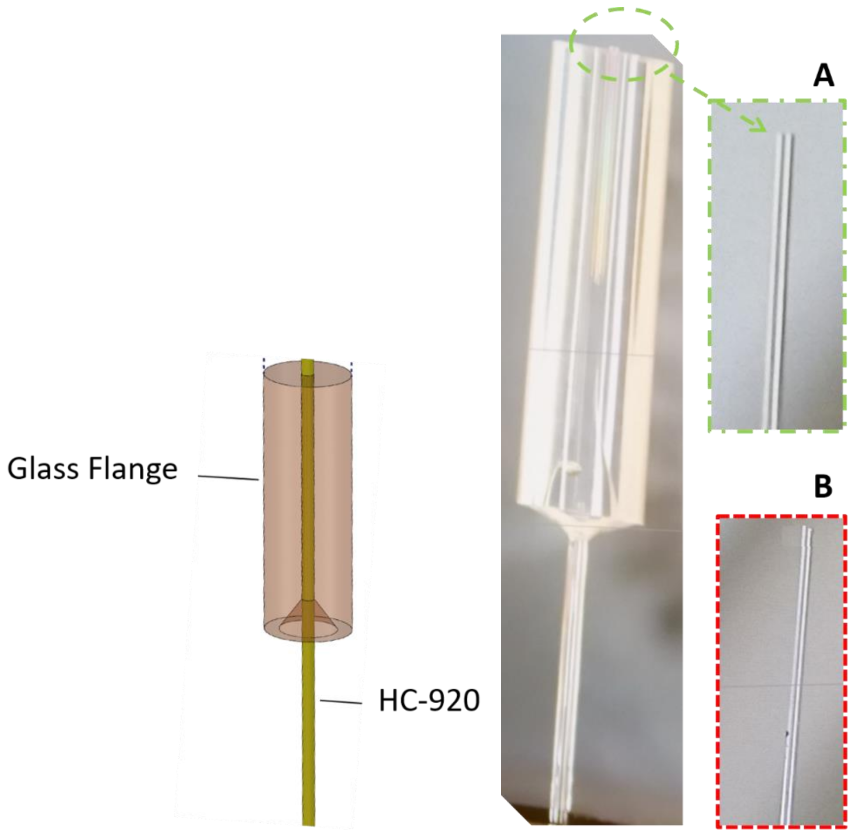



*Figure 4: Schematic of HC-920 fiber inserted into glass flange with tip sticking out on the top side of the Glass Flange (left). Illustration of (A) a good fiber tip; (B) a bad (not flat) ending tip.*

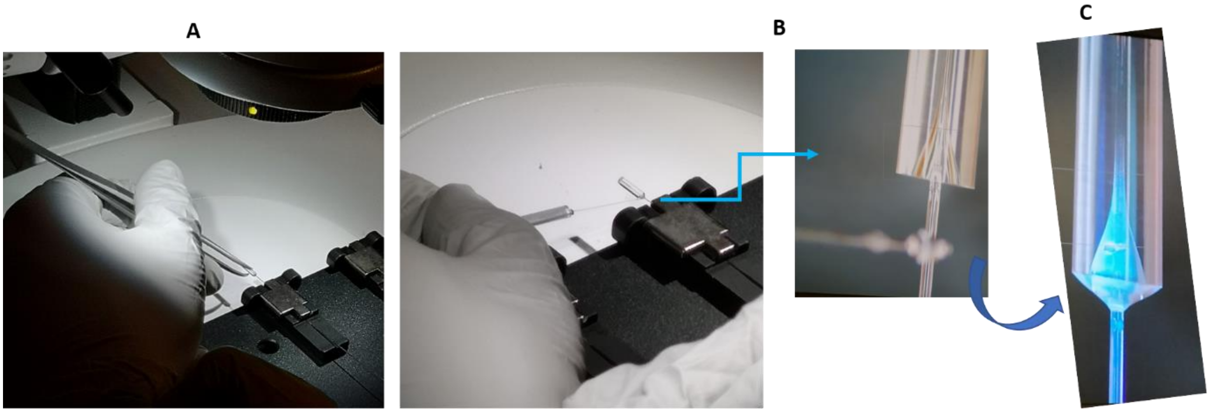



*Figure 5: Assembling of a glass flange to one of the ends of the HC-920 fiber: (A) Insertion of a cleaned glass flange around the prepared end of the fiber using a curved tip tweezer; (B) gluing of the flange to the fiber by adding glue to the funnel at the root of the glass flange using a small fiber tip; (C) curing process with UV light.*

## Preparing the fiber collimator

**The video tutorial can be found on the link:**
**https://www.youtube.com/watch?v=HjAtoPbDu8E**

**Step21.** Fix the collimating lens (item 43) into the collimator holder (item 26) by using optical adhesive (item l) (see Fig. 6)

**Caution!** Pay attention not to drop glue on top of the collimating lens. Also, do not use too much glue because it can flow down to the hold for the glass flange. To prevent this, flip the collimator holder and cure the adhesive on the other side as well (item e).

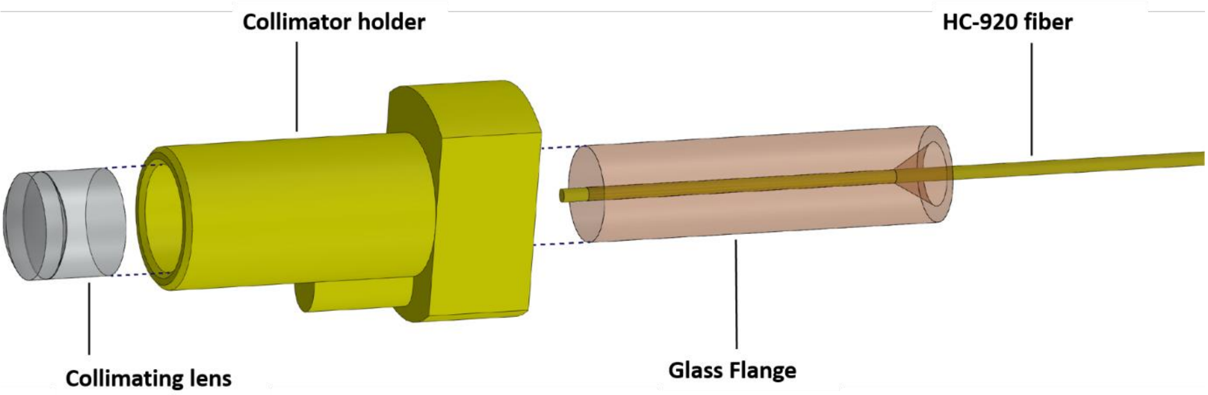



*Figure 6: Schematic showing the four different components making up the assembling of collimator to the HC-920 fiber. A distance between the HC-920 fiber tip and collimator lens is optimized to attain collimated light when the laser beam that originates from the HC-920 fiber passes through the lens.*

**Step22.** Temporarily fix the collimator holder on the fiber coupling stage with collimator assemble tool (items 10 and 25) as shown in Fig. S8E-F in *Zong, et al.,2022* & Fig.7 in this document. At the same time, fix the glass flange in the clamp (item 11). Align the center of the glass flange with the center of the collimator holder, and slowly slide the glass flange into the collimator holder, by using the 3-axial adjustment on the stage (item 8) (Fig.S8E in *Zong, et al.,2022*).

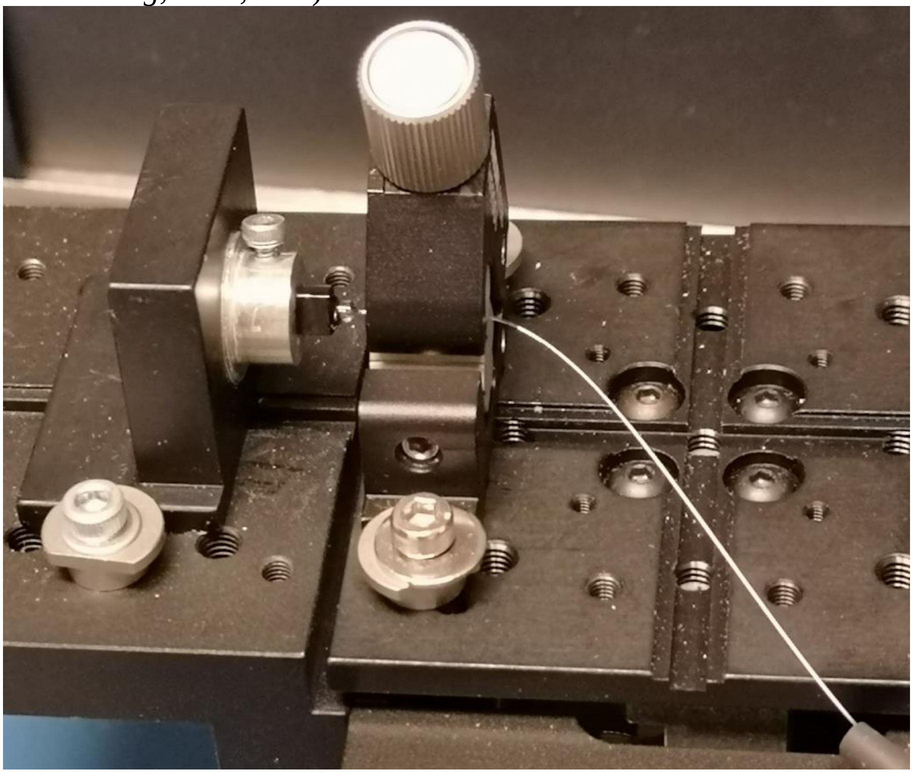


*Figure 7: Collimator holder fixated on the fiber coupling stage (left) with end of HC-920 fiber inserted in the clamp and glass flange inserted in the collimator holder. This also illustrates a section of the wider jacket (bottom right side).*

**Couple the laser light into HC-920 fiber (the video tutorial will be provided soon…).**

**Step23.** Place another fiber coupling stage in front of the coupling holder (item 11) which is attached to the coupling box (Fig.S8D in *Zong, et al.,2022* & Fig.8 in this document).

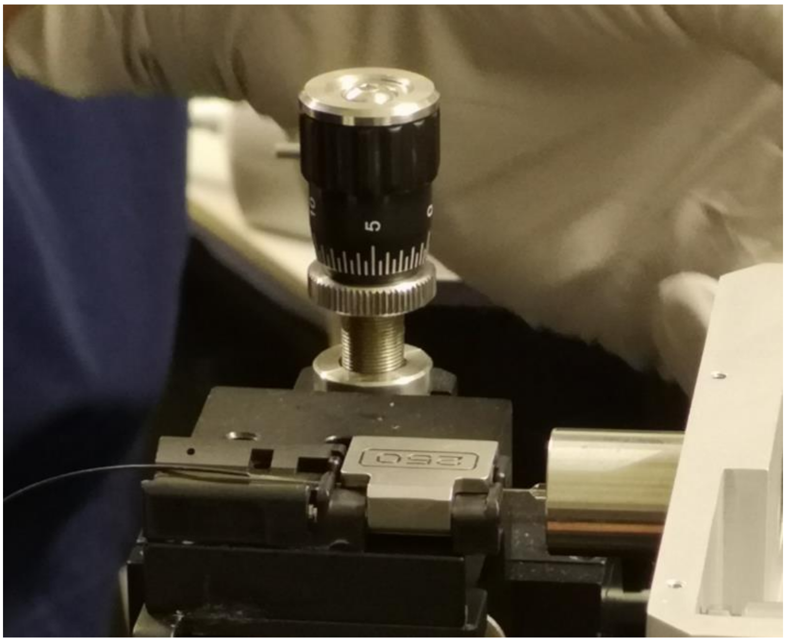


*Figure 8: Fixation of glass flange into the clamp and insertion of the flange into the collimator holder.*

**Step24.** Monitor the laser power by using a power meter (item g) from the output of the collimator. Adjust the mirror mounts in the coupling box and the distance between the glass flange and the coupling lens until the output power reaches about 70% of the input power (measured in front of the coupling lens) – see more details of this on Steps 24-25 of Laser alignment.

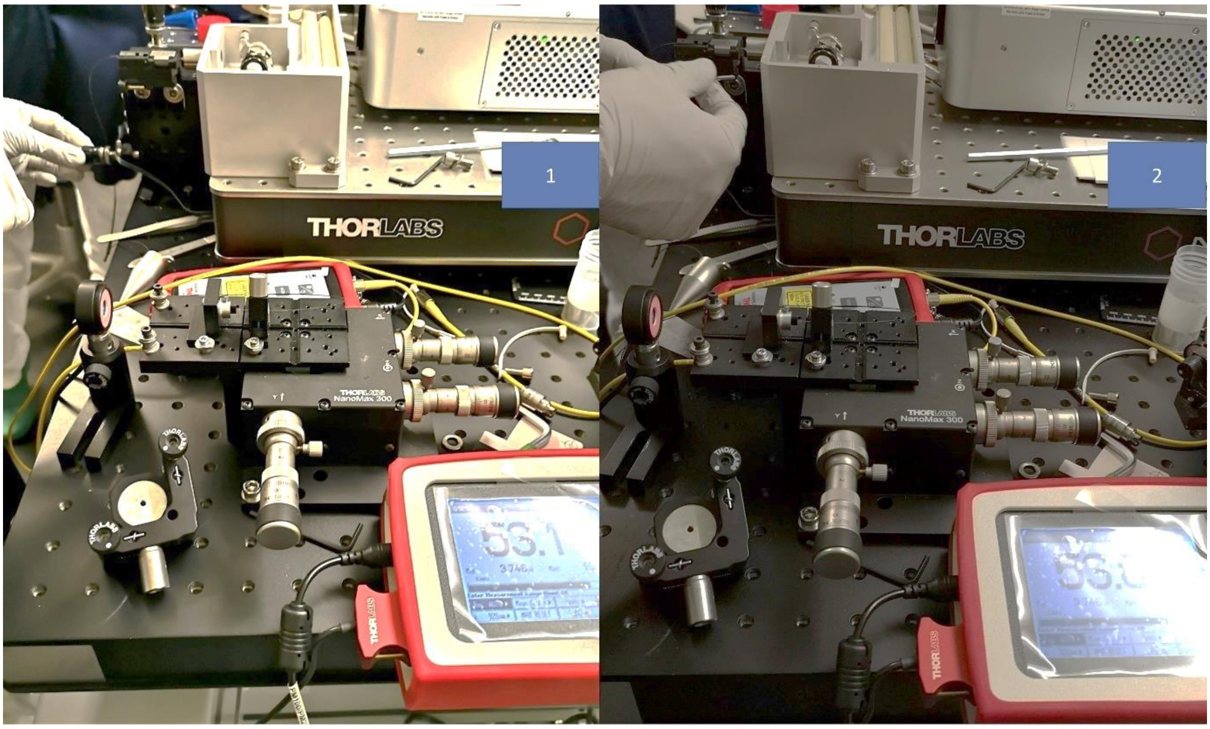



*Figure 9: Illustration of both ends of HC-920 fiber placed on stages: first end in front of the coupling holder (top left side of both figures) and the other end aligned to the collimator holder. This figure also demonstrates the monitoring of the laser power using a power meter in front of the collimating setup. In this step, by adjustment of the distance between the glass flange and the coupling lens (Step22-1, left figure) and of the mirror mounts (Step 22-2, right figure), an ≈6% increase of power is demonstrated*

## Laser alignment

**The video tutorial can be found on the link: https://www.youtube.com/watch?v=HjAtoPbDu8E)**

**Step25.** Mount SM05 threaded adapter (item 13) into stage mount (item 10). Connect patch cable of the handheld red laser source (item f) to threaded adapter.

**Step26.** Adjust the two mirror mounts (MM1, MM2 of Fig.1) of the coupling box back and forth until both red laser (item 13) and green laser output overlap all the way to the coupling box entrance hole. Remove then handheld laser source and replace it by the photodiode power sensor (item g).

**Step27.** Adjust the stage that supports the HC-920 glass flange. Slide the flange inside the coupling holder and stop when the maximum power is reached (see Fig.9-1).

**Step28.** Adjust the two mirror mounts (MM1, MM2 of Fig.1) of the coupling box back and forth, i.e., start with one of the mirror mounts and adjust the top screw while the power increases, and stop when it decreases (see Fig.9-2). Continue with the other mirror mount, also adjust the top screw, and repeat this process to maximize the power.

**Step29.** Repeat Step 27, but for the latter adjust bottom screws of both mirror mounts.

**Step30.** Adjust the HWP to further maximize the output power after the HC-920 fiber.

**Step31.** Glue the glass flange to the coupling holder by initially adding small amounts of optical adhesive (item l) to cover the gap between the two. Cure it with UV light (item c) for 60s to fixate it. Fill the gap with more adhesive and cure it again for 100s.

**Step32.** Open the clamp and move the stage z-axis down, away from the fiber. Slide heat shrinkage tube 2 (item 7) up, until it covers the glass flange, and use heat gun (item q) to shrink it. Add epoxy (item p) to the contact points.

**Caution!** When moving the stage down, first do this with the small cursor while monitoring the power, to make sure it is stable. When the stage is far enough from the HC-920 fiber coupling end, move it away.

**Step33.** Slide the coupling protector (item 24) over and fixate it to the coupling box with M2 screws (item t). Then slide heat shrinkage tube2 such that it enters the coupling protector and use epoxy to fixate it well to the coupling protector (see Fig. 11-1).

**Step34.** Move the stage under the glass flange (collimator side) away or closer, until the output beam is collimated (see Fig.S8-G).

**Note!** Check the shape of the spot at the exit of the collimator (Fig.10-A) by following the green laser beam with a NIR detector card and fixate the latter ≈2m away from the collimator. A very sharp hexagonal-star-shape spot with a gaussian-profile-like intensity distribution in the center, as show in Fig. 10-B, should be visible.

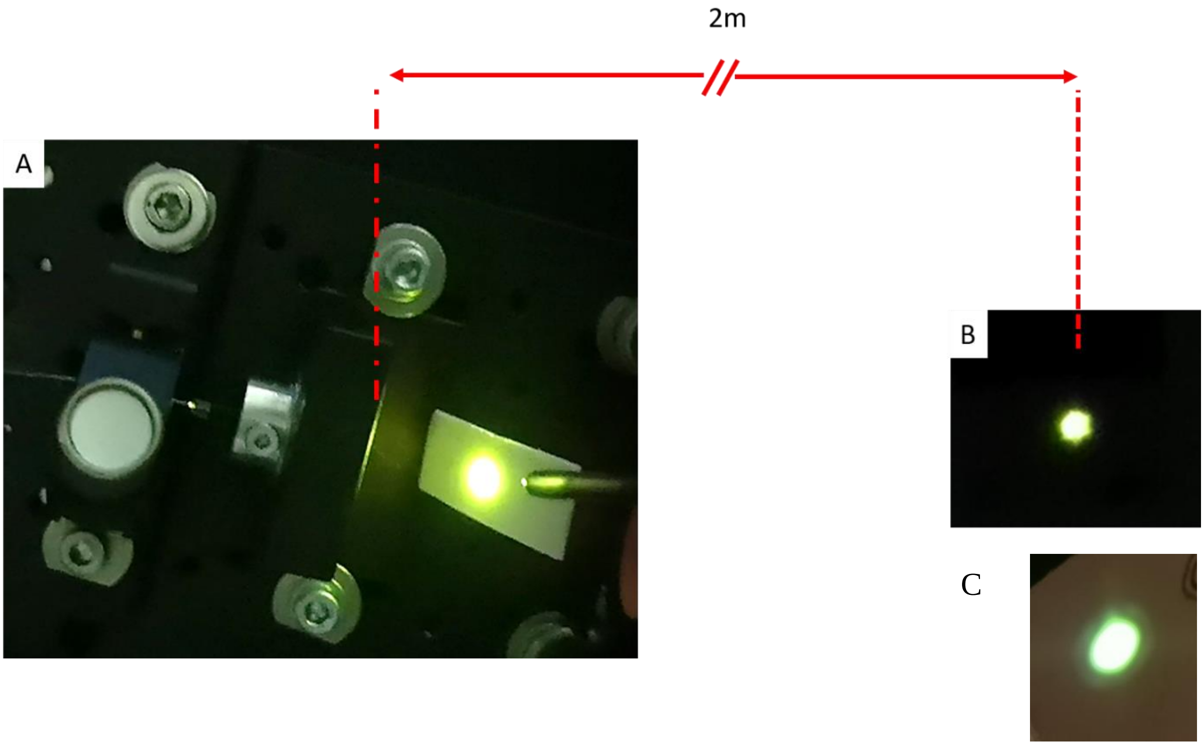



*Figure 10: Laser output shining on a NIR detection card placed in front of the collimator (A) and 2m away on a nearby wall (B), confirming the light is collimated after passing through the coupled HC-920 fiber and collimator for K50-060-70;* ***(C) output after K60-60-PM (new version of HC-920 provided by NKT fiber in 2023).***

**Step35.** Fixate the glass flange to the collimator holder adding small amounts of optical adhesive (item l) and cure it for 60s.

**Caution!** Ensure that the laser spot described in Step31 does not change during gluing.

**Step36.** Repeat Step34, adding more adhesive. Cure it for 100s.

**Step37.** Slide the three thickness heat shrinkage tubes (item 7) of different thicknesses towards the collimator holder and add epoxy to hold them: 1$^{st}$ tube1 to cover the fiber without the jacket; 2nd tube2 to cover the glass flange; (3rd) tube3 to cover the gap of HC-920 end and the collimator holder base (see Fig.11-2).

**Note!** For more details, check video tutorial HC-920 assembly, especially regarding the technique to heat shrink the three thickness tubes.

**Step38.** After the epoxy is dry (overnight), remove the collimator stage.

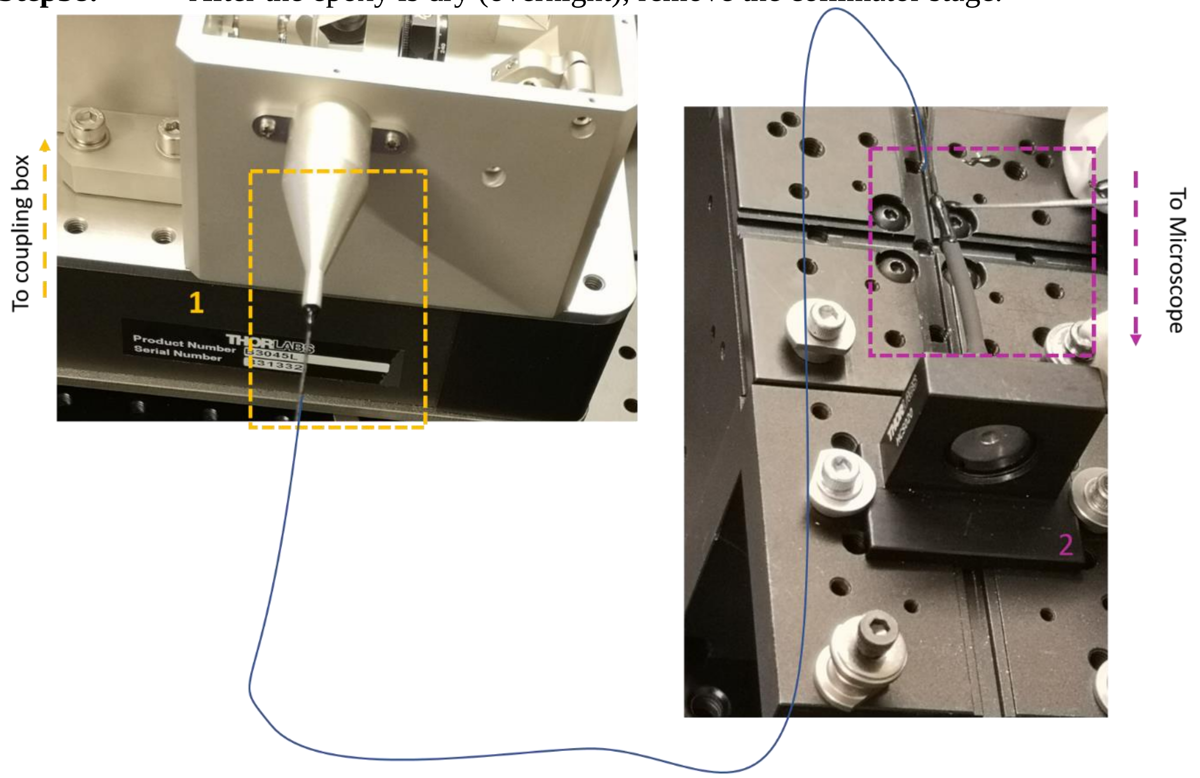
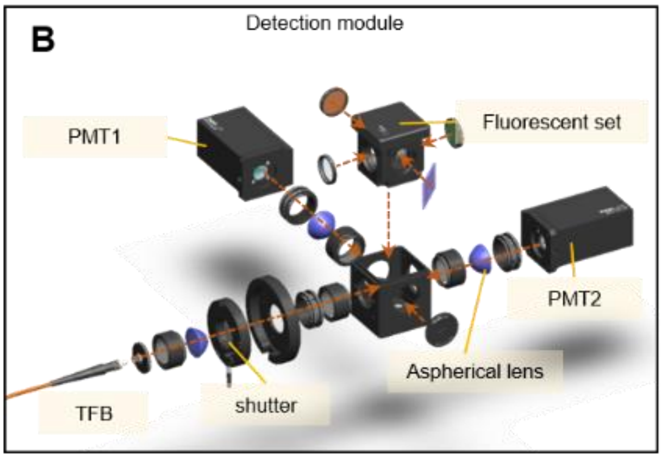



*Figure 11: Illustration of the fiber's ends protected with jackets and connected to (1) coupling box, covered also with coupling protection; (2) collimator holder and epoxy is placed on top to join the jackets.*

## Assembly of the detection module.




Taken from Fig.S7-C in Zong et al., 2022

Prepare the Cage Cube for Fluorescence Filter Sets

**Step39.** Separate the removeable insert (item 14) from its base (item 15) (see Fig.S7-C in *Zong et al., 2022*)

**Step40.** Open the Cube Top removed on Step38 by untightening the two retaining screws (3/32" hex) and remove half-part of the cage

**Step41.** Untighten the top two screws with a Phillips #00 screwdriver to loosen the leaf spring and slide the dichroic mirror (item 38) under the clamps (see arrows in Fig.12-A). Place the coated side of the mirror facing the direction from where the laser beam comes from. Tighten the screws enough to hold without breaking the mirror (see Fig.12-B).

**Caution!** Do not touch the mirror, so best is to place it on the slit holding a tweezer from the corner.

**Caution!** To confirm which side is coated, place a tweezer with the tip very close to the mirror surface and choose the side which does not have a ghost reflection on top of the real reflection.

**Caution!** Only light force should be applied to tighten the mirror to prevent it from moving.

**Step42.** Mount back the other half of the cage and prepare the filters to assemble on the next steps (see Fig.12-C)

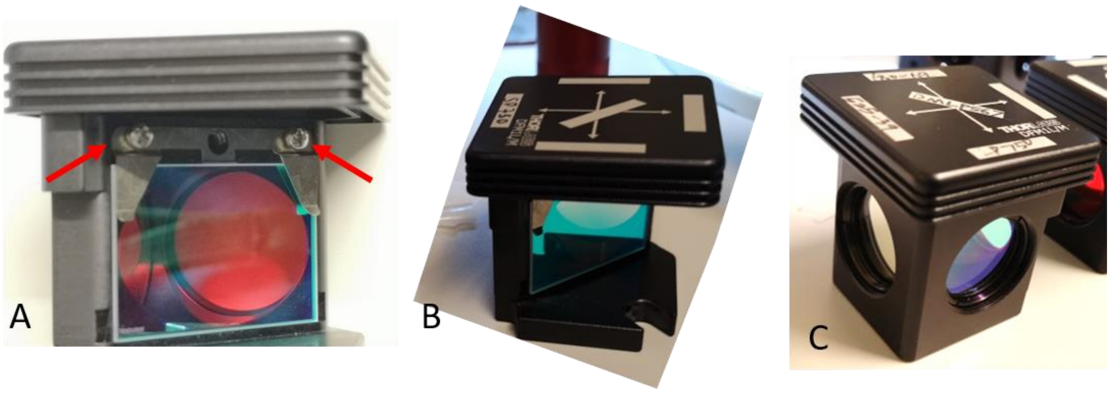



*Figure 12: Kinematic cage mounting steps for assembling the dichroic mirror inside the removeable cube top (A-B) and filters on the side ports (C)*

**Step43.** Place the cage rotated 90 degrees from its standing position with the frontside filter port facing up.

**Step44.** Remove the threaded retaining ring using either a tweezer or Spanner Wrench 2 (item d) by rotate it anti-clockwise.

**Step45.** Insert the shortpass filter (item 37) with the coated side facing up and outwards, i.e., direction of beam light.

**Caution!** Follow tips of Step40 to identify the coated side.

**Step46.** Place the threaded retaining ring on top of the filter and with the Spanner Wrench 2 slightly tilted, rotate it clockwise until the ring is all the way down, in contact with the filter.

**Caution!** Make sure there is no gap between the ring and the filter. And that the filter does not wiggle if shacked, meaning it is secure.

**Step47.** Mount the 525nm and 630nm emission filters (item 35,36) on the ports as labeled in Fig.13. For each filter, follow Steps 40-42.

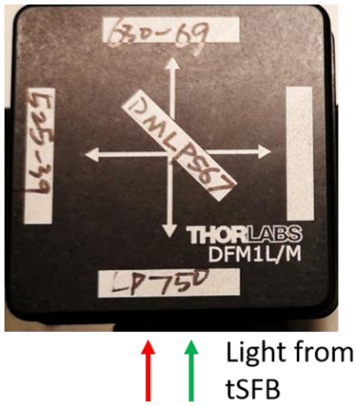



*Figure 13: Top view of a possible Kinematic cage configuration. The labels indicate the assignment of the filters relative to the incoming beam from the TFB. LP750 stands for 750nm lowpass filter; 525-39 and 630-69 correspond to the emission filters with centered wavelength on 525nm and 630nm with FWHM of 39nm and 69nm, respectively.*

**Step48.** Close the empty filter port (right side of Fig.13) with an End Cap for machining (item 17)

<u>Mounting TFB to kinematic cage</u>

**Step49.** Prepare a SM1 Coupler for external Threads (item 18) and place the two rotating halfway. Attach it to the shortpass filter port.
**Step50.** Thread the shutter mounting adapter (item20) to the Coupler of Step48.
**Step51.** Mount the optical beam shutter (item 19) after Step49 by screwing it clockwise.
**Step52.** Mount an aspherical lens (item 36) on a two connected lens tubes (item 16) and place a threaded retaining ring on top to prevent it from moving and crash with the shutter of Step49.
**Caution!** The flat surface of the lens must face the TFB (outwards of cage) to collimate the beam in the direction of the kinematic cage (convergent point from the light guider to parallel laser beam rays entering the filters cage) – See Fig. 14.

**Step53.** Confirm that the light coming out of Lens tube of Step51 is collimated. For this, attach a green LED (item h) to the mount and follow visualize spot changes by approaching and distancing the assemble relative to a white wall (see Fig.15)

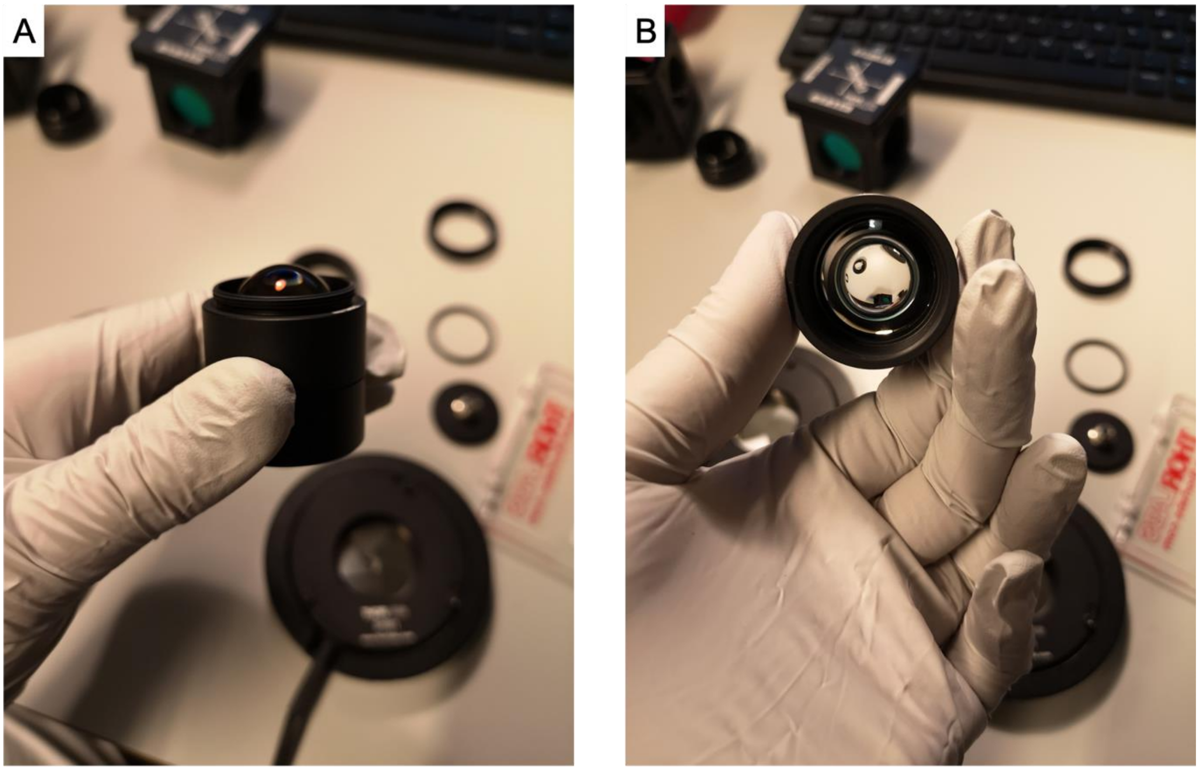



Figure 14: Top (A) and bottom view (B) of two Tube Lens attached together and an aspherical lens mounted inside showing its curved and flat surfaces, respectively. (A) is the side facing the shutter and (B) facing the TFB.

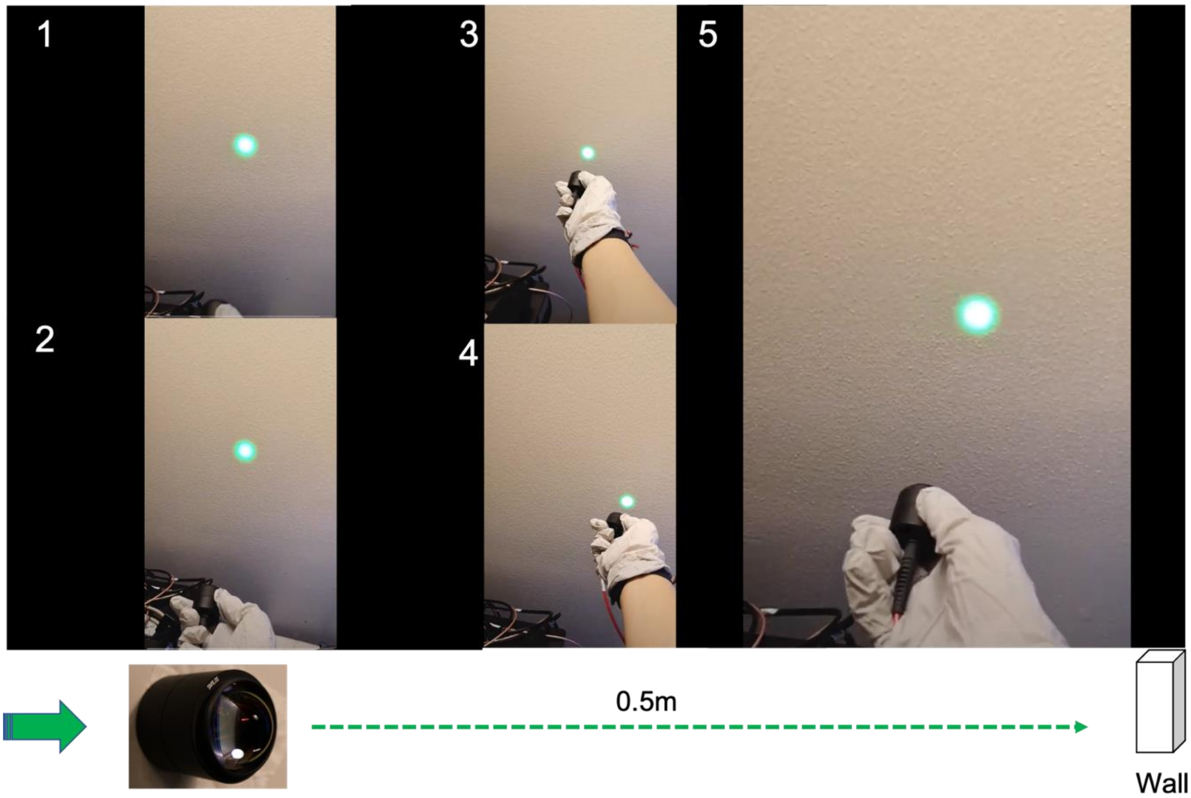



*Figure 15: Checking output light from TFB after passing through aspherical lenses and before entering the kinematic filter cage.*

**Step54.** Assemble the mounted lens on Step51 after the shutter (see Fig.16-A).
**Step55.** Thread the sprayed TFB (item 30) at last.
**Caution!** Before adding the TFB, coat it with black rubber spray (item x) because of its grey coating. One way to ensure its uniformity is by holding it vertically and spraying very fast around 8-10x times. Wait until it dries and repeat once or twice.

**Step56.** Fixate the assembled kinematic cage on top of the coupling box as shown in Fig.16(A-B).

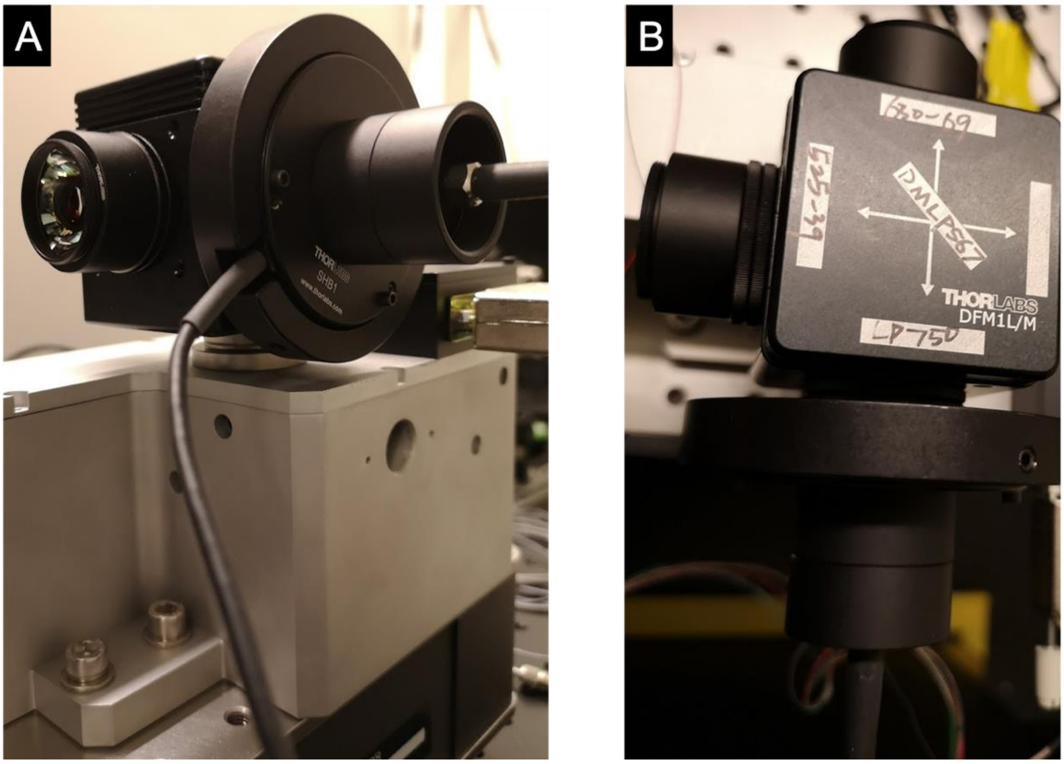



*Figure 16: Side (A) and top view (B) of the kinematic cage connected to TFB via LP750 filter port. The cage is placed on top of the coupling´s box cap and the Tube Lenses with aspherical lenses inside are also mounted on the emission filters ports, to be connected to the PMTs (see P5.3).*

Connecting PMTs to kinematic cage

**Step57.** Mount another aspherical lens on Lens Tube (item 36) with flat surface facing the PMT (outwards of kinematic cage port). Add a threaded ring to hold it in place.
**Step58.** Assemble the prepared lens tube on Step56 to the Kinematic cage threading it on the 525-emission filter port (left side – see Fig.16-B).
**Step59.** Thread SM1 Coupler (item 18) to the Lens Tube with external side facing out.
**Step60.** Turn off any room light. Connect PMT1 (item 52) (green channel) after coupler of Step58. Rotate it clockwise until it is fixated. Adjust the position of the middle coupler such the PMT is in a vertical position.
**Caution!** When opening the PMT lid, there must not be any light source in the room to avoid damage of the PMT anode and shorten its lifetime.

**Step61.** Repeat steps 56 to 59 for the 630nm emission filter port and respective PMT2 (item 52) (red channel). The result is show in Fig.17

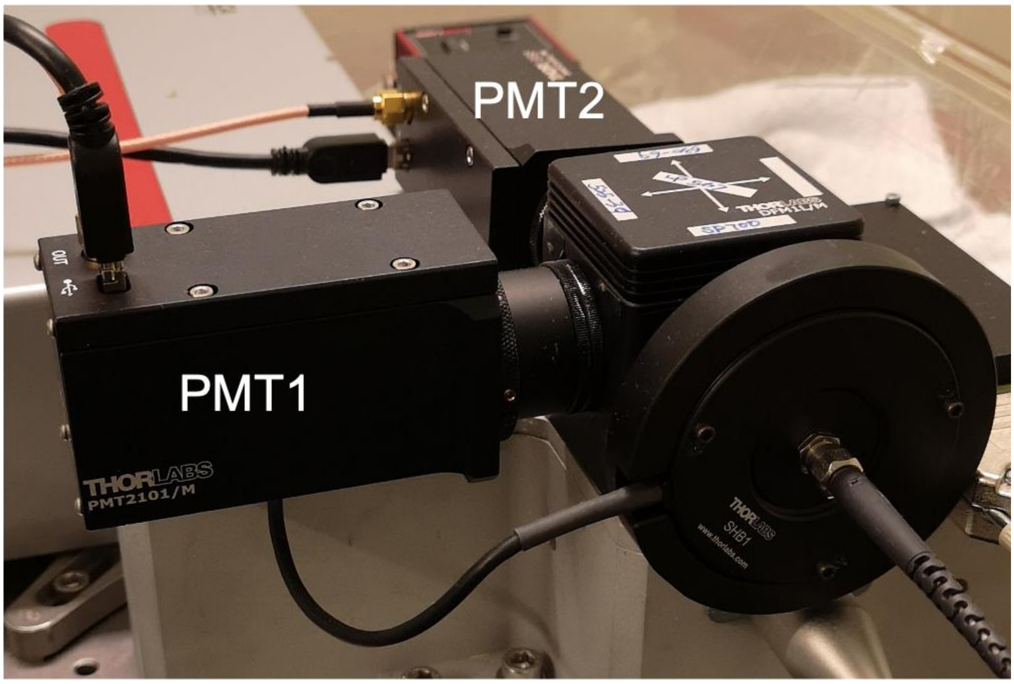



*Figure 17: Kinematic cage connect to PMT shutter, TFB and PMTs assembled on top of the coupling box´s cap.*

Mount the µTlens driver and controller for Shutter

**Step62.** Fixate the Table mounting plate of the µTLens Driver (item 51) onto the core optics breadboard (item 1), next to the Coupling box (item 21). Fixate it with two M6 cap screws (item 6), each with a washer as shown on the left side of Fig.18.

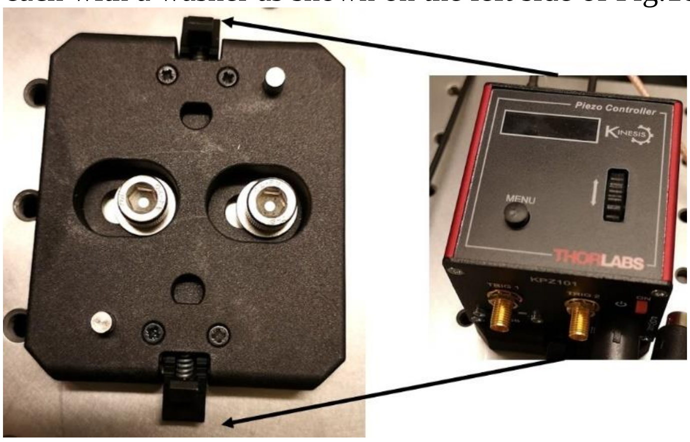



*Figure 18: Placement of µTLens base on the breadboard and how its Driver is fixated to it.*

**Step63.** Mount the µTLens Driver unit on top of the magnetic plate and hold it in place by clipping both ends inwards.

**Step64.** Place the shutter controller after the µTLens Driver as shown in Fig.S7-A in *Zong et al., 2022*.

## Assembly of the controlling module

There are 8 input signals (BNC cables, see Fig.S9-A) connecting to the controlling box (black box in Fig.19) which then give a signal to the MEMS controller (item 44) and has as output six wires that are connected to the miniscope.

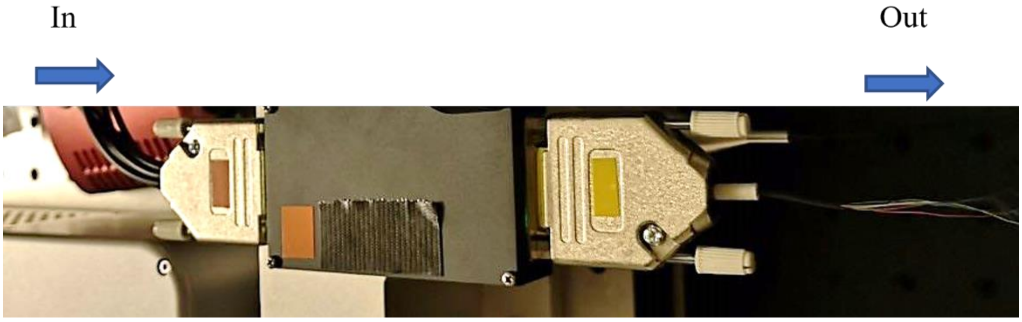



*Figure 19: Illustration of the coupling box with both Input Cable from the vDAQ (left) and Output cable to the miniscope (right). An arrow on the box side indicates the direction of the signal.*

**Step65.** Place the MEMS controller (item 44) inside the control box shell (item 28) and split the grey connector on the input side (connector J1) by splitting each wire.

**Step66.** Attach each input DSUB15 (item 46) to the J1 connector (see Fig.S7-D in *Zong et al., 2022*) to, i.e. three grounds connected to one another on the input side and to the J1 connector – Pin4 (green lines on Fig.S7-D in *Zong et al., 2022*).

**Step67.** Link also the other pins from Input DSUB15 to J1 connector, as illustrated on Fig.S7-D in *Zong et al., 2022* (black lines).

**Step68.** Connect the other side of MEMS controller to 6 pins of J2 connector. The later then connects to 6 wires bounded with a connector, to be attached to the MEMS. This is the output signal (see Fig.S7-C, right side & Fig.S7-E in *Zong et al., 2022*)

**Step69.** Establish direct connections from input to output, without linking to J1 or J2 connectors. This is illustrated on Fig.S7-C in *Zong et al., 2022* (uTLens and LED).

**Step70.** Mount the connector socket (item 48) into the backshell (item 47).

**Step71.** Attach 8 BNC cables (item 95) for Signal-Input to the DSUB15.

**Step72.** Repeat Step70 for output side, but merging the 6 single wire cables (item 50) that will connect to the miniscope

**Step73.** Add a 6-pin connector for MEMS (item 49) at the other end of the gathered wires of Step67.

**Step74.** Close the control box with lid (item 29).

**Step75.** Mount controlling module next to the detection module, on top of coupling box lid as shown in Fig.20 using a M4 capscrew.


*Figure 20: Placement of controlling module next to the detection module.*

The core optics module has been successfully assembled and the result is summarized on Fig. 21.

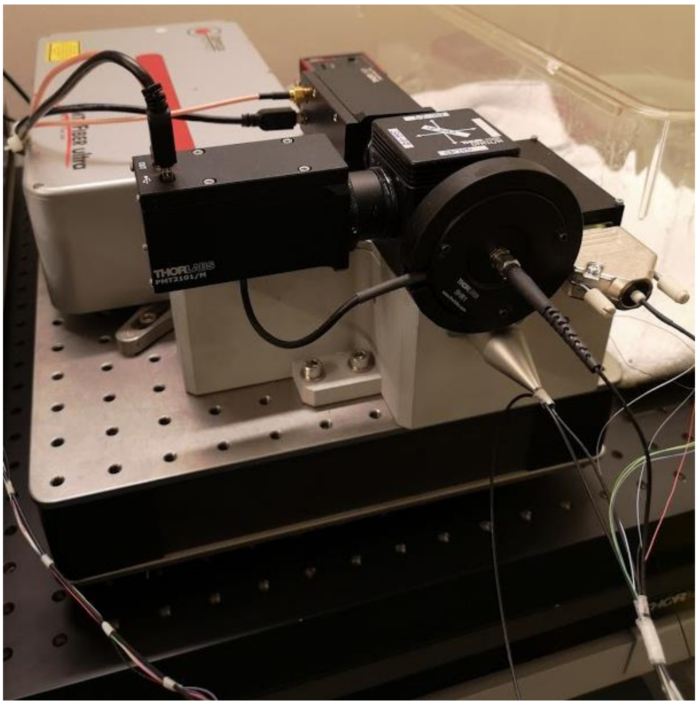
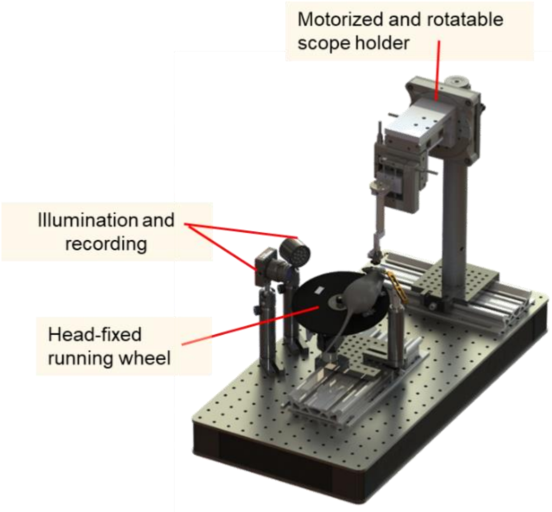



*Figure 21: Overview of core optics module with coupling box placed in front of laser seed, detection and controlling modules assembled on top of the coupling box cap.*

## Part 2- Scope Mounting Assembly

The assembling of the Scope Mounting module is divided in three parts: A) Motorized and rotatable scope holder; B) Head-fixed running Wheel; C) Illumination and Recording. This Protocol includes the main steps to build such module as illustrated in Fig.S7E in *Zong et al., 2022*.




Taken from Fig.S7E in Zong et al., 2022

**Equipment:**

- Hex keys (M2.5, M3, M4, … M6)
- Screwdrivers (Cross Slot/Phillips, Hexagon)
- Components of the Scope Holder (Table 1)
- Components of the Head-fixed Running Wheel (Table 2)
- Components of the Illumination and Recording (Table 3)

**Table 1: Components of the Scope Holder setup**

| **Component Name** | **Amount** | **Item Number (see Protocol S1)** |
|---|---|---|
| Breadboard | 1 | 54 |
| Sorbothane Feet | 1 | 55 |
| Construction Rail | 1 | 56 |
| Rail carriage | 1 | 57 |
| Precision construction Rail | 1 | 58 |
| Post mounting clamp | 1 | 59 |
| Manual rotation stage | 1 | 60 |
| MINI2P Holder P1 | 1 | 72 |
| DC Motors | 3 | 82 |
| MINI2P Holder P2 | 1 | 73 |
| MINI2P Holder P3 | 1 | 74 |
| Optical Post | 1 | 62 |
| Base for item 58 | 1 | 61 |
| MINI2P Holder P4 | 1 | 75 |
| Hex Screw M2, M2.5 | 1 | 113 |

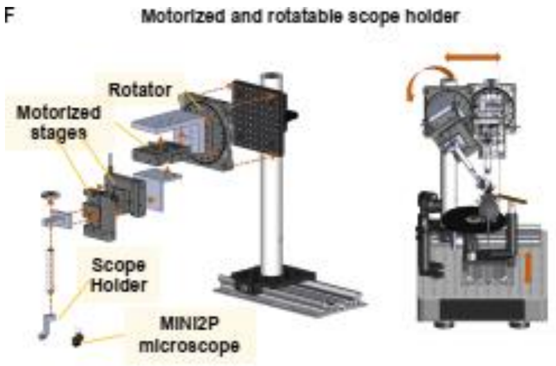
**Table 2: Components of the Head-fixed Running Wheel**

| | | |
|---|---|---|
| Running wheel (wheel spread + spray) | 1 | 63 |
| Spacer1 | 2 | 64 |
| Spacer2 | 2 | 65 |
| Bearing | 1 | 66 |
| Wheel Holder | 1 | 76 |
| M6 cap screw | 1 | 6 |
| Right-angle clamp | 1 | 67 |
| Pillar posts | 2 | 68 |
| Construction Rail | 1 | 56 |
| Rail Carriage | 1 | 57 |
| Headbar holder | 1 | 77 |

**Table 3: Components of the Illumination and Recording setup**

| | | |
|---|---|---|
| Universal Post Holder | 2 | 69 |
| Zelux CMOS camera | 1 | 79 |
| C-Mount Adapter Lens | 1 | 71 |
| Lens 4.5 or 8.5mm focal length | 1 | 80 |
| Locking Ball and Socket Mount | 9 | |
| LED array lights | 1 | 78 |
| LED Power supply | 1 | 78 |
| Basler camera | 1 | 81 |

**Assembling of Motor Stages**




Taken from Fig. S7F in Zong et al., 2022

**Step1.** Fix the construction rail (item 56) to the breadboard (item 54) with four M6x12 mm cap screws (see Fig.22-A) and add the rail carriage on top (see Fig.22-B).

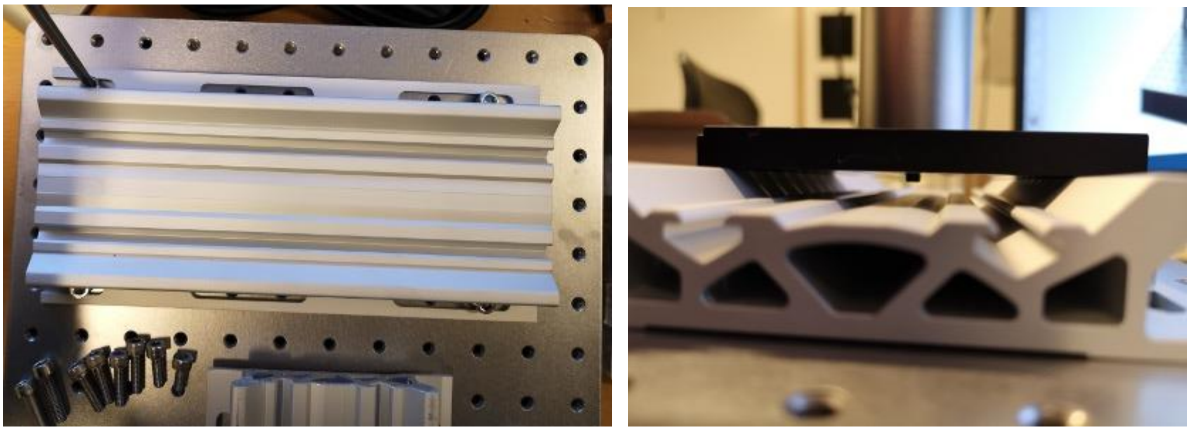


*Figure 22: Mount of (A) construction rail to the breadboard; (B) rail carriage and mounting post on top of each previous components.*

**Step2.** Mount all components for the scope holders on the construction rail (See Fig. S7-F in *Zong et al., 2022*).

**Caution!** Before assembling the PI motorized stages (item 82), connect the elongated cables to the Motor controller (see Fig. 23), and move the motor to one end of its range by the software to reach the screw holes.

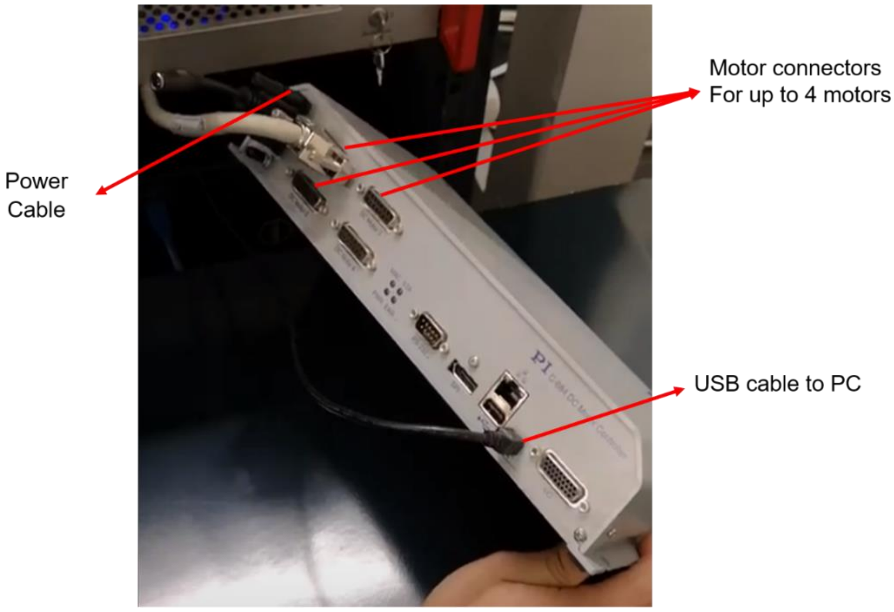



*Figure 23: Photography of the DC Motors controller. Only one motor is connected (DC motor 1), yet this controller can allocate up to four motors.*

**Step3.** Fix the precision construction rail (item 57) to its base (item 61). Adjust the height of the scope holder by releasing the M6 screw of the rail carriage on the precision construction rail. Adjust the angle of the scope holder with the rotator (see Fig.24)

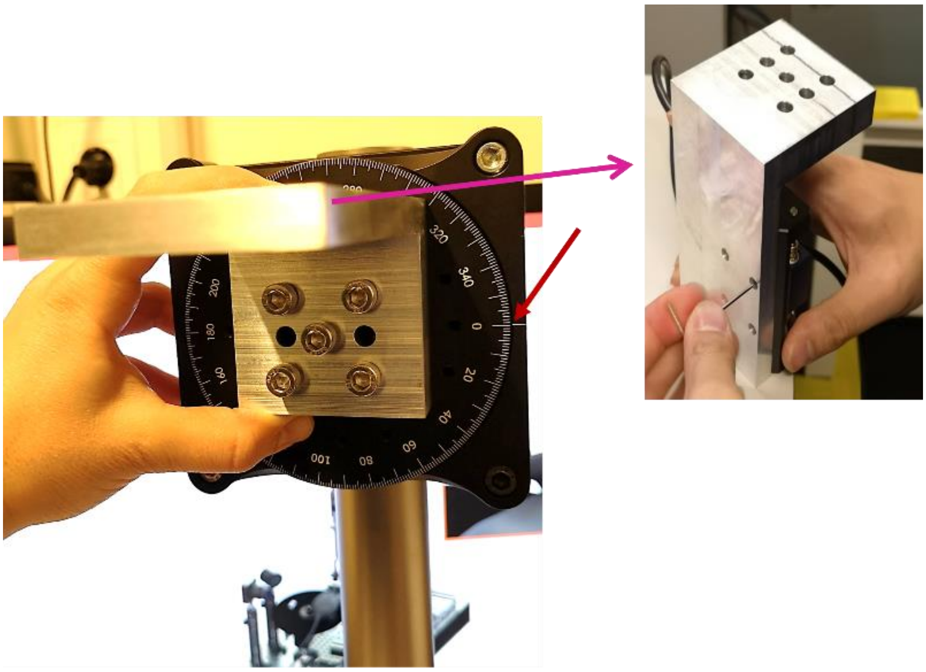


*Figure 24: Placement of MINI2P Holder P1 (item 72) relative to the manual rotation stage (item 60) and alignment of the latter to the reference line on the right. The top right figure illustrates the assembly of DC motor1(item 82) to the longer side of MINI2P Holder P1.*

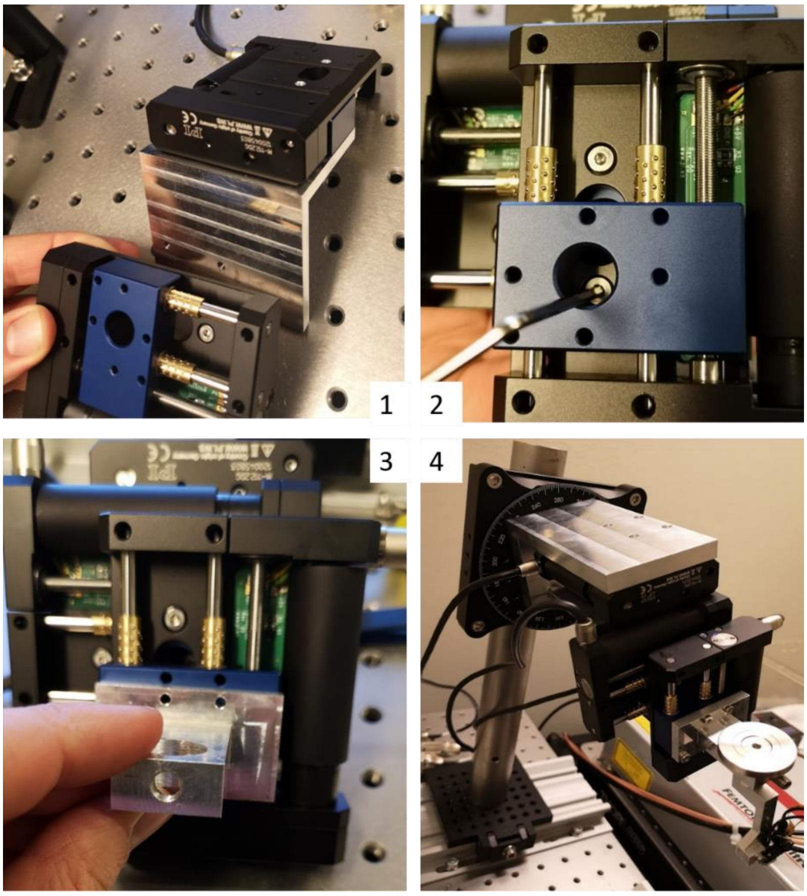
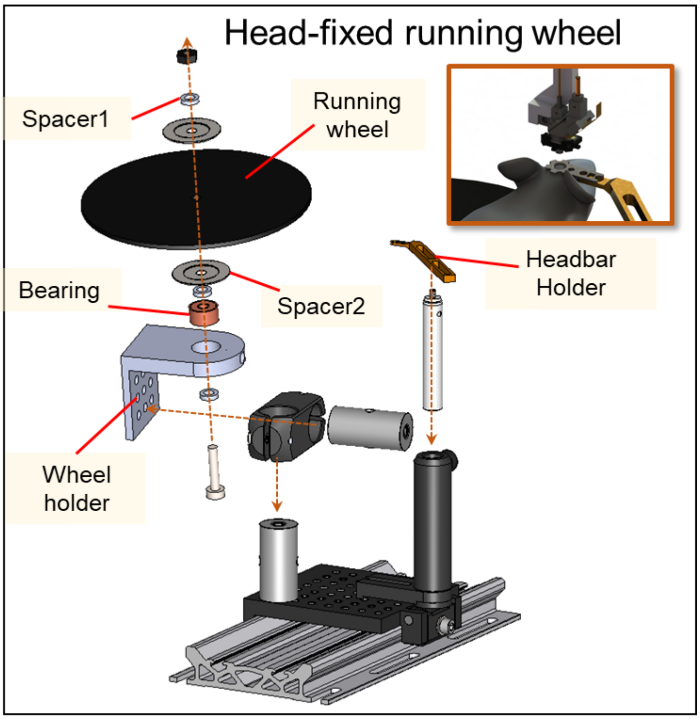



*Figure 25: Illustration of assembling the stage (1-2), MINI2P Holder P3 (3) and MINI2P Holder P4(4).*

## Assembling of the Running Wheel




Taken from Fig.S7-G in *Zong et al., 2022*

**Step4.** Make the wheel spread

**i.** Cut hardboard (Item 80) to a circular shape (diameter Ø = 17 cm). One of the methods used can be a Jigsaw machine which is dragged in a compass-like movement to make the cut smoother (see Steps i) and ii) in Fig.26).

**ii.** Create a hole for M6 screws in the center of the running wheel prepared in i. and for that, use a Drill Tower with a 6mm drill bit placed above the hardboard, as shown in Fig.27.

**iii.** Spray the wheel with black rubber spray approximately ten times, until the surface is uniformly opaque black. Between spray sessions, wait ~ 10 min until it dries (see Fig.28).

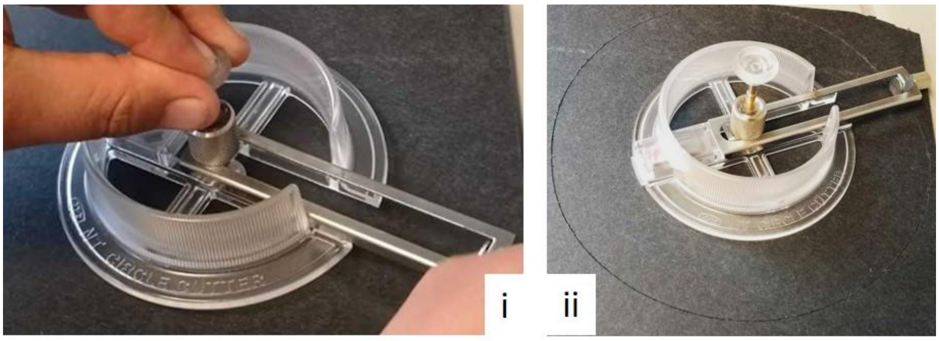



*Figure 26: Hardboard cutting method to make a running wheel. By using a Jigsaw machine with a blade on the end-tip (i), slide it circularly with the desired diameter (in this case Ø = 17 cm) and dragging its blade, pressing the center with the fingers. Repeat one or twice until the circle is well separated from the main hardboard (ii) and then remove it.*

*Figure27: Illustration of process to create a hole centered on the running wheel hardboard to fit M6 screws. The circular hardboard is placed under the 6mm drill bit of a Drill Tower.*

*Figure 28: Spraying setup: a hanging wheel between two holes on opposite sides of a paper box.*

**Step5.** Fix the other construction rail (item 56) to the breadboard and mount all components for the head-fixed running wheel on the construction rail, as illustrated on Fig.S7-G in *Zong et al., 2022* and Fig.29 to Fig. 32.

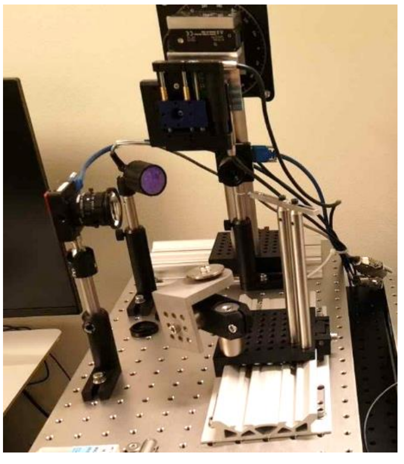


*Figure 29: Schematic overview showing the disposition of Running Wheel, with construction rail positioned beneath the Motors setup. The Illumination and recording stand on the side.*

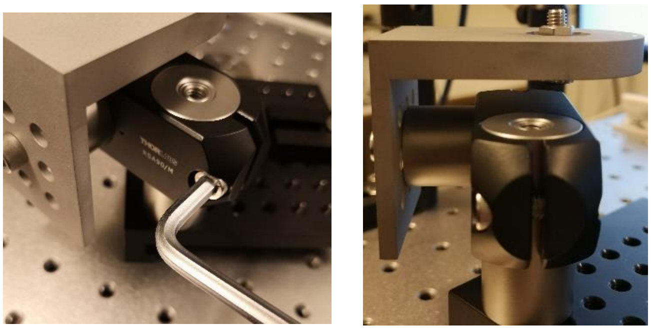


*Figure 30: Schematic of the base to assemble the Running Wheel*

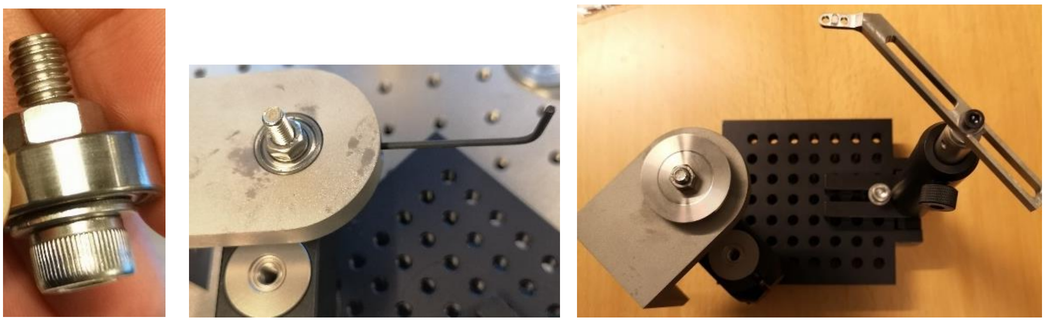


*Figure 31: Bearing (left) mounted on Wheel Holder (middle) and Top view of the Running Wheel before mounting the wheel itself (right).*

**Step6.** Mount the NIR camera (item 92) and NIR LED (item 91). Adjust the positions of the camera mount and LED mounts (item 87) so the illumination and the camera focus are on the animal`s head.

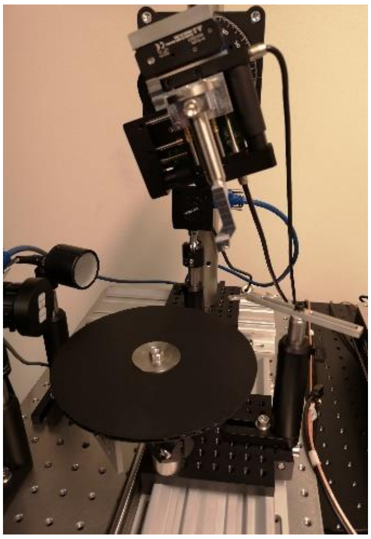
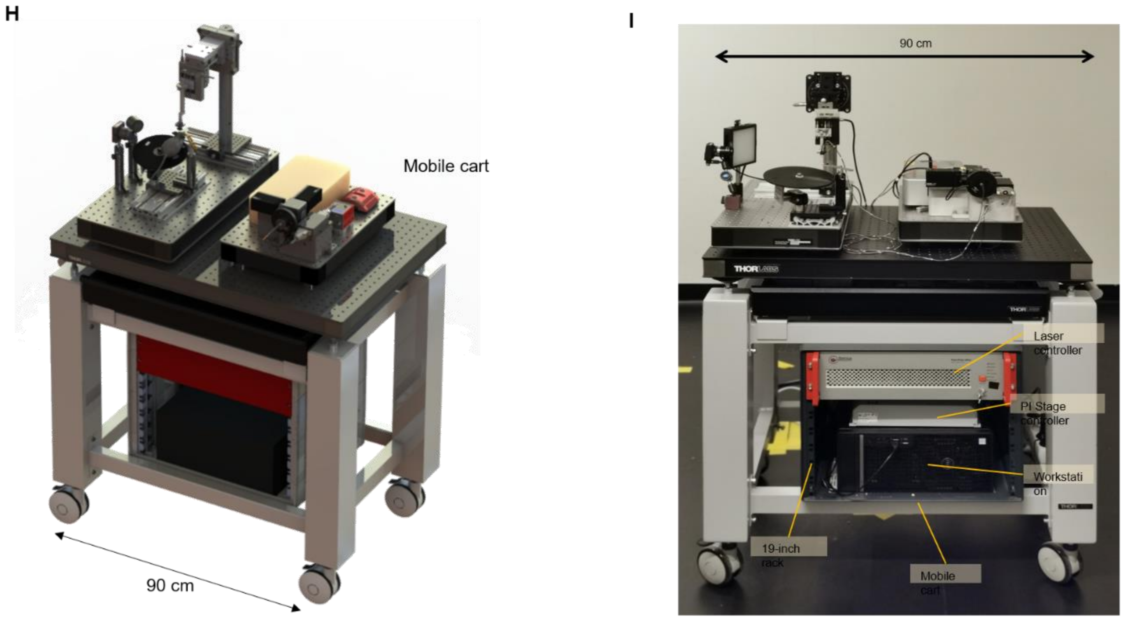


*Figure32: Running Wheel result.*

## Part 3- Mobile Cart Assembly

The Mobile Cart Module comprises a breadboard cart which supports a drawer to store microscope objectives and other useful tools (screw drivers, spacers, clean lens tissue, swabs, etc), a rack that has a workstation (desktop computer), acquisition breadboard and controllers for both laser and DC-motors. Furthermore, it supports all the other units (Core Optics and Scope Mounting Assemblies) which are available on top of the larger breadboard. It is the basis of this setup and facilitates the transport and maneuver of the whole system, and so one can compare it to the pillars of a house: without it, the whole structure is not as strong and complete.




Taken from Fig.S7 (H,I) in *Zong et al., 2022*

Equipment:
- Hex keys for M4, M6 screws
- Philips screw drivers for M4
- Bubble level
- M4, M6 Cap screws
- Components of the Mobile Cart (see Table 1)
- Components for Connecting Cables (see Table 2)

**Table 1: Components of the Mobile Cart**

| Component Name | Amount | Item Number (see Protocol S1) |
|---|---|---|
| Mobile Cart | 1 | 83 |
| Main Optical breadboard | 1 | 84 |
| Storage Drawer | 1 | 85 |
| 19-inch rack | 1 | 86 |
| Span-In Nuts | 10 | 87 |
| Breadboards for modules | 2 | Items 1, 54 |
| Workstation | 1 | 88 |
| Monitors | 2 | 89 |

| | | |
|---|---|---|
| vDAQ – acquisition card | 1 | 90 |
| vDAQ breadboard | 1 | 91 |
| Motion Controller for PI motors | 1 | 92 |
| Controller for 920nm laser | 1 | 93 |

**Table 2: Components for Connecting Cables**

| | | |
|---|---|---|
| <u>Controlling box</u> | | |
| BNC cables | 8 | 95 |
| BNC to BNC adapters | 8 | 95 |
| | | |
| <u>µTlens controller</u> | | |
| BNC to SMA male connector | 1 | 96 |
| BNC adapter Female-Female | 1 | 95 |
| SMC connector | 1 | 97 |
| Power supply | 1 | 99 |
| | | |
| <u>Lasers Controller</u> | | |
| Power In Connector | | |
| USB B connection cable to workstation | 1 | Included with item 93 |
| Ethernet | 1 | |
| D-sub15-connector to Seed Laser | 1 | |
| TTL in | 1 | |
| Analog In (SMA-connector) | 1 | |
| Trigger Out | 1 | |
| Keys for Key Switch | 1 | |
| | | |
| <u>Laser Head</u> | | |
| AOM connector (SMA cable) | 1 | Included with item 29 |
| Optical Fiber Input | 1 | |
| Laser Control Unit Connector | 1 | |
| | | |
| <u>PMT</u> | | |
| USB Male A to USB 2.0 | 1 | Included with item 52 |
| SMA to SMB cable | 1 | Included with item 52 |
| | | |
| <u>Monitor to Workstation</u> | | |
| HMI cable | 1 | |
| Ethernet cable | 1 | |
| USB B (screen) to USB 3.0 | 1 | |
| | | |
| <u>Workstation</u> | | |
| USB 3.0 & 2.0 cables | 4 | |
| USB-hub (7) ports | 1 | 94 |
| Basler power cable | 1 | |
| USB 3.0 Micro-B for Basler camera | 1 | |

**Assembling of Mobile Cart**

**Step1.** Lock the casters to assemble the Mobile Cart (item 83) without moving it.
**Step2.** Mount a 19-inch Rack (item 86) following supplier instructions (see Protocol S1).
**Step3.** Remove the frontal frame from the Mobile Cart and insert the Rack positioned on the bottom frames as shown in Fig.33-A.

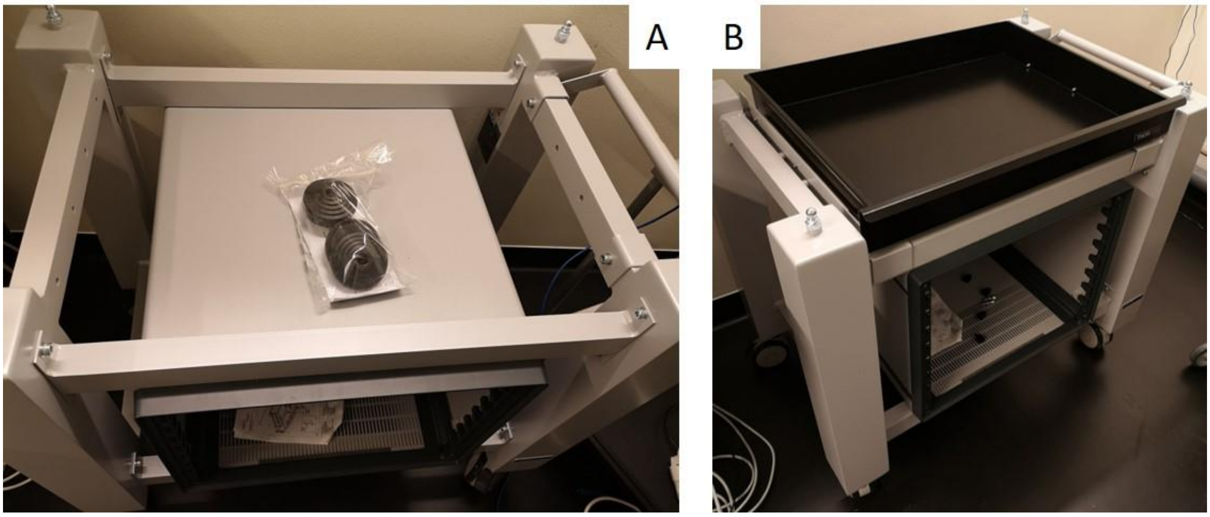



*Figure 33: Top view of Rack inserted into Mobile Cart (A) and Drawer mounted on top (B).*

**Step4.** Mount the Users tools Drawer (item 85) on the mobile cart following instructions given by Thorlabs (see Protocol S1).
**Step5.** Add Span-In nuts (item 87) on the front and back rack's side to later fixate Laser Controller and vDAQ breadboard, respectively (see how these are placed in Fig.S7-I)
**Step6.** On each of the four corners of the Mobile Cart, place an adjustable anti-slip support pad aligned to each of the height adjusters. Using body weight, press the pad down until hearing a click.
**Step7.** Annex a breadboard (item 84) on top of the four circular adjustable mounts.
**Step8.** Level the mounts to minimize the breadboard tilt. Use a bubble level to adjust.
**Step9.** Place the two smaller breadboards (items 1 and 54) on top of breadboard mounted on Step7.
**Step10.** Add Scope Mounting and Core Optics assemblies to each breadboard.
**Step11.** Attach the Laser Controller (item 93) to the upper section of the front rack. Fixate it with four M6 screws (see Fig.S7-I).
**Step12.** Attach the vDAQ breadboard (item 91) to the back side of the Rack and beneath the position of Laser Head. Insert M6 screws to the Snap-In Nuts to hold it in place.
**Step13.** Place Workstation (item 88) near the bottom rack grids to maximize ventilation.
**Step14.** Lay the motion controller for PI motors (item 92) on top of the workstation.

**Connecting Cables / Main Connections**

For more details of Steps 15-18 & Step 21, please consult vDAQ Datasheet Installation guide (see link of Items 90,91 on Protocol S1).

**Step15.** Slide workstation cover and remove its second back grid as shown in Fig. 34.

**Step16.** Insert acquisition card (see Fig.34-A) through the inside of the CPU with the connectors sticking out through the open grid´s rack (see Fig.34-B1). Place it between blue and white boards and press it downwards and to the left until it clicks.

**Step17.** Before closing the workstation, link the acquisition card to one of the CPU PCI Express Power Connector (Molex connector) which is a six-pin connector as described in Datasheet Installation Guide.

**Step18.** Connect the breakout breadboard to the acquisition card. For that, link two cables from the ports of the former, labelled A and B to the top and bottom ports of the latter (see Fig.34-C and Fig.35).

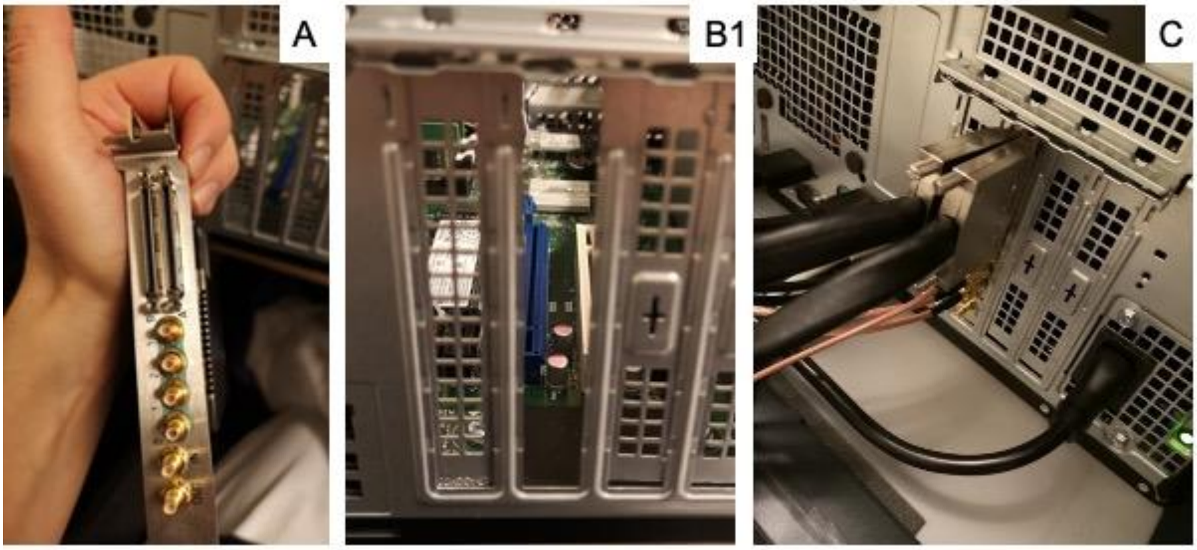



*Figure 34: Assembling of acquisition card (A) in workstation showing front view (B1) and top view (B2) of section where the card is mounted into: on the second grid (B1), placed between the blue and white board. (C) illustrates the power cables connected from A and B channels (left and right) of the acquisition card to the vDAQ breadboard.*

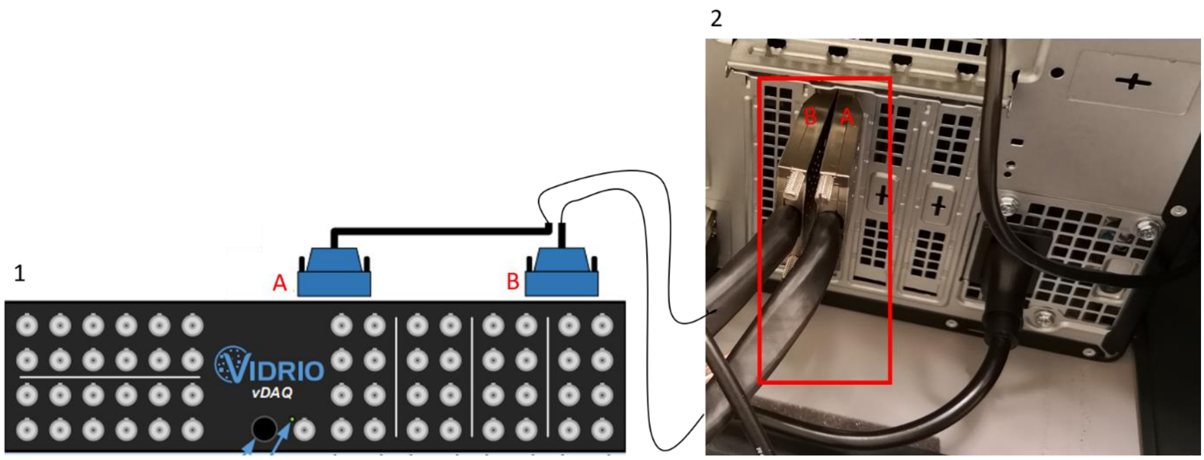



*Figure 35: Schematic to illustrate connecting A,B - power cables to the vDAQ breadboard backside, top(A) and bottom (B) (1) from the acquisition card (2, B-left and A-right side, as labelled).*

**Step19.** Connect three USB 3.0 and a USB 2.0 cables from Scope Mounting Module monitoring camera (item 79), tracking camera (item 81) and motion controller for PI motors (item 92), respectively, to the workstation ports.

**Caution!** As there are not enough ports, add extra USB-hub (item 94) on top of the workstation.

**Step20.** From the backside of the workstation, connect also to the monitor/screen(s): HDMI port(s), ethernet cable and USB 3.0 to USB B.

**Step21.** Link the connections shown in Fig.S9-A that go from/to vDAQ breadboard to/from Controlling Module (Signal Input), ***μ***TLens Driver, Laser and Shutter Controllers and Tracking Camera.

**Step22.** Connect also BNC7 of controlling module to HV Out of ***μ***TLens Driver (short cable SMA to BNC). Moreover, connect the Power of the Driver on the front side.

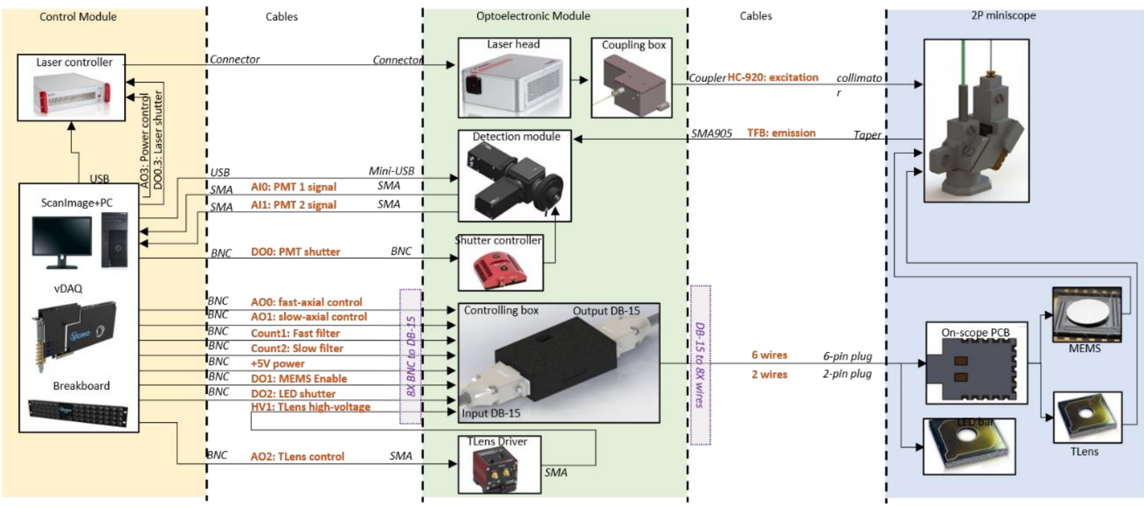
**Caution!** The ***μ***TLens Driver power cable has pins with specific orientation, be careful to place those pins with the correct alignment before pushing them in.

**Step23.** Connect a BNC-to-BNC cable from TTL input (front side) of the Shutter Controller to vDAQ (Digital channel D02). To this unit, link also the Power cable (5V DC, 2.4A) from the backside of the Controller.

**Step24.** Finally, attach the Shutter controller to Shutter Head via the jacket next to the Power, also backside.

**Step25.** Link each PMT from its USB Port to the backside of the workstation.

**Step26.** Connect the two PMTs SMA output cables (item 52) to the acquisition card channels 0 and 1. The MINI2P system should be now completed.




Taken from Fig. S9-A in Zong et al., 2022

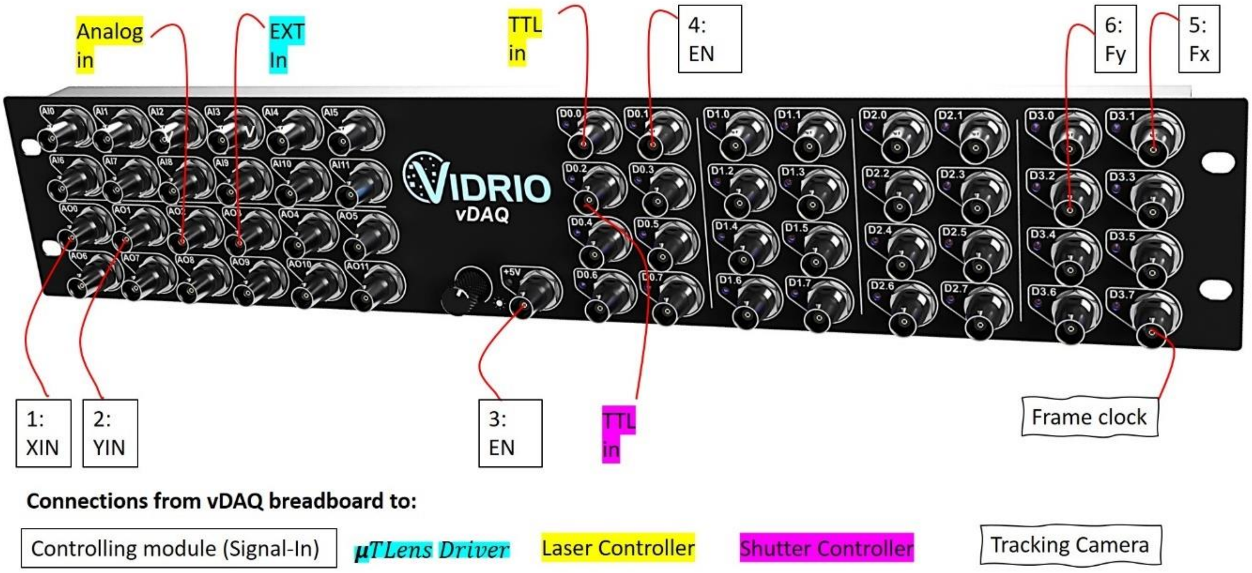



*Figure 36: Diagram showing main connections from vDAQ breadboard to the controlling box (BNCs 1 to 6), $\mu$TLens Driver (EXT in), Laser Controller (Analog In and TTL in) and Tracking Camera (via pink BNC connector, to input-2 – see Wiki section of [AnimalTracker](AnimalTracker)).*


*Figure 37: Connections to the back (A) and front (B) of the $\mu$TLens Driver, as well as top view (C). HV OUT connects to BNC7 of the controlling module/box (SMC connector to BNC), whereas EXT IN is linked to the vDAQ (SMA to BNC, Analog output AO3).* ***IMPORTANT: When connecting the piezocontroller, select HV of 75V, not higher!***


*Figure 38: Top view of Shutter Controller with power on (bottom green LED light) and shutter opened (top green LED light right under enable button). The controller rear view (zoomed-in inlets) shows the main connections: TTL Input to the vDAQ (External Modulation Input), Sutter Head Jacket to the Shutter Head and Power (DC input jacket).*

### Scan Image configuration*

| Controlling module | vDAQ channel | ScanImage (MDF of MEMS-L 2000Hz) |
|---|---|---|
| 1_X «fast axis» | AO0 | Dabs.generic.GalvoPureAnalog (slow axis)*<br><br>Control channel/…/AO0 |
| 2_Y «slow axis» | AO1 | Dabs.mirrorcle.ResonantAxis (fast axis)*<br><br>Zoom control channel/vDAQ0/AO1<br><br>Filter clock DO channels<br>X – D3.2 250000<br>Y – D3.1 100000 |
| 3 +5V | +5V | |
| 4 EN | D0.1 | |
| 5 Fx «fast filter» | D3.1 | Defined in Dabs.mirrorcle.ResonantAxis |
| 6 Fy «slow filter» | D3.2 | |
| 7 TLens | HV out piezo controller | |
| 8 | - | LED not in use |

Note: MDF parameters for slow and fast axes vary depending on the MEMS scanner, if MEMS-L (2000Hz) or MEMS-F (5600Hz).