

A protocol for the assembly of miniBB2p headpiece

Part A: Evaluating the micro-axicon fabricated on the fiber tip

Reagents:

- Air duster
- Lens cleaning wipe
- 100% ethanol

Equipment:

- ~1.5-meter LMA-12 fiber (NKT Photonics) with customized micro-axicon tip (Comcore)
- Tweezers
- Fiber stripping tool (T06S13, Thorlabs)
- Fiber cleaver (CT-101, Fujikura)
- Stereoscope (M125C, Leica)
- Beam profiler (CMOS-1202, CINOGY Technologies)
- Power meter (PM130D, Thorlabs)
- Others (Table 1)

Table 1 Main components used to evaluate the micro-axicon fabricated on the fiber tip

Component Number	Component Name	Part Number	Amount	Company
#1	Post-Mountable Fiber Clamp	T711/M-250	1	Thorlabs
#2	3-Axis Stage	MAX313D/M	1	Thorlabs
#3	Fiber Holder for 3-Axis Stage	HFV002	1	Thorlabs

Procedure:

Step 1. Examine the fiber tip under a stereoscope to confirm the micro-axicon's quality (Figure 1.1). Should dust be present, carefully clean the tip with either an air duster or a lens cleaning wipe.

Caution! The fiber tip is fragile. Clean it with extreme caution, particularly when using a lens cleaning wipe moistened with 100% ethanol.

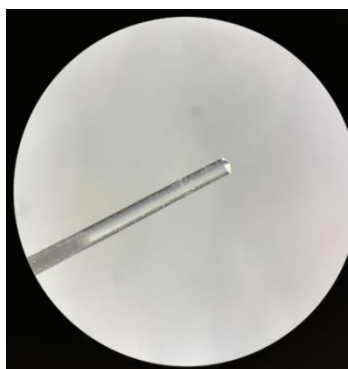


Figure 1.1 Visual inspection of the fiber tip under the stereoscope

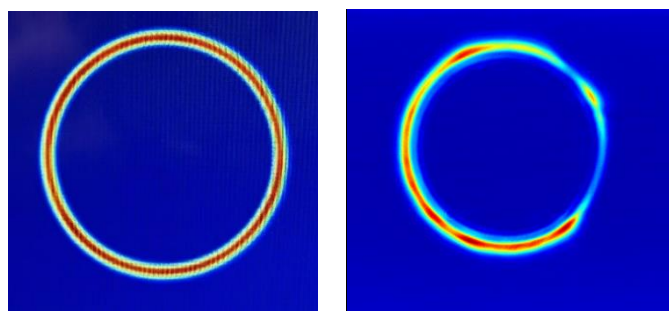


Figure 1.4 Representative images showing ring beam with high (left) and low (right) qualities.

Part B: Assembling the fiber collimator

Reagents:

- Air duster
- Lens cleaning wipe
- 100% ethanol
- UV-Curing Optical Adhesives (NOA61, Thorlabs)
- Black epoxy glue (9005B, LEAFTOP)

Equipment:

- Tweezers
- UV lamp
- Ultrasonic cleaner
- ~1.5-meter length LMA-12 fiber (NKT Photonics) with fabricated micro-axicon tip (Comcore)
- Stereoscope (M125C, Leica)
- NIR Detector Card (VRC4, Thorlabs)
- Video camera
- Beam profiler (CMOS-1202, CINOGY Technologies)
- Others (Table 2)

Table 2 Main components of the fiber collimating setup

Component Number	Component Name	Part Number	Amount	Company
#4	Glass Flange	TUB-1.8x6-0.127	1	Sunlight
#5	Collimating Lens Holder	/	1	Customized
#6	Collimating Lens	45-956	1	Edmund
#2 and #7	3-Axis Stage	MAX313D/M	2	Thorlabs
#8	Adjustable Fiber Clamp	HFF001	1	Thorlabs
#9	Compatible Flexure Stage Mount	HCS020	1	Thorlabs
#10	Collimator Holder	/	1	Customized
#11	Fixed Mounting Bracket	AMA007/M	1	Thorlabs

Procedure:

Step 6. Clean the glass flange (#4) and collimating lens holder (#5) in an ultrasonic cleaner for 15 minutes, and spray all the cleaned components with air duster.

Step 7. Visually inspect the fiber end with micro-axicon if the length of the removed protective cladding is longer than the length of glass flange.

Note! A piece of protective cladding at the fiber end was removed during micro-axicon fabricating. Please customize this length in advance, as remove of the protective cladding near micro-axicon may lead to damage of the micro-axicon tip. If the glass flange is longer, we suggest cutting the glass flange instead of re-removing protective cladding of the fiber.

Step 8. Carefully slide the glass flange on the micro-axicon tip end of the fiber with a tweezer under the stereoscope, until micro-axicon tip is slightly sticking out at the other end of the flange (Figure 2.1).

Caution! Insertion itself may break down the micro-axicon tip.

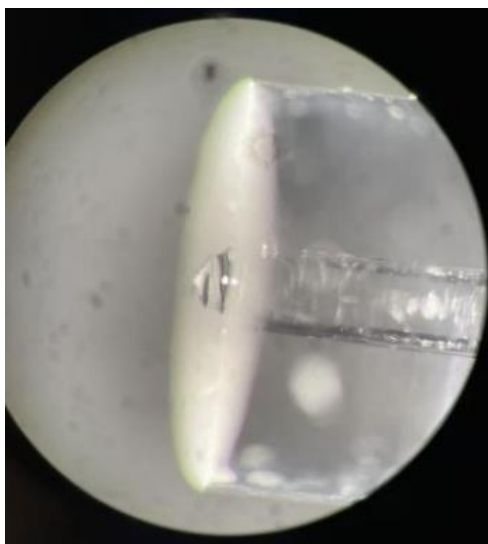


Figure 2.1 Photo of the fiber inserted into glass flange

Step 9. Add UV-curable adhesive to the funnel at the root of the glass flange (Figure 2.2). Wait until adhesive fill the gap between the glass and fiber, then cure the adhesive with UV lamp for ~1 min.

Caution! Adhesive should be added slowly, and the filling process should be monitored under the stereoscope to avoid the micro-axicon tip contaminated by the adhesive.

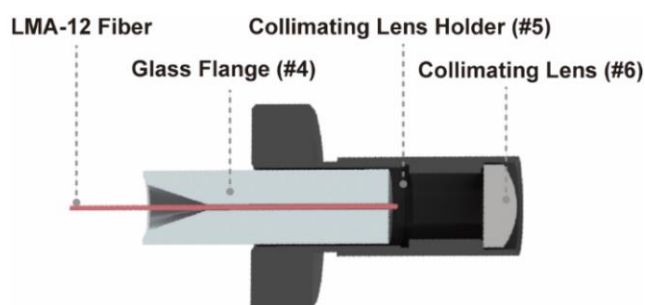


Figure 2.2 Schematic of the fiber collimator

Note! We need to double check the laser output from the micro-axicon tip by following a protocol similar to that in Steps 4–5. The output ring pattern should be still uniform.

Step 10. Fix the collimating lens (#6) into the lens holder (#5) with UV-curable adhesive following a protocol similar to Step 9 (Figure 2.2).

Caution! Pay attention not to get adhesive on the surface of the collimating lens. Apply glue only to the gap between the collimating lens and lens holder.

Step 11. Set up the fiber coupling stage according to Figure 2.3. Align the V-groove of the adjustable fiber clamp (#8) and the center of the collimator holder (#10) by adjusting the 3-axis stage (#7).

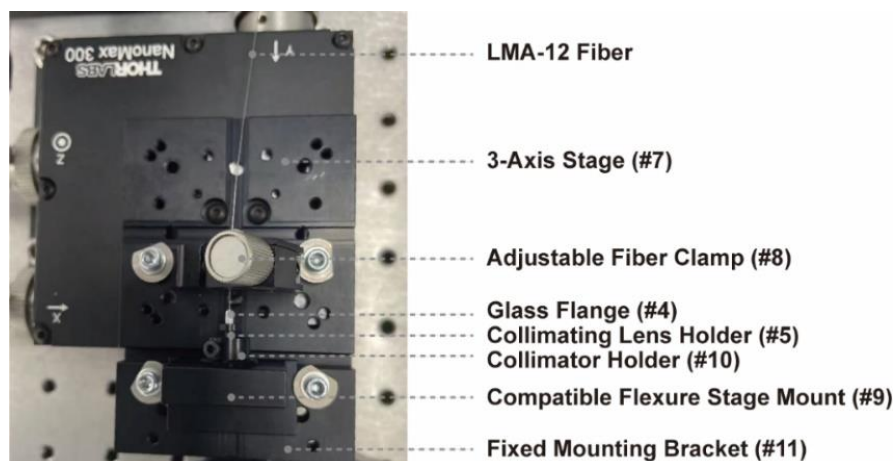


Figure 2.3 Fiber coupling stage

Step 12. Open the adjustable fiber clamp and carefully insert the glass flange (with fiber) into the collimating lens holder.

Caution! Insertion itself may break down the fiber. Insert the glass flange to the bottom of the collimating lens holder, and then move glass flange back for about 1–2 mm to reserve enough space for further adjustment.

Note! If a longer protruding tip was prepared in Steps 8–9, do not insert the glass flange all the way to the bottom of the collimating lens holder in this step. This prevents the micro-axicon tip from colliding with the collimating lens.

Step 13. Secure the LMA-12 fiber by tightening the adjustable fiber clamp (#8). Ensure the glass flange can move smoothly back and forth within the hole of the collimating lens holder by adjusting the 3-axis stage.

Note! If the glass flange cannot move smoothly, make slight adjustments using the 3-axis stage. Exercise caution during adjustment to avoid fiber damage. Loosen the adjustable fiber clamp if a large adjustment is required.

Step 14. Mount the other end of the fiber onto the second 3-axis stage and couple the laser into the fiber following a protocol similar to Step 4 (Figure 2.4).

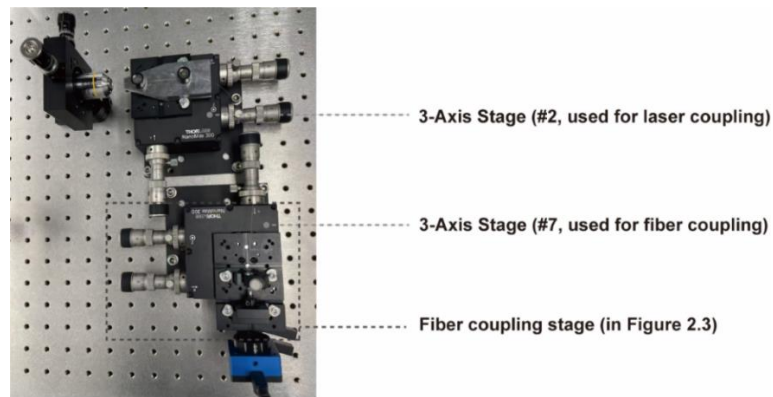


Figure 2.4 Two 3-axis stages used in the setup; a short-length fiber used here for visualization.

Step 15. Carefully adjusting the distance between the glass flange and collimating lens using the 3-axis stage until a near-collimated ring pattern could be seen with the NIR detector card (Figure 2.5).



Figure 2.5 Visual inspection of the beam with a NIR detector card

Step 16. Replace the NIR detector card with a camera in Step 15. Finely adjust the 3-axis stage until the ring pattern's diameter remains constant when moving the camera forward and backward.

Note! Since the ring pattern is only observable within a very short working distance (Figure 2.6), position the camera sensor as close as possible to the fiber tip.

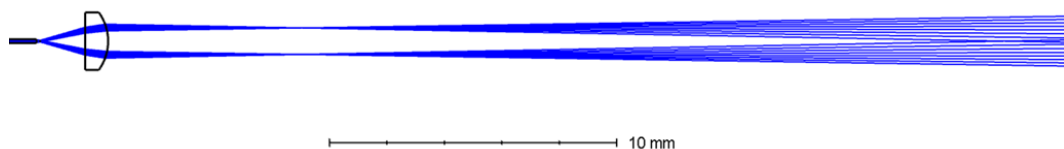


Figure 2.6 Simulation result of the collimated Bessel ring derived from the collimator.

Step 17. Firmly attach the glass flange to the collimating lens holder using black epoxy glue.

Caution! Avoid contact with both the fiber and glass flange during adhesive application, as physical disturbance may alter the critical distance between the fiber tip and collimating lens.

Step 18. After the epoxy has fully cured, gently detach the collimating lens holder (#5) from the collimator holder (#10) and verify the quality of the ring pattern from the collimator.

Part C: Assembling other optical and electrical components

Reagents:

- Air duster
- Lens cleaning wipe
- 100% ethanol
- UV-Curing Optical Adhesives (NOA61, Thorlabs)
- Black epoxy glue (9005B, LEAFTOP)
- Soldering tip thinner & cleaner

Equipment:

- Tweezers
- Scissor
- Stripping tool
- Soldering iron
- UV lamp
- Ultrasonic cleaner
- Stereoscope (M125C, Leica)
- Main components of the scan and tube lens setup (Table 3.1)
- Main components of the objective setup (Table 3.2)
- Main components of the MEMS mirror setup (Table 3.3)
- Main components of the SiPM detector setup (Table 3.4)

Table 3.1 Main components of the scan and tube lens setup

Component Number	Component Name	Part Number	Amount	Company
#12	Scan and Tube Lens Holder	/	1	Customized
#13	Scan and Tube Lenses	48-704	4	Edmund

Table 3.2 Main components of the objective setup

Component Number	Component Name	Part Number	Amount	Company
#14	Objective Holder	/	1	Customized
#15	Objective Lens ($\varnothing 2.5$ mm)	65-300	1	Edmund
#16	Objective Lens ($\varnothing 4.0$ mm)	47-850	1	Edmund

Table 3.3 Main components of the MEMS mirror setup

Component Number	Component Name	Part Number	Amount	Company
#17	MEMS Mirror	A3I12.2-1200AL	1	Mirrorcle
#18	MEMS PCB	/	1	Customized
#19	Multi-Core Cable	EN4C-FEP	~1.5 m	METOP

Table 3.4 Main components of the SiPM detector setup

Component Number	Component Name	Part Number	Amount	Company
#20	SiPM Detector	S13360-3075PE	1	Hamamatsu
#21	Coaxial Cable	44AWG	~1.5 m	METOP
#22	Filter (size of 6 × 6 mm ²)	FGB37-A	1	Thorlabs

Procedure:

Step 19. Clean the mechanical components #12 and #14, following the same protocol as Step 6.

Part C1: Assembling scan and tube lens

Step 20. Install the four lenses (#13) sequentially into the scan and tube lens holder (#12) using UV-curable adhesive, following the same protocol as Step 10 (Figure 3.1)

Note! The scan and tube lenses theoretically form an ideal 4f system in the design. However, in practice, manufacturing tolerances of both the lenses and holder may cause slight convergence or divergence in the output beam. This primarily affects the working distance of the objective lens.

Scan and Tube Lens Holder (#12) Scan and Tube Lenses (#13)

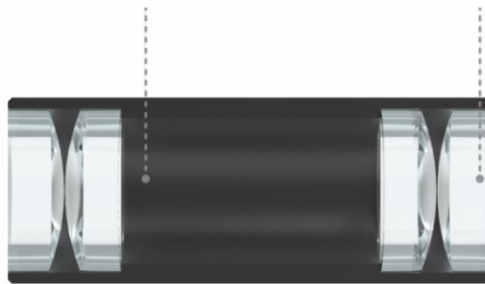


Figure 3.1 Schematic of the scan and tube lens

Part C2: Assembling objective

Step 21. Install the lens (#15) into the objective holder (#14) using UV-curable adhesive, following the same protocol as Step 20 (Figure 3.2).

Caution! Seal the objective by applying sufficient UV-curable adhesive to fill the interface gap between the lens and its holder.

Step 22. Install the second lens (#16) into the objective holder (#14) using UV-curable adhesive, following the same protocol as Step 20 (Figure 3.2).



Figure 3.2 Schematic of the objective

Part C3: Assembling MEMS mirror

Step 23. Solder the MEMS PCB (#18) to the base of the MEMS mirror (#17) and the multi-core cable (#19) to the MEMS PCB (#18) as shown in Figure 3.3. (Zong *et al.* have detailed the soldering methods in their work, <https://doi.org/10.1016/j.cell.2022.02.017>).

Caution! Do not remove the protective glass of the MEMS mirror at this step.

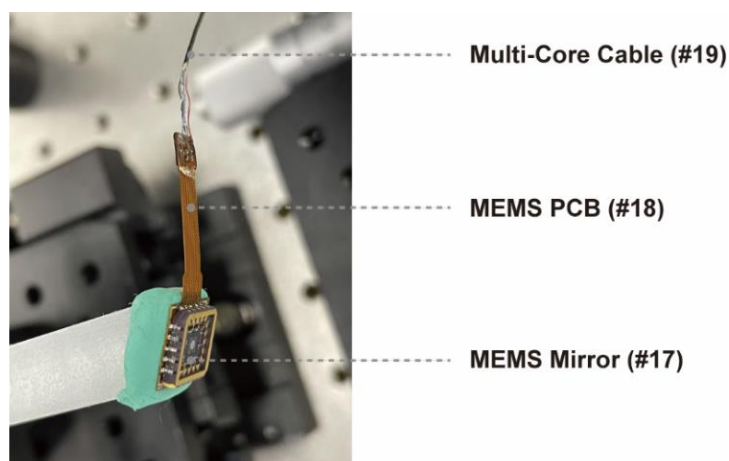


Figure 3.3 Photo of the MEMS mirror attached to its holder

Part C4: Assembling SiPM detector

Step 24. Connect the coaxial cable (#21) to the SiPM detector (#20) using similar soldering protocol described in Step 23.

Step 25. Position the SiPM detector with its photosensitive surface facing downward onto the center of the filter (#22), then secure it using UV-curable adhesive.

Caution! Only add a minimal amount of adhesive at the edge of the SiPM detector to prevent seepage into the detector-filter interface, which could contaminate the photosensitive surface.

Step 26. Apply black epoxy adhesive around the perimeter of the SiPM detector (Figure 3.4).

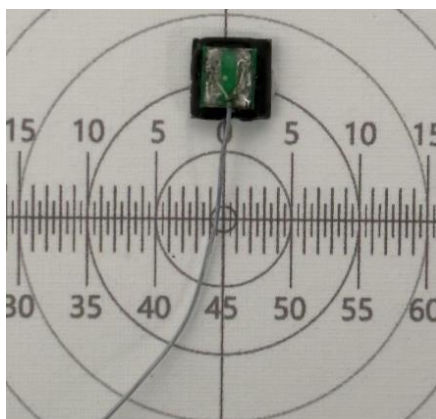


Figure 3.4 Fixing the SiPM detector onto the filter using black epoxy adhesive

Step 27. Apply black epoxy adhesive on the top of the SiPM detector (Figure 3.5).

Note! Steps 26–27 are critical for preventing scattered light from entering the SiPM detector.



Figure 3.5 Sealing the SiPM detector using black epoxy adhesive

Part D: Assembling the main body of the miniBB2p headpiece.

Reagents:

- Air duster
- Lens cleaning wipe
- 100% ethanol
- UV-Curing Optical Adhesives (NOA61, Thorlabs)
- Black epoxy glue (9005B, LEAFTOP)
- Black plasticine
- Black tape
- Tack-It (Faber Castle)

Equipment:

- Tweezers
- UV lamp
- Ultrasonic cleaner
- Stereoscope (M125C, Leica)
- Power meter (PM130D, Thorlabs)
- Optical mirror (UM10-AG, Thorlabs)
- NIR Detector Card (VRC4, Thorlabs)
- Oscilloscope (Tektronix, MDO34 3-BW-200)
- Fluorescent beads slide
- Fiber collimator (assembled in Part B)
- Scan and tube lens (assembled in Part C1)
- Objective (assembled in Part C2)
- MEMS mirror (assembled in Part C3)
- SiPM detector (assembled in Part C4)
- Main components of the main body setup (Table 4.1)

Table 4.1 Main components of the main body setup

Component Number	Component Name	Part Number	Amount	Company
#23	Main Frame	/	1	Customized
#24	Dichroic Mirror (size of $7 \times 7 \text{ mm}^2$)	DMR-805SP	1	LBTEK
#25	Filter (size of $6 \times 6 \text{ mm}^2$)	FIL-800	1	Sunlight
#26	Main Frame Holder	/	1	Customized
#27	MEMS Mirror Holder	/	1	Customized
#28	6-Axis Kinematic Mount (Frame)	K6XS	1	Thorlabs
#29	Rotation Stage	XRR1/M	1	Thorlabs
#30	Dovetail Rail	XR25DR3	1	Thorlabs
#31	6-Axis Kinematic Mount (MEMS)	K6XS	1	Thorlabs
#32	XY Translation Stage	PT1/M	2	Thorlabs
#33	Mounting Adapter	PT101/M	1	Thorlabs
#34	Single-Axis Translation Stage	PT1/M	1	Thorlabs

Procedure:

Step 28. Clean mechanical components #23, #26, and #27 following the same protocol as Step 6.

Part D1: Assembling optical components

Step 29. Assemble the dichroic mirror (#24), the scan and tube lens (assembled in **Part C1**), and the objective lens (assembled in **Part C2**) into the main frame (#23) in sequence, using UV-curable adhesive. Assemble the filter (#25) into the main frame using black epoxy adhesive.

Caution! The coated surface of the dichroic mirror and filter should face downward. When inserting the dichroic mirror into the narrow slit on the main frame, take care to avoid damaging the coated surface. Seal the slit using black tape or black epoxy adhesive.

Part D2: Assembling and evaluating the SiPM detector

Step 30. After the epoxy has fully cured, temporarily secure the SiPM detector (assembled in **Part C4**) into the main frame using black plasticine.

Step 31. Mount the main frame and align the free-space laser beam with the principal optic axis of the scan and tube lens. A focal spot can be observed after the objective using the NIR detector card.

Note! The beam can be aligned imperfectly at this step.

Step 32. Place an optical mirror behind the objective and increase the laser power to a high level (we used 100 mW post-objective).

Step 33. Turn off all the light in the room, and monitor the signal of the SiPM detector using an

oscilloscope. Similar signal levels should be observed whether we turn on or off the laser.

Note! In Steps 32–33, the 920-nm laser reflected from the mirror simulates the scattered/reflected excitation light during imaging, which should be fully blocked by the filters.

Troubleshooting! If an increasing signal level is observed when turning on the laser (indicating that the gaps in Steps 26 and 29 were not fully filled with black epoxy adhesive), re-sealing these gaps with black epoxy adhesive is critical to ensure effective background light rejection.

Part D3: Assembling the MEMS mirror

Step 35. Set up the assembling stage (Figure 4.1). Align the center of two 6-axis kinematic mounts (#28 and #31) by screwing z-axis adjustments of the mounts.

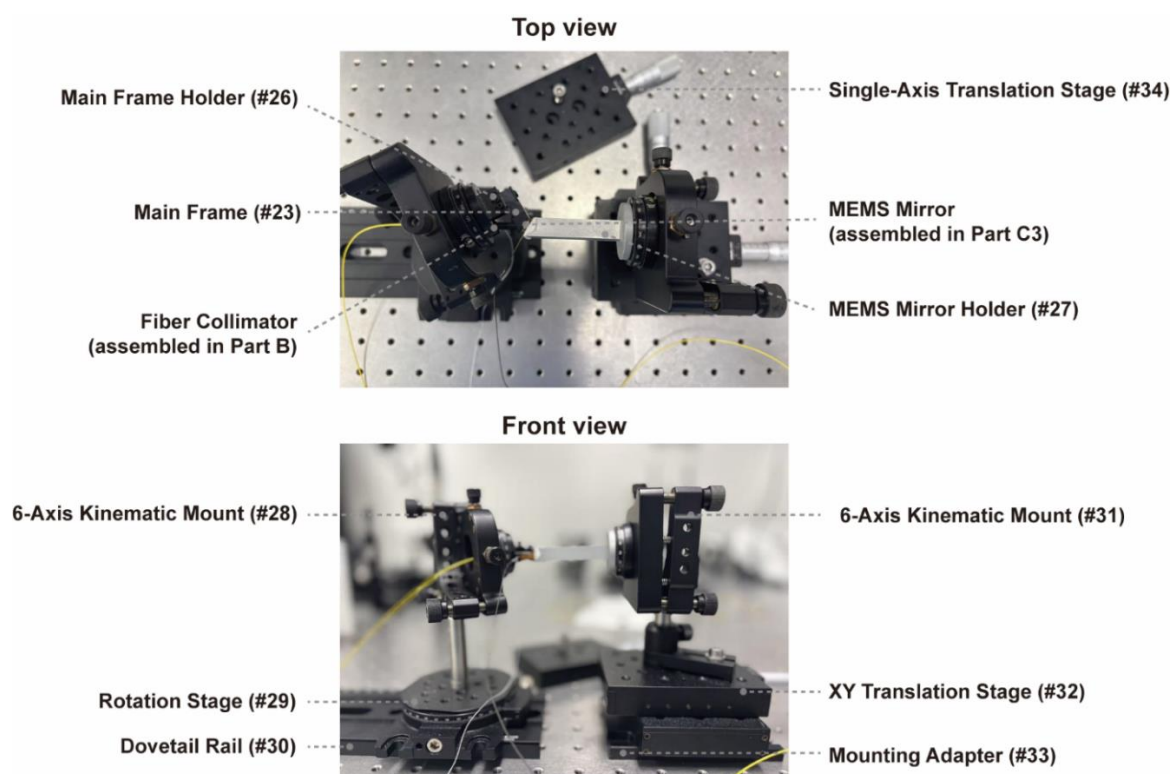


Figure 4.1 Assembling stage

Step 36. Unlock the dovetail locking screw of the rotation stage (#29) and move the rotation stage away from the MEMS mirror holder (#27). Insert the fiber collimator (assembled in **Part B**), through the 6-axis kinematic mount (#28), to the main frame. Fix the main frame to the main frame holder (#26) using screws.

Step 37. Mount the MEMS mirror (assembled in **Part C3**) onto the MEMS mirror holder by using Tack-It (Figure 4.1). Carefully remove the protective glass of the MEMS mirror with a tweezer.

Caution! Pay attention to mounting direction of the MEMS mirror.

Step 38. Move the rotation stage close to the MEMS mirror. Align the mounting surface of the main frame to the package of the MEMS mirror (Figure 4.2) by adjusting the 6-axis kinematic mount (#28) and rotation stage (#29). Lock the dovetail locking screw of the rotation stage.

Caution! The rotation stage needs to be slightly moved forward or backward several times during aligning.

Caution! After this step, keep all the pitch, yaw, and rotation adjustment unchanged, and only adjust x-, y-, or z-translation.

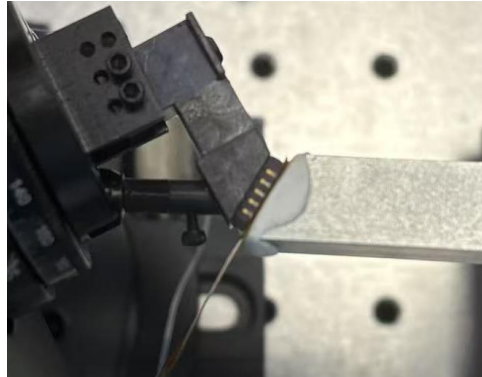


Figure 4.2 Align the main frame and MEMS mirror

Step 39. Couple the laser into the fiber following the same protocol as Step 4, monitor the beam after the objective using the NIR detector card, and adjust the 6-axis kinematic mount (#31) until the outgoing laser is fully ring-shaped and not trimmed (Figure 4.3).

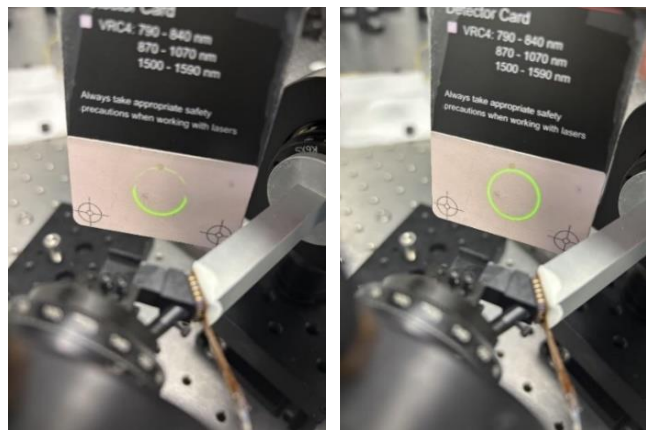


Figure 4.3 Trimmed (left) and untrimmed ring beam (right)

Step 40. Place a fluorescent bead slide on the single -axis translation stage (#34) (Figure 4.4).

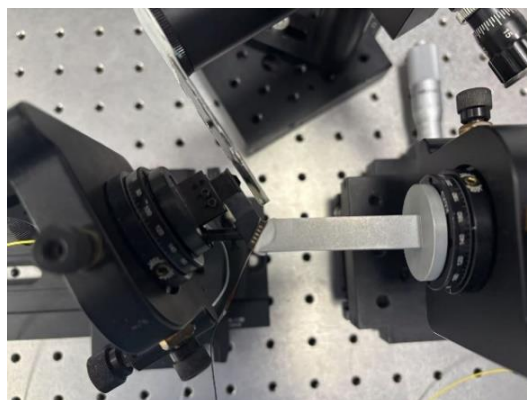


Figure 4.4 Imaging fluorescent beads on a slide

Step 41. Fill the imaging medium between the objective and slide. Turn off all lights in the room, then adjust the single -axis translation stage (#34) to position the slide at the focal plane of the miniBB2p.

Step 42. Slightly adjust the XY translation stage (#32) and 6-axis kinematic mount (#31) until sharp spots (point spread functions) could be observed across the field of view (Figure 4.5).

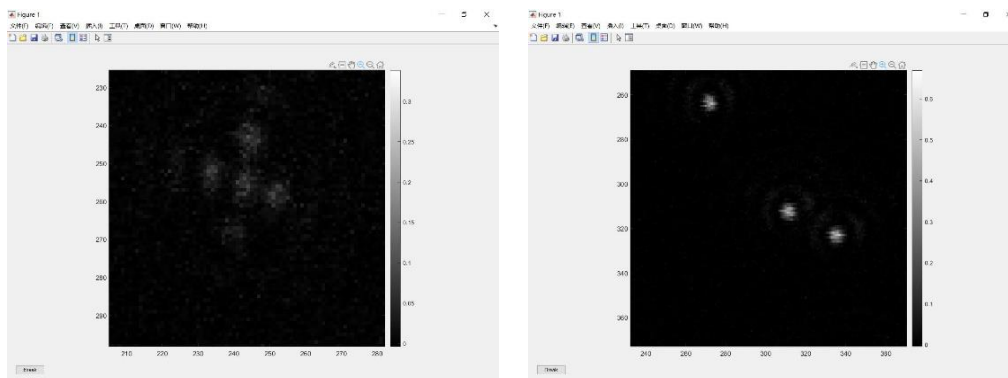


Figure 4.5 Representative images showing blurred (left) and sharp spots (right).

Step 43. Lock all the screws of the mounts and stages. Apply a small amount of UV-curable adhesive to the gap between the MEMS package and the attached main frame surface (Figure 4.6), following the protocol described in Step 10.

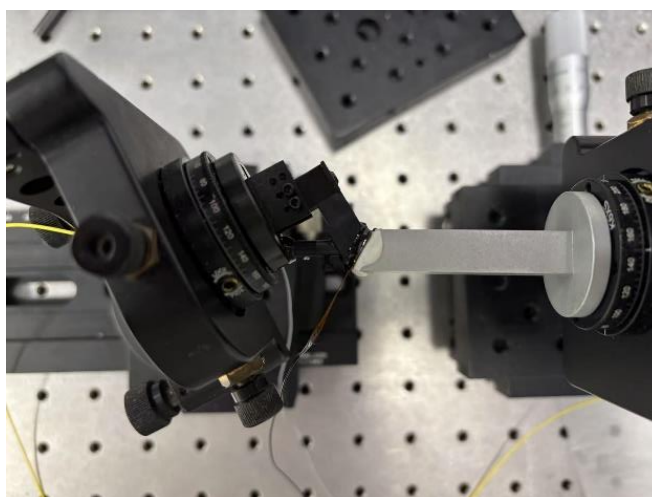


Figure 4.6 Bond the MEMS mirror to the main frame.

Caution! Avoid using too much adhesive, as excess adhesive can bond to the MEMS surface and cause damage.

Step 44. Apply black epoxy adhesive around the gap and allow at least 24 hours curing time for the MEMS-to-main frame bond to stabilize (Figure 4.6).

Caution! Take care not to adjust any mounts or stages during Steps 43–44.

Step 45. After the black epoxy adhesive has cured, separate the MEMS mirror from its holder by removing Tack-It, remove the fiber collimator from the main frame, and detach the main frame from its holder.

Part E: Fiber protecting and connecting

Reagents:

- Air duster
- Lens cleaning wipe
- 100% ethanol
- UV-Curing Optical Adhesives (NOA61, Thorlabs)
- Black epoxy glue (9005B, LEAFTOP)

Equipment:

- Scissor
- Tweezers
- UV lamp
- Stereoscope (M125C, Leica)
- Fiber stripping tool (T06S13, Thorlabs)
- Power meter (PM130D, Thorlabs)
- NIR Detector Card (VRC4, Thorlabs)
- Fiber collimator (assembled in Part B)
- Main components of the fiber protecting and connecting setup (Table 1)

Table 5.1 Main components of the fiber protecting and connecting setup

Component Number	Component Name	Part Number	Amount	Company
#35	Furcation Tubing	FT900Y	~1 m	Thorlabs
#36	Fiber Connector	30126C9	1	Thorlabs
#37	Connectorized-Fiber Holder	OFB02-FPC2	1	LBTEK

Procedure:

Step 46. Carefully insert the fiber into the furcation tubing (#34), advancing it until the furcation tubing reaches the collimating lens holder (#5).

Caution! Double-check the fiber end prepared in Step 3. If the surface is not flat, re-cut the fiber using a protocol similar to Step 3 before insertion.

Caution! First measure both the fiber and furcation tubing lengths. The furcation tubing should be ~4 cm shorter than the fiber for proper assembly with fiber connector (#36).

Step 47. Secure the furcation tubing to the collimating lens holder using black epoxy adhesive.

Step 48. Assemble the fiber connector (#36) according to the Thorlabs FN96A guide (Chapter 2).

Caution! The LMA-12 fiber cannot be polished after connector assembly, so ensure the stripped fiber has the appropriate length before assembling the connector.

Step 49. Secure the other end of the furcation tubing to the connector boot using UV-curable adhesive.

Step 50. Mount the assembled fiber onto the connectorized-fiber holder (#37), and optimize the laser-to-fiber coupling using the protocol described in Step 4 (Figure 5.1).

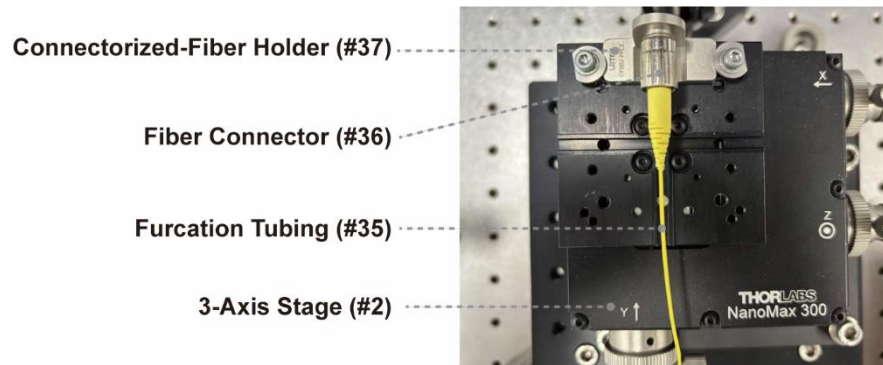


Figure 5.1 Mount the assembled fiber onto the connectorized-fiber holder.

Step 51. Reinsert the fiber collimator into the main frame and secure it using black epoxy adhesive.

Caution! Verify the imaging performance before applying the adhesive.