Electronic Supplementary Information (ESI)

Fully Automated Spotting Device for Thin-Layer Chromatography based on the Modification of a 3D Printer

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Building instructions

3D printer setup

All 3D-printed parts were designed with Autodesk Inventor Professional 2022. The exported STL-files were sliced with the software simplify3D.

All parts were printed in PETG (polyethylene terephthalate glycol-modified) 1.75 mm from Fiberlogy (Bordeaux red) on a Qidi X-CF Pro from Qidi. The filament roll was stored in a sealed box with silica gel bags inside to ensure proper drying of the PETG. The following settings were used:

Print speed = 60 mm/s

Extrusion multiplier = 1.00

Nozzle temp. = 230 ° C

Bed temp. = 80 °C, printed on a flexible PEI metal sheet, coated with DimaFix pen.

Layer height: 0.3 mm (indication in the parts list if a part was printed with 0.2 mm layer height)

The STP and STL files of all printed parts can be found in the Documentation section. To be able to print all relevant parts, a printer with a printing area of at least 300 x 250mm required.

Detailed step-by-step assembly instruction

The individual parts of the TLC robot are shown in **Figure S1;** the complete parts list can be found in **Table S1**.

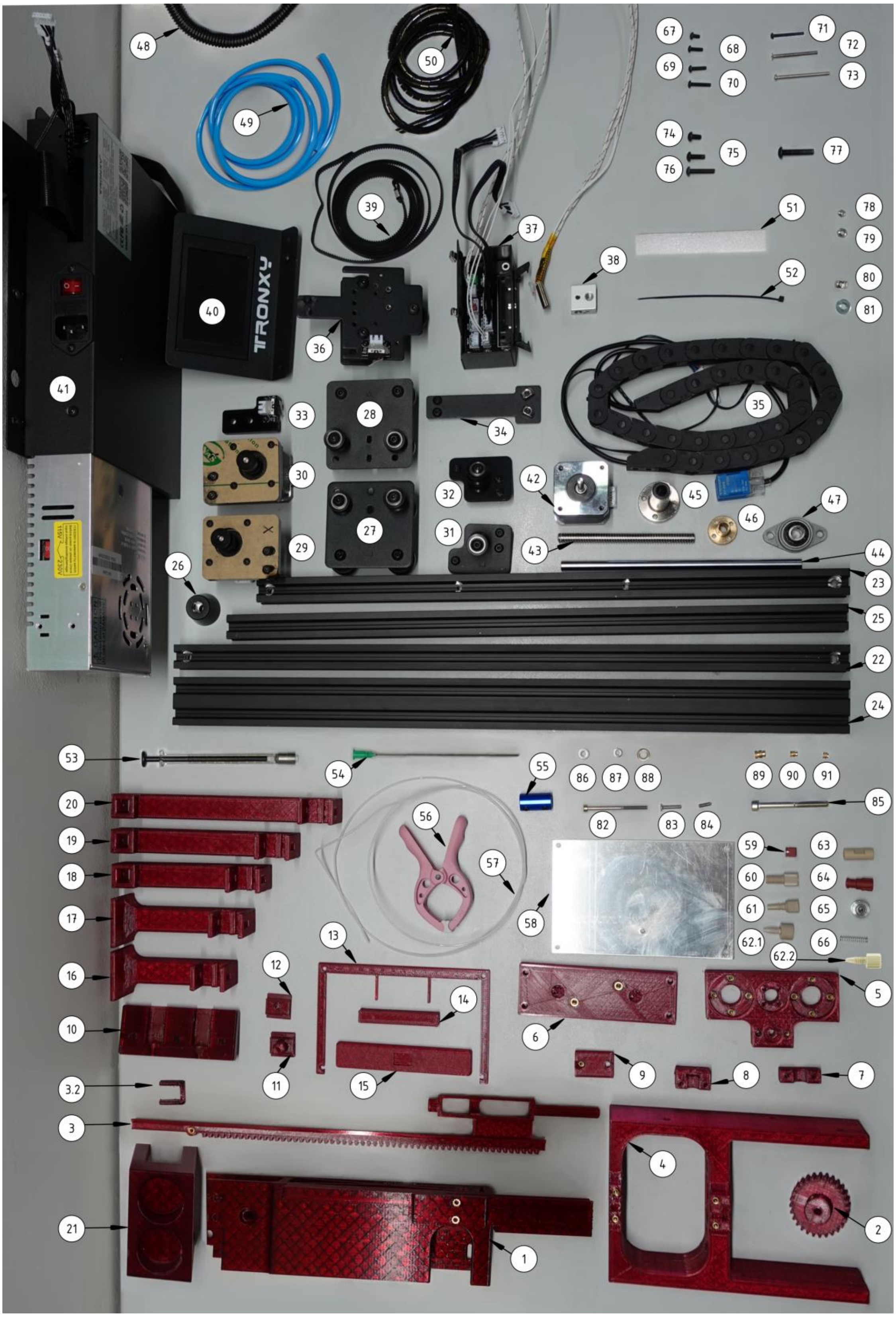


Figure S1: All parts needed for the assembly of the TLC robot.

Table S1: Parts list for the TLC robot (The links were checked on 05.06.2024)

No.	Part	Source	Quantity.
1	Z-axis mounting (01_Z_Mounting)	3D-printed	1
2	Gear wheel (02_Z_Gear_Wheel)	3D-printed	1
3	Gear rack with Capillary holder (03_Z_Gear_Rack)	3D-printed	1
3.2	Fitting fixation (03.2_Z_Gear_Fixation)	3D-printed	1
4	Top part of syringe pump (04_SP_Top)	3D-printed	1
5	Middle part of syringe pump (05_SP_Middle)	3D-printed	1
6	Bottom part of syringe pump (06_SP_Bottom)	3D-printed	1
7	Barrel clamp 1 (07_SP_Clamp1)	3D-printed	1
8	Barrel clamp 2 (08_SP_Clamp2)	3D-printed	1
9	Plunger plate (09_SP_Plate)	3D-printed	1
10	Main part of belt tensioner (10_BT_main)	3D printed	1
11	Left part of belt tensioner (11_BT_left)	3D printed	1
12	Right part of belt tensioner (12_BT_right)	3D printed	1
13	Top part of TLC holder (13_TH_Top)	3D-printed	1
14	Legs of TLC holder (14_TH_Leg)	3D-printed	4
15	Clamp plate for TLC holder (15_TH_Plate)	3D-printed	1
16	Y-axis positioner of rack (16_PS_Rack_Y)	3D-printed	1
17	X-axis positioner of rack (17_PS_Rack_X)	3D-printed	2
18	X-axis positioner of TLC holder (18_PS_TH_X)	3D-printed	1
19	X-axis positioner of table (19_PS_Table_X)	3D-printed	1
20	Y-axis positioner of TLC holder (20_PS_TH_Y)	3D-printed	1
21	Y-axis positioner of TLC holder (21_Table)	3D-printed	1
22	Aluminium profile 1: 20x20x530 mm	Tronxy X5SA	4
23	Aluminium profile 2: 20x20x460 mm	Tronxy X5SA	4
24	Aluminium profile 3: 20x40x530 mm	Tronxy X5SA	4
25	Aluminium profile 4: 20x20x484 mm	Tronxy X5SA	1
26	Rubber feet	Tronxy X5SA	4
27	Left return board	Tronxy X5SA	1
28	Right return board	Tronxy X5SA	1
29	X-motor	Tronxy X5SA	1
30	Y-motor	Tronxy X5SA	1
31	Left pulley component	Tronxy X5SA	1
32	Right pulley component	Tronxy X5SA	1
33	Y-axis switch	Tronxy X5SA	1
34	Towline holder	Tronxy X5SA	1
35	Cable drag chain with Z-axis sensor	Tronxy X5SA	1
36	Hotend carriage with X-axis switch	Tronxy X5SA	1
37	Terminal block	Tronxy X5SA	1
38	Heating block	Tronxy X5SA	1
39	Tension belt	Tronxy X5SA	2
40	Display	Tronxy X5SA	1
41	Host box	Tronxy X5SA	1
42	Z-motor	Tronxy X5SA	2

43	Lead screw	Tronxy X5SA	1
44	Sliding rod	Tronxy X5SA	2
45	Round flange linear bearing	Tronxy X5SA	2
46	Trapezoidal lead nut	Tronxy X5SA	1
47	Flange bearing	Tronxy X5SA	1
48	Corrugated pipe	Tronxy X5SA	1
49	Aluminum profile seal	Tronxy X5SA	1
50	Cable spiral hose	Tronxy X5SA	1
51	Packaging foam piece: 105x15 mm	Tronxy X5SA	1
52	Cable ties	Tronxy X5SA, in-house workshop	approx. 20
53	Hamilton 1 mL gastight syringe model 1001, consisting of a barrel (53.1) and a plunger (53.2)	Fisher Scientific	1
54	B.Braun Sterican® cannula 21G x 4 3/4", 0.80 x 120 mm	Fisher Scientific	
55	Aluminum alloy coupler 5 to 8 mm	Amazon	1
56	Wolfcraft spring clamp microfix, span = 30 cm	Amazon	1
57.1	PTFE-tubing, ID = 0.750 mm, OD = 1/16", length = 120cm	Techlab	1
57.2	PTFE-tubing, ID = 0.750 mm, OD = 1/16", length = 30cm	Techlab	1
58	Aluminium plate 9x14cm	in-house workshop	1
59	2-Pin jumper bridge	in-house workshop	3
60	IDEX H&S XP-230: flangeless flat-bottom fitting, 1/4"-28 UNF (M) for 1/16" OD tubing	Techlab	1
61	IDEX H&S P-656 Adaptor: Luer Lock (M) to 10-32 UNF (F)	Techlab	1
62.1	IDEX H&S F-120 fingertight fitting: 10-32 UNF (M) to 1/16'' OD tubing	Techlab	2
62.2	Fitting PEEK one-piece natural combi-head; VICI JR-55020-1: 10-32 UNF (M) to 1/16'' OD tubing	Techlab	1
63	PEEK connector: 10-32 UNF (F) to 10-32 UNF (F)	Techlab	1
64	IDEX H&S P-658 Adaptor: Luer (F) to 1/4"-28 UNF (F)	Techlab	1
65	GT2 Idler pulley for 16 teeth (without teeth): bore = 3mm, width = 6mm	Amazon	2
66	Compression spring, 0.3x5x20mm	Amazon	1
67	Screw M3x6	Tronxy X5SA	4
68	Screw M3x10	Tronxy X5SA	6
69	Screw M3x12	Tronxy X5SA	8
70	Screw M3x16	Tronxy X5SA	8
71	Screw M3x25	Tronxy X5SA, obtained from filament sensor	1
72	Screw M3x30	Tronxy X5SA	2

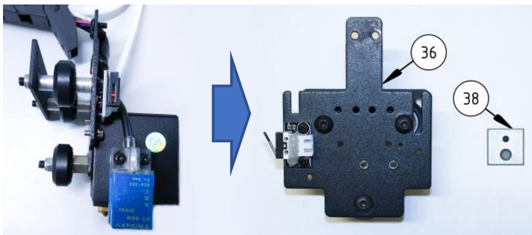
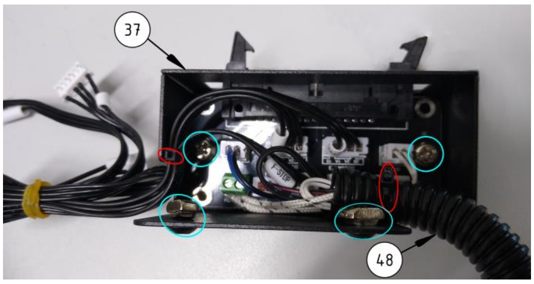
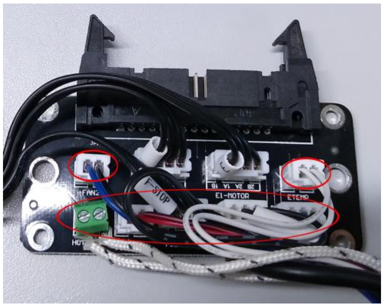
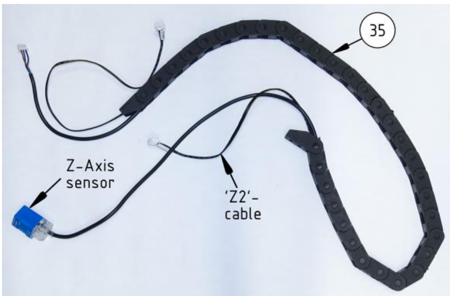
73	Screw M3x40	Tronxy X5SA, obtained from mounting plates of z-motors	4
74	Screw M4x8	Tronxy X5SA	19
75	Screw M4x12	Tronxy X5SA	14
76	Screw M4x20	Tronxy X5SA	4
77	Screw M5x25	Tronxy X5SA	16
78	M3 Nut	Tronxy X5SA	6
79	M4 Nut	Tronxy X5SA	4
80	M4 T-Nut	Tronxy X5SA	23
81	M4 Washer	Tronxy X5SA	13
82	Screw M3x45	Amazon	4
83	Countersunk screw M3x16	in-house workshop	1
84	Grub screw M3x10	Amazon	2
85	Screw M5x50	Amazon	2
86	M3 Washer: ID = 4 mm, OD = 9 mm	in-house workshop	4
87	M3 Washer: ID = 3.5 mm, OD = 7 mm	in-house workshop	5
88	M5 Washer	in-house workshop	4
89	Threaded insert nut brass M4x8x6	Amazon	6
90	Threaded insert nut brass M3x6x5	Amazon	19
91	Threaded insert nut brass M3x4x5	Amazon	2

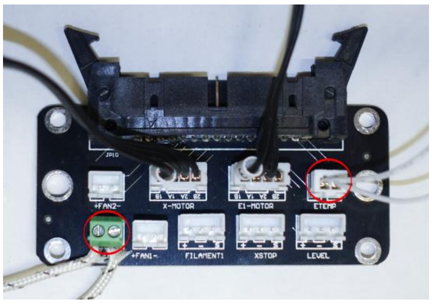
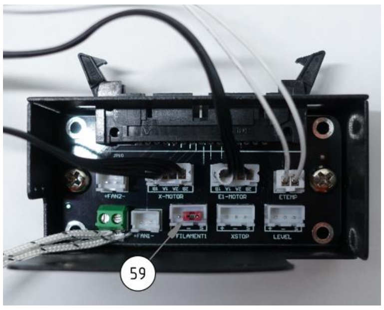
Detailed assembly instructions with example photos are provided in the following that explain how to build the individual components of the TLC robot and how to mount them together. After all necessary parts are 3D printed, the TLC robot can be comfortably assembled within 1-2 day(s).

As the steps for the base frame assembly and the Tension belt installation of the TLC robot are similar to those during the assembly of the Tronxy X5SA printer, additional information on these steps can be found in the installation instructions for the Tronxy X5SA or in the official installation video.

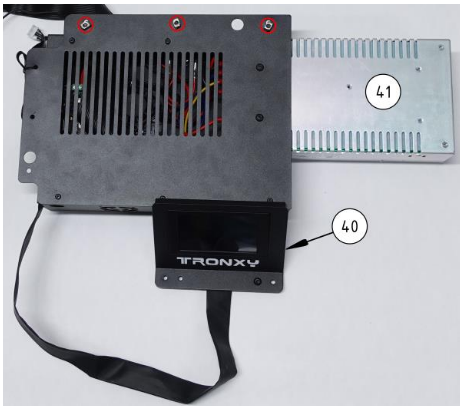
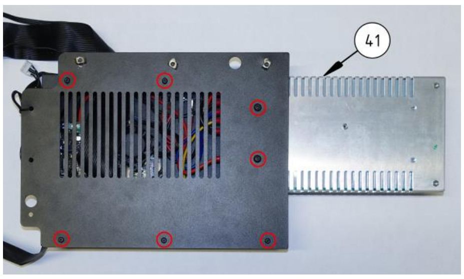
Using the example photos and the additional exploded CAD views of the individual components, assembling the TLC robot should be possible without special tools. However, an angle grinder is recommended for cutting the Sliding rods to the correct length and a bench drill with a vise is advised for drilling accurate holes into the aluminum plate. Furthermore, it is recommended to shorten the cannulas with a rotary tool with a cut-off wheel and a vice to avoid obstructing them.

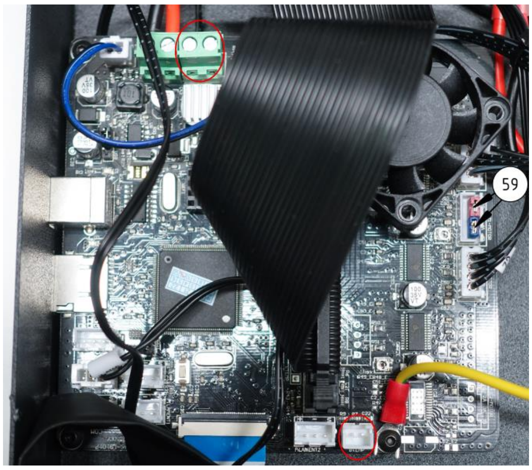
Disassembly of the Extruder Head

	**Step 1:** First, the printing head component was disassembled to retrieve the hotend carriage (36) with the X-axis switch and the heating block (38).
	**Step 2:** The cable ties (red ellipsis) were cut and the corrugated pipe (48) was carefully removed from the terminal block (37). **Step 3:** Four screws and two M4 T-nuts (turquoise ellipsis) were unfastened to remove the adaptor board from the metal case.
	**Step 4:** The cables in the sockets 'FAN1', 'FAN2', 'FILAMENT1', 'XSTOP', 'LEVEL', 'HOTEND' and "ETEMP" of the adaptor board were unplugged (red ellipsis).
	**Step 5:** The cables of the heating cartridge and the temperature sensor were carefully pulled through the cable drag chain (35). When the plug at the end of the 'ETEMP' cable got tangled up inside the cable drag chain, tweezers were used to release it. **Step 6:** Step 5 was repeated with the 'FAN1' and 'FAN2' cables. In the end, the cable drag chain only contained the cable of the Z-axis sensor and the 'Z2'-cable.

	**Step 7:** The cables of the Heating cartridge and the temperature sensor were reconnected to the sockets "HOTEND" and "ETEMP" (red circles) on the adaptor board.
	**Step 8:** A 2-pin jumper bridge (59) was placed inside the 'FILAMENT1' socket. **Step 9:** The adaptor board was repositioned inside the metal case and the four screws and two M4 T-nut removed in Step 3 were reattached.

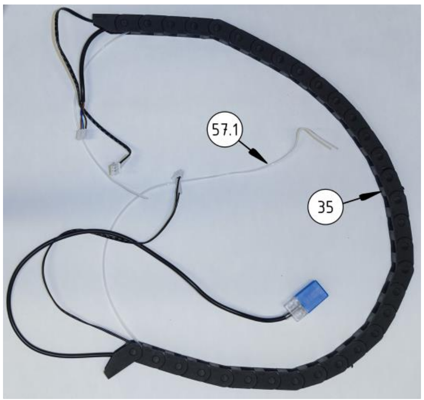
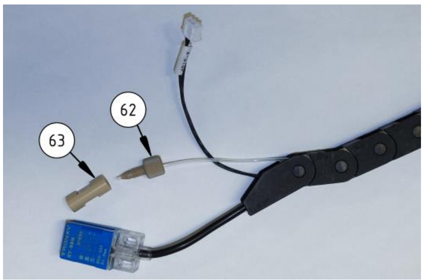
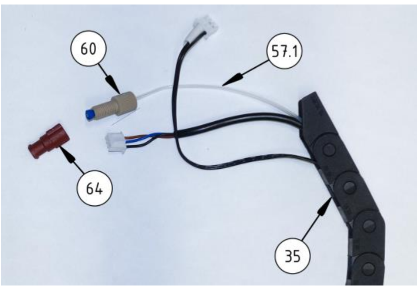
Electronic Setup of the Host Box

	**Step 1:** The screw connecting the display (40) to the Host box (41) was loosened. After both components were separated, an M4x8 screw (74) and an M4 T-nut (80) were connected to the right side of the display. **Step 2:** The three M4 screws and T-nuts at the top of the host box were readjusted as shown in the picture (red circles).
	**Step 3:** The host box (41) was opened by removing the seven screws (red circles) on its back side.

<table>
<tr><td>
</td><td>Step 4: The cables in the sockets 'HOTBED' and "BTEMP" were unplugged (red ellipsis).

Step 5: The 'Z1-Motor' cable was removed and two 2-pin jumper bridges (59) were placed inside the 'Z1-Motor' socket.</td></tr>
</table>

Assembly of the cable Drag Chain

<table>
<tr><td>
</td><td>Step 1: The longer piece of PTFE-tubing (57.1) was carefully pulled through the cable drag chain (35). If the tubing got tangled up inside the cable drag chain, tweezers were used to release it.</td></tr>
<tr><td>
</td><td>Step 2: The end of the PTFE-tubing adjacent to the Z-axis sensor was connected to a fingertight fitting (62.1) and a PEEK connector (63).</td></tr>
<tr><td>
</td><td>Step 3: The other end of the PTFE-tubing was connected to a flat-bottom fitting (60) and a P-658 adaptor (64).</td></tr>
</table>

Assembly of the Z-axis

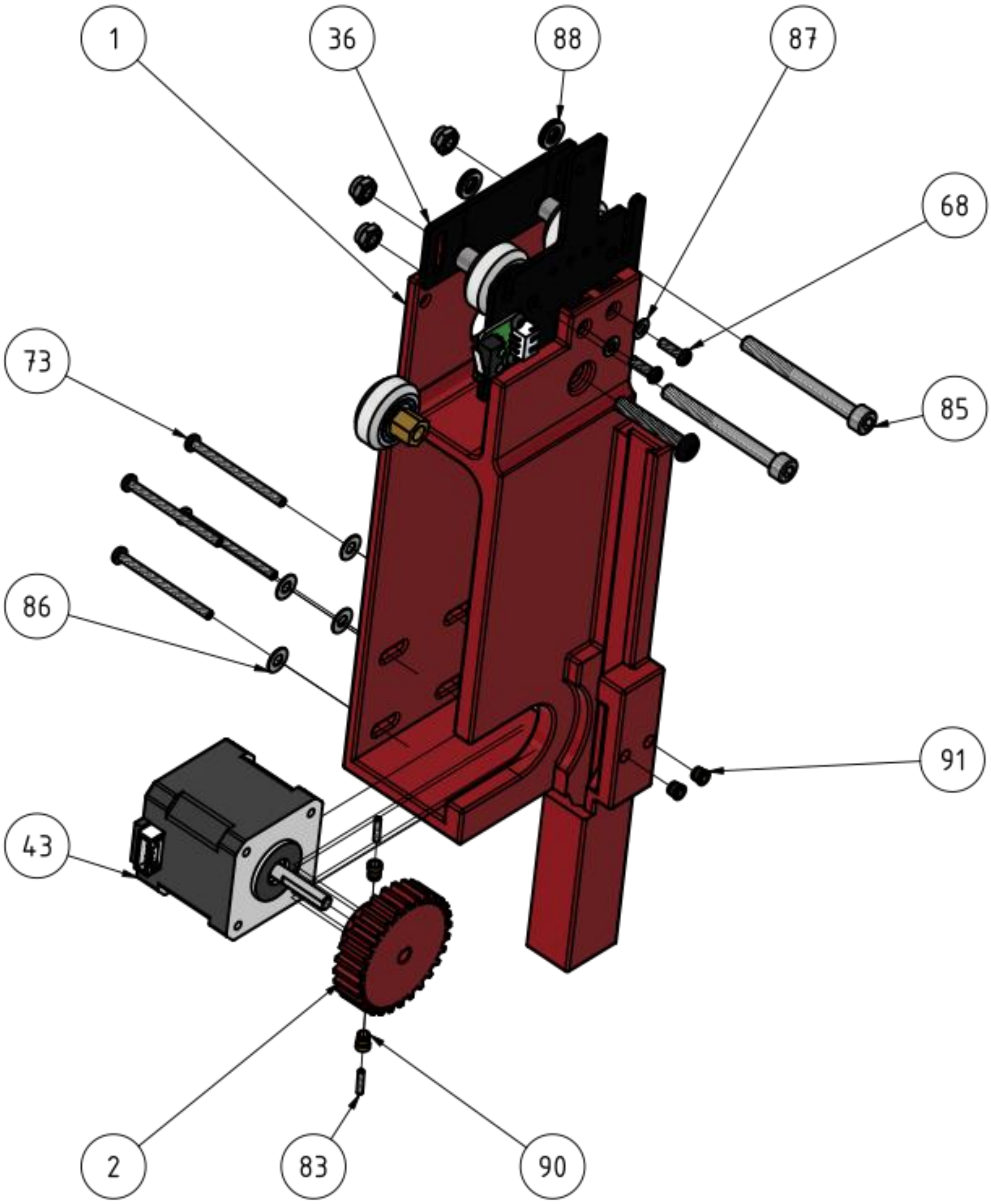
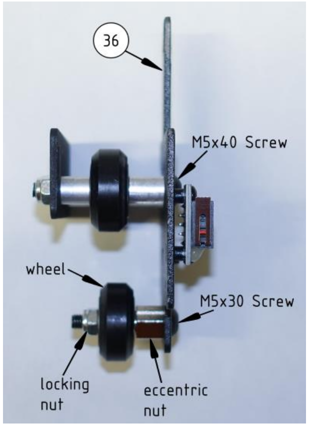
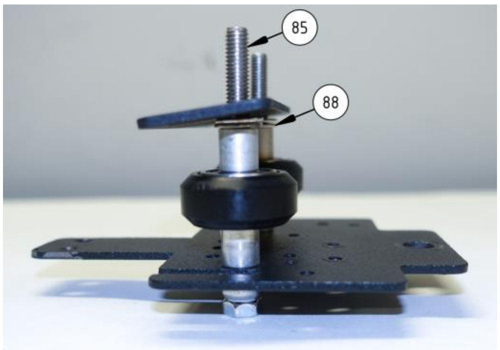
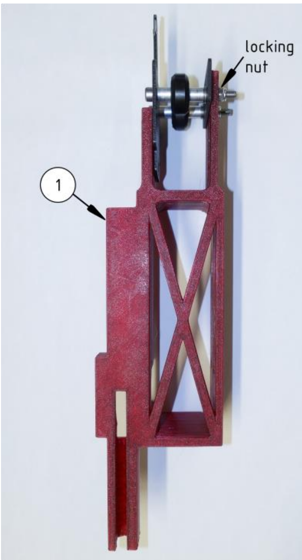
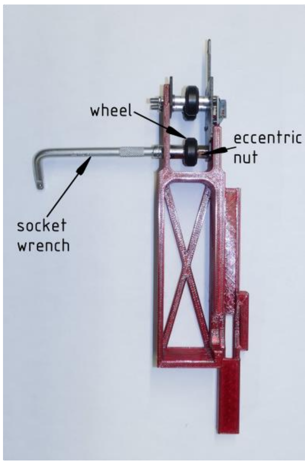
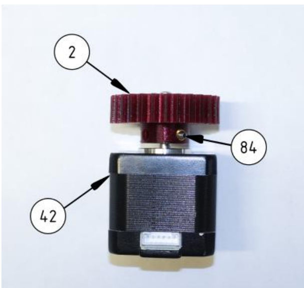


Figure S2: Exploded-view CAD drawing of the Z-axis[1-3]

	**Step 1:** Using a soldering iron, two M3 inserts (91) were inserted into the Z-axis mounting (1). Two M3 inserts (90) were inserted into two opposing holes in the gear wheel (2). **Step 2:** The M5x30 Screw in the middle of the hotend carriage (36) was removed and the individual components (M5x30 Screw, locking nut, wheel, eccentric nut) were put aside.
	**Step 3:** The locking nuts at the back side of the hotend carriage were loosened and put aside. **Step 4**: The two M5x40 screws at the top of the hotend carriage were replaced by two M5x40 screws (85). **Step 5:** Two M5 washers (88) were placed on each M5 Screw between the spacer sleeve and the back of the hotend carriage.
	**Step 6:** The Z-axis mounting (1) was slid onto the hotend carriage and fixed with the two locking nuts on the back.

	**Step 7:** Two M3x10 screws (68) and two M3 washers (87) were used to connect the Z-axis mounting to the front of the hotend carriage.
	**Step 8:** The M5x30 screw was inserted through the holes in the front side of the Z-axis mounting and the hotend carriage. **Step 9:** The eccentric nut and the wheel were added from the side and placed on the M5 screw. **Step 10:** A socket wrench was used to place the lock nut on the M5 screw through the hole in the back side of the Z-axis mounting tightening it. **Step 11:** It was verified that the three wheels of the hotend carriage were aligned in one vertical plane.
	**Step 12:** Two M3x10 grub screws (84) were used to connect the gear wheel (2) to one of the Z-motors (42).

	**Step 13:** Four M3 washers (86) and four M3x45 screws (73) were used to connect the Z-motor with the gear wheel to the Z-axis mounting of the hotend carriage. The screws should not be fully tightened to readjust them later.

Assembly of the Capillary Holder

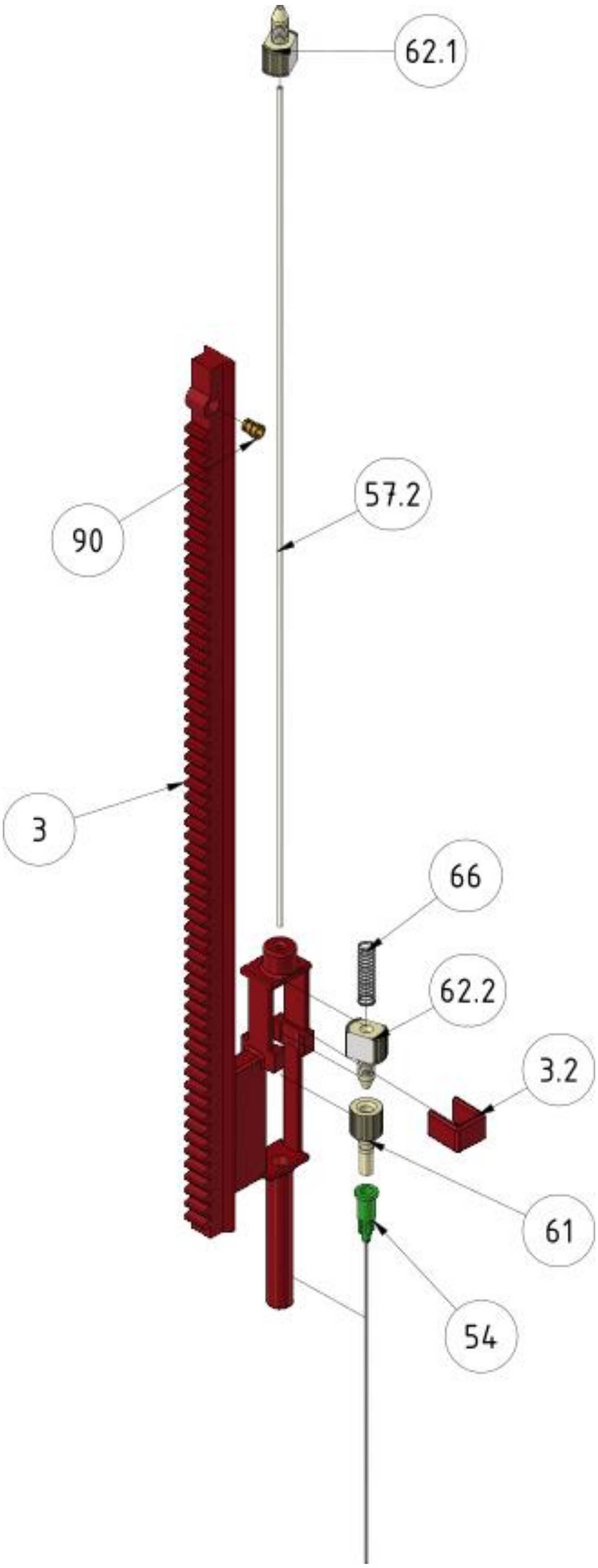
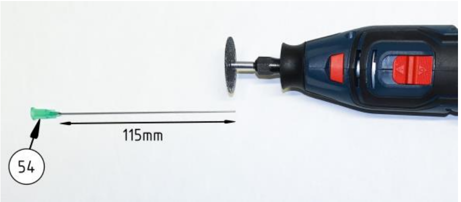
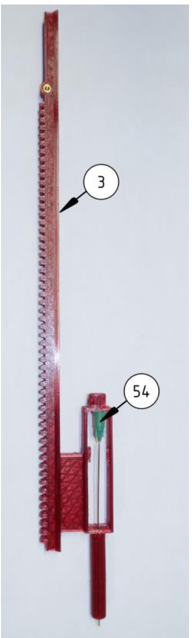
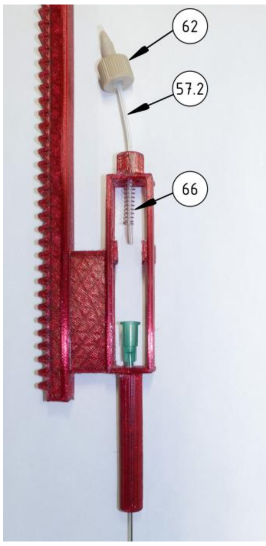
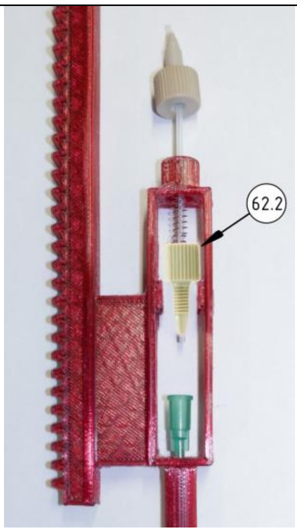
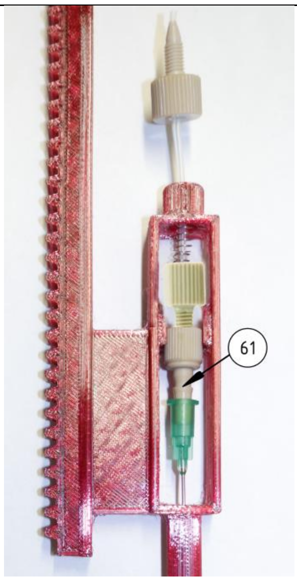
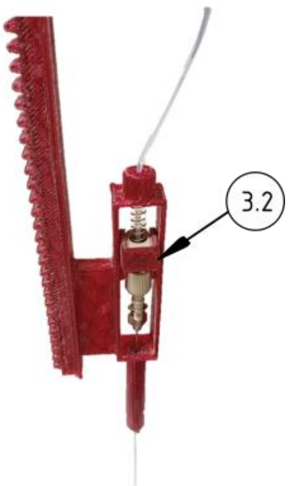


Figure S3: Exploded-view CAD drawings of the Capillary holder.

	**Step 1:** A rotary tool with a cut-off wheel was used to shorten the Capillary (54) to 115 mm. **Step 2:** The edges at the bottom of the Capillary were smoothed with sandpaper.
	**Step 3:** An M3 threaded insert (90) was inserted into the gear rack (3) using a soldering iron. **Step 4**: The Capillary (54) was carefully inserted through the front opening at a slight angle. Care should be taken so that the Capillary is not bent permanently.
	**Step 5:** One end of the shorter PTFE-tubing piece (57.2) was connected to a fingertight fitting (62.1). **Step 6:** The other end of the tubing was inserted through the hole in the top of the Capillary holder. **Step 7:** A Compression Spring (66) was placed on the PTFE-tubing.

<table>
<tr><td>
</td><td>Step 8: Subsequently, the free end of the PTFE-tubing was connected to another fingertight fitting with combi-head (62.2).

Step 9: The fitting was then inserted into the upper segment of the Capillary holder through the front opening.</td></tr>
<tr><td>
</td><td>Step 10: A P-656 Adaptor (61) was inserted into the Capillary holder and screwed onto the fitting.

Step 11: Afterward, the P-656 adaptor was connected to the capillary. While the fitting was tightened, the capillary was held in place with tweezers to ensure a tight connection.</td></tr>
<tr><td>
</td><td>Step 12: The fitting fixation (3.2) was sanded on both sides (grain size 200-1000) to ensure easy sliding up and down. After sanding, it was inserted over the fitting (61.2). This fixation is required to prevent the fitting and thus the needle from rotating</td></tr>
</table>

Assembly of the syringe pump

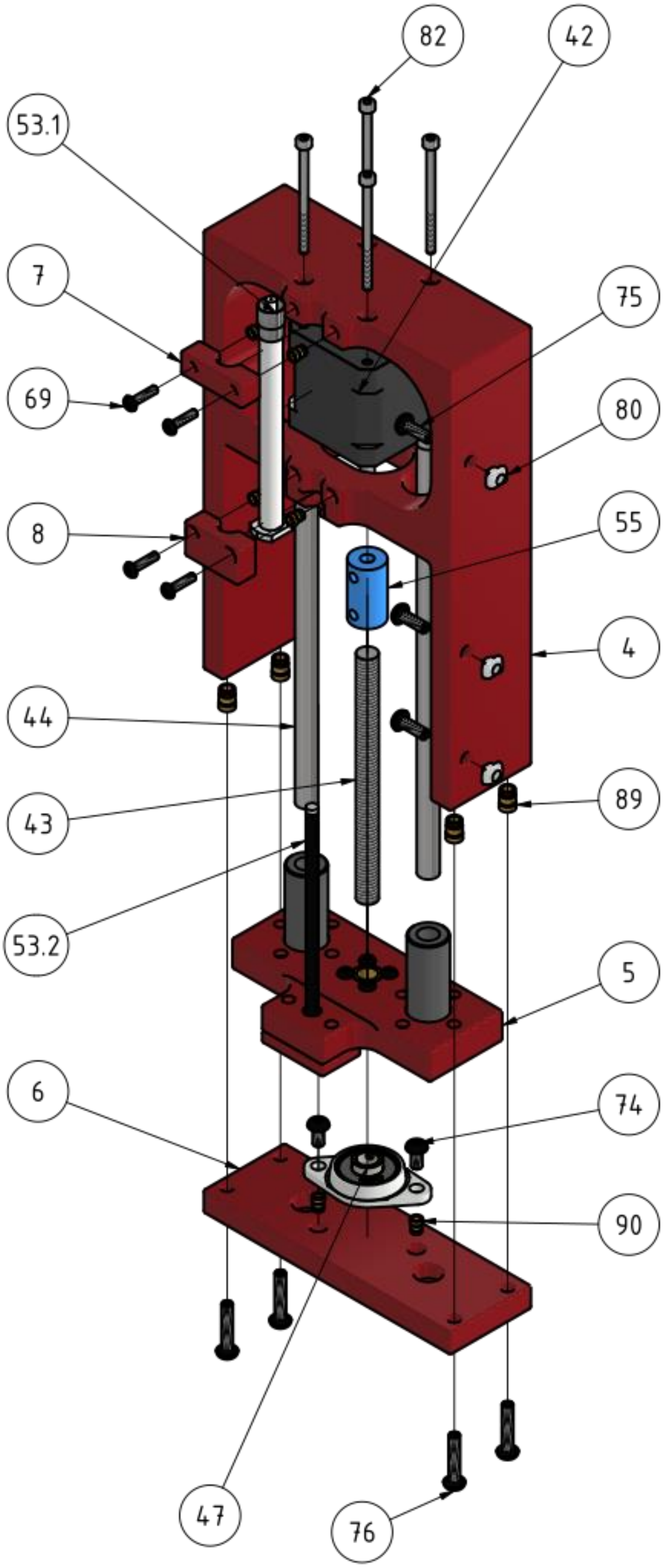


Figure S4: Exploded-view CAD drawing of the syringe pump (Flange Bearing: Adapted from Schmidt, 2021).

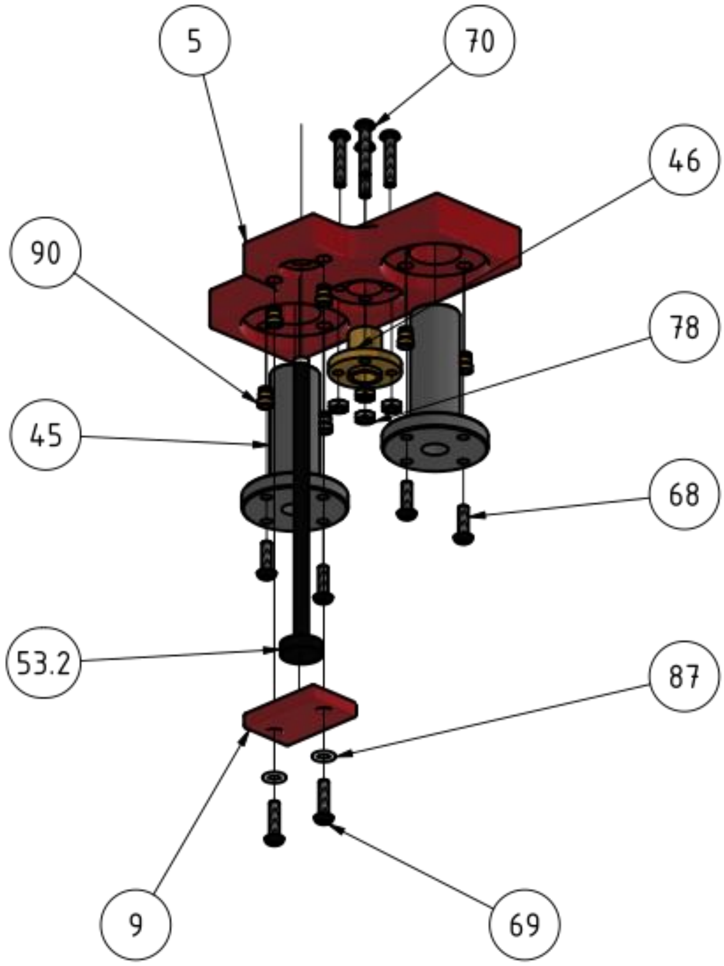
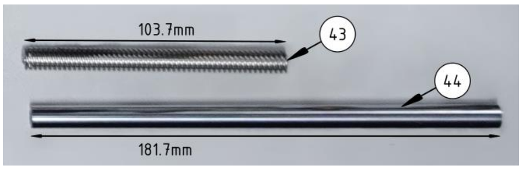
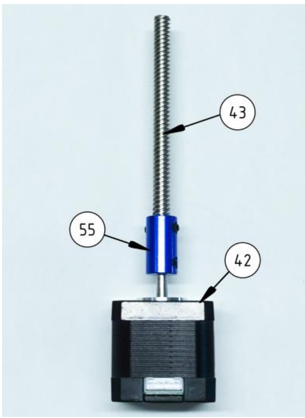
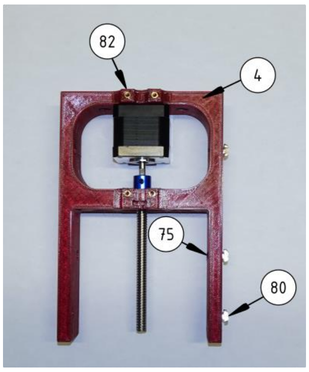
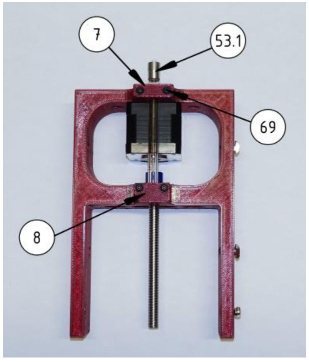
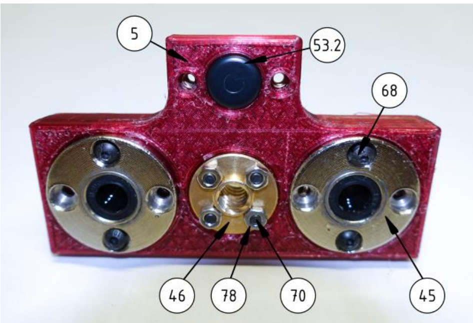
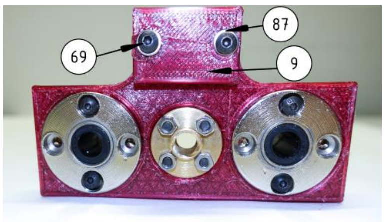
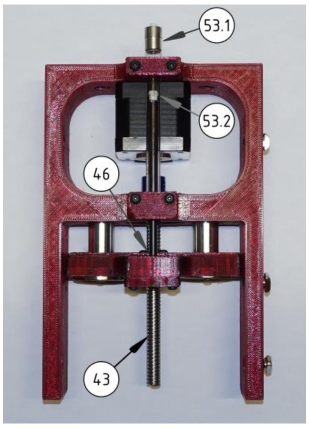
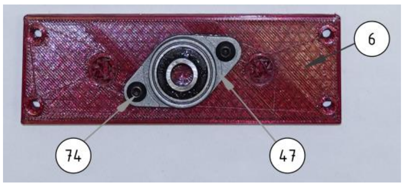
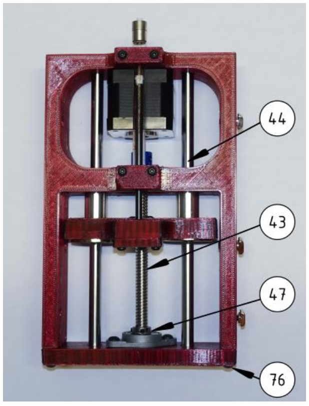


Figure S5: Exploded-view CAD drawing of the middle part of the syringe pump.

	**Step 1:** The lead screw (43) was shortened to 103.7 mm. **Step 2:** An angle grinder was used to cut two Sliding rods (44) to a length of 181.7 mm.
	**Step 3:** Ten M3 threaded inserts (90) and six M4 inserts (89) were inserted into the compounds of the syringe pump using a soldering iron. **Step 4:** The shaft of the other Z-motor (42) and the lead screw (43) were placed inside an aluminum coupler (55). Afterward, the four grub screws of the coupler were tightened to fix the connections.

	**Step 5:** Three M4x12 Screws (75) were inserted through the holes in the right side of the Top part (4) and connected to three M4 T-nuts (80). **Step 6:** The unit consisting of the motor, the coupler and the lead screw was inserted through the front opening in the top part at an angle. The motor was connected to the top part with four M3x45 screws (82).
	**Step 7:** The top collar of the syringe barrel (53.1) was placed in the corresponding indentation in the top part. **Step 8:** Four M3x12 screws (69) were used to connect the two barrel clamps (7,8) to the top part of the syringe pump and fix the barrel of the syringe.
	**Step 9:** Two round flange linear bearings (45) were connected to the middle part (5) of the syringe pump with two M3x10 screws (68) each. To avoid tilting of the middle part in the syringe pump, it should be verified that both bearings are aligned equally. **Step 10:** The trapezoidal lead nut (46) was connected to the Middle part with four M3x16 screws (70) and four M3 nuts (78). **Step 11:** The syringe plunger (53.2) was inserted through the hole in the middle part and the plunger button was placed inside the corresponding indentation (as shown in the picture).
	**Step 12:** Two M3x12 screws (69) and two M3 washers (87) were used to connect the plunger plate (9) to the middle part of the syringe pump and fix the syringe plunger.

	**Step 13:** The trapezoidal lead nut (46) in the middle part of the syringe pump was screwed onto the lead screw (43). The syringe plunger (53.2) was carefully inserted into the barrel of the syringe (53.1).
	**Step 14:** Two M4x8 screws (74) were used to connect the flange bearing (47) to the bottom part (6) of the syringe pump.
	**Step 15:** The two sliding rods (44) were placed in the indentations in the top and bottom part of the syringe pump and the lead screw (43) was placed inside the flange bearing (47). **Step 16:** Four M4x20 screws (76) were used to connect the bottom part to the top part of the syringe pump.

Assembly of the TLC Holder

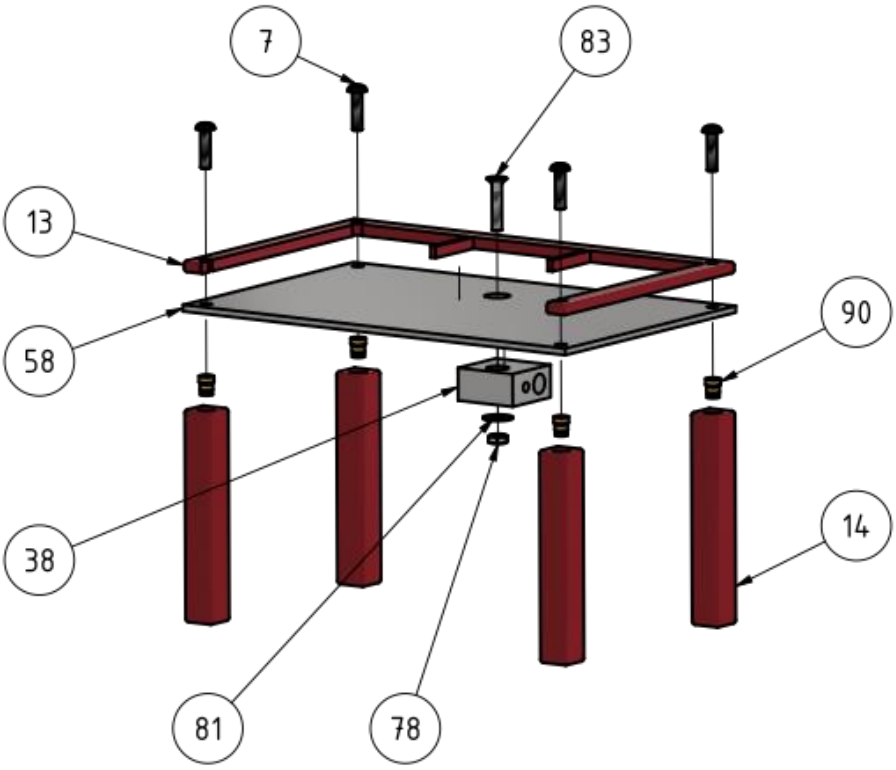
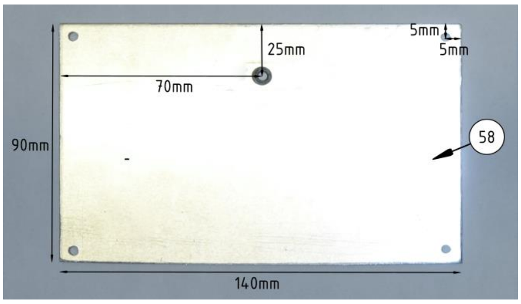
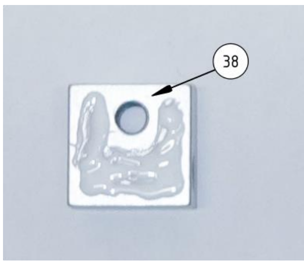
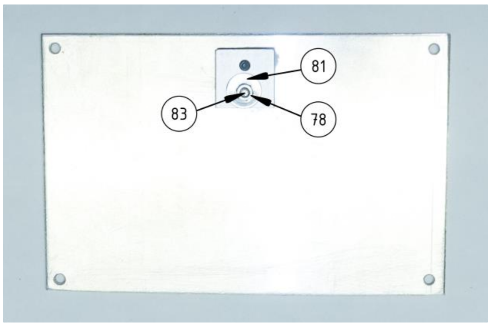
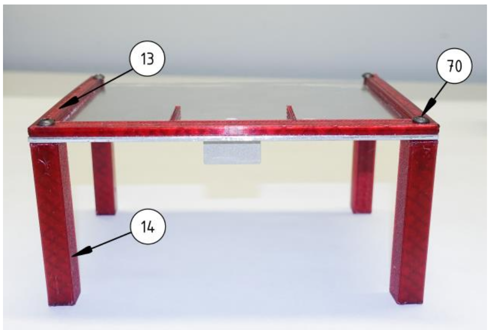
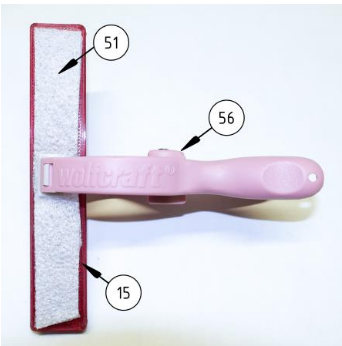


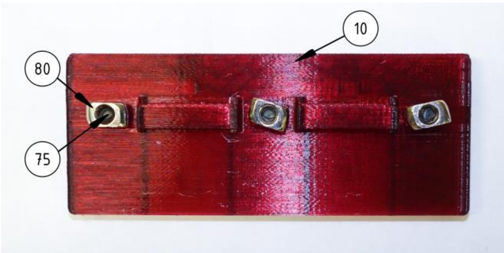
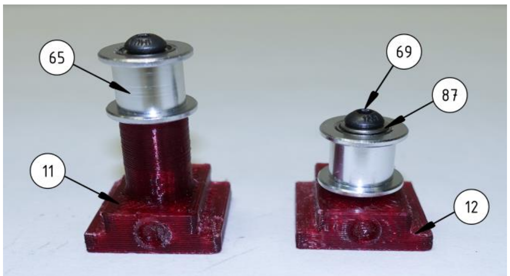
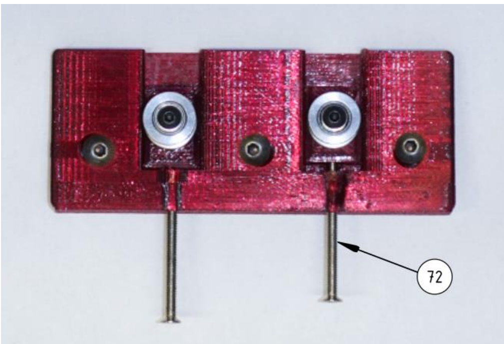
Figure S6: Exploded-view CAD drawings of the TLC holder.

	**Step 1:** Five 3.5 mm diameter boreholes were drilled into the aluminum plate (58). **Step 2:** The drilled hole in the middle of the aluminum plate was chamfered with a countersink so that the M3x16 countersunk screw (83) could be later countersunk in it.
	**Step 3:** The heating block (38) was covered with a thin layer of thermal paste (ARCTIC MX-4).

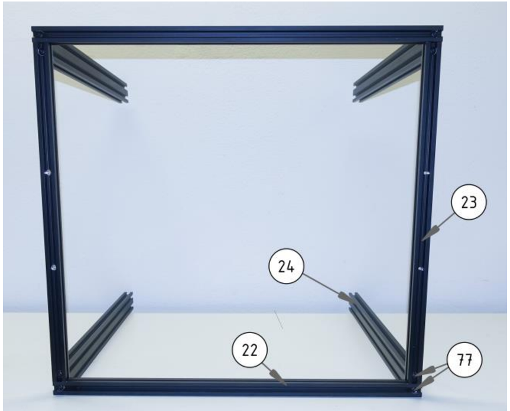
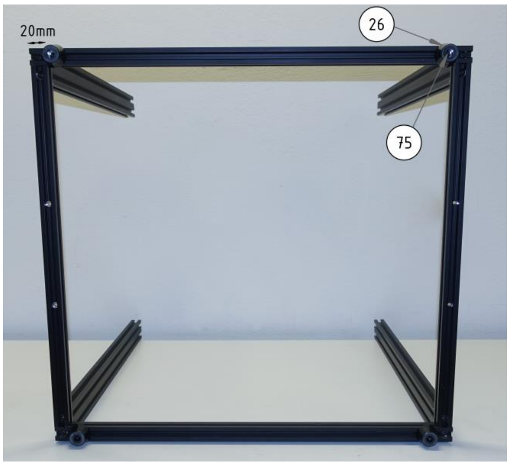
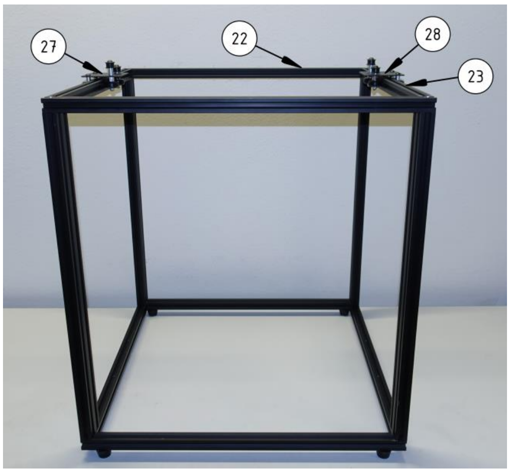
	**Step 4:** An M3x16 countersunk screw (83), an M4 washer (81) and an M3 nut (78) were used to mount the heating block to the aluminum plate.
	**Step 5:** Four M3 threaded inserts (90) were inserted into the legs (14) of the TLC holder using a soldering iron. **Step 6:** Subsequently, four M3x16 screws (70) were used to connect the aluminum plate, the Top part (13), and the legs (14) of the TLC holder.
	**Step 7:** A piece of packaging foam (51) from the packaging of the 3D printer was cut to the correct size (105x15 mm) and glued to the bottom side of the clamp plate (15) without the indentation. **Step 8**: The contact area at one side of the clamp (56) was glued to the indentation in the clamp plate.

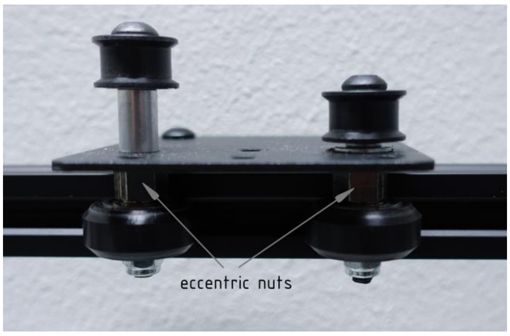
Assembly of the Belt Tensioner

The belt tensioner was built following an already-existing design.[4]

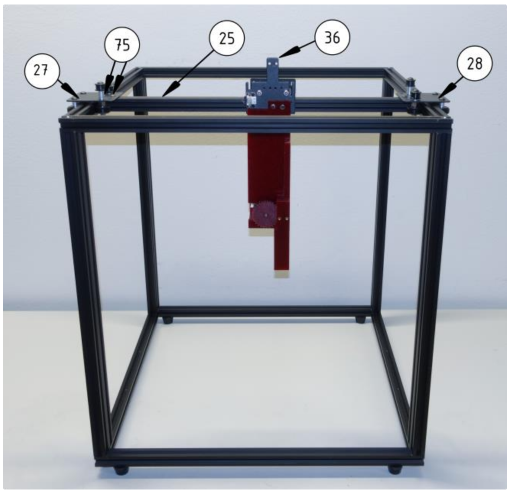
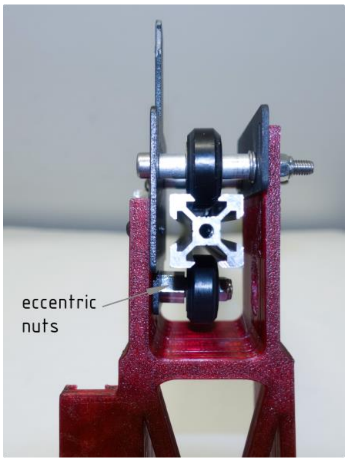
	**Step 1:** Two M3 threaded inserts (90) were inserted into the main part and one M3 nut (78) was inserted into the right part of the belt tensioner using a soldering iron. **Step 2:** Three M4x12 screws (75) and three M4 T- nuts (80) were connected to the main part (10) of the belt tensioner.
	**Step 3:** Two M3x12 screws (69) were used to connect the two Idler pulleys (65) to the left (11) and the right part (12) of the belt tensioner. An M3 washer (87) was placed between the idler pulley and the head of the screw of the right part beforehand.
	**Step 4:** Two M3x30 screws (72) were connected to the main part of the belt tensioner. **Step 5:** The left and the right part of the belt tensioner were inside the guide rails in the main part.

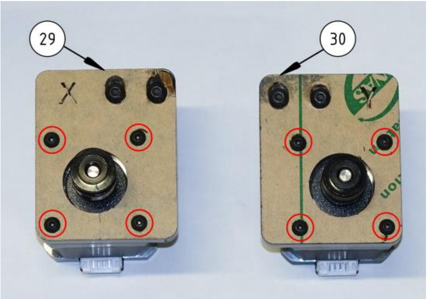
Assembly of the base frame

	**Step 1:** Two aluminum profiles 1 (22), two aluminum profiles 2 (23) and four aluminum profiles 3 (24) were mounted together with eight M5x25 screws (77). The screws were not tightened fully so that the profiles could later be adjusted.
	**Step 2:** Four M4x12 screws (75) and four M4 T-nuts (80) were connected to four rubber feet (26). These rubber feet were then fixed to the two aluminum profiles 1 (22) at the bottom of the base frame. To mount the rubber feet to the base frame, the T-nuts were first aligned with the groove in the aluminum profiles and placed inside of it. A screwdriver was used to loosen the M4 screws inside the rubber feet and to tighten them subsequently. Afterward, it was verified that the T-nuts were aligned perpendicular to the aluminum profile groove. If not, the process of loosening and tightening the M4 screw was repeated.
	**Step 3:** The frame was positioned on its four rubber feet. Two aluminum profiles 1 (22) were placed at the top of the frame and connected to it with four M5x25 screws (77). **Step 4:** The left (27) and the right return board (28) were each slid onto one aluminum profile 2 (23). **Step 5:** Four M5x25 screws (77) were used to connect these two aluminum profiles 2 to the base frame.

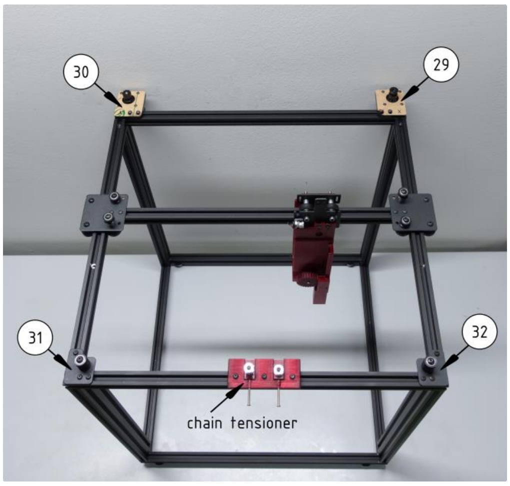
	**Step 6:** An open-ended wrench was used to adjust the two eccentric nuts of each return board and to optimize the resistance of these boards.

Assembly of the TLC robot

	**Step 1:** The aluminum profile 4 (25) was inserted into the hotend carriage (36). **Step 2:** The return boards (27, 28) were pushed against the aluminum profile at the front. Then, two M4x12 screws (75) were used to connect each side of the aluminum profile 4 (25) to the middle of the return boards. Afterward, it was verified that the return boards could be moved freely and that the min. distance between the two return boards and the aluminum profile at the front is approx. equal. **Step 3:** All M5x25 screws were tightened. Afterward, it was verified that the return boards could still move with little resistance.
	**Step 4:** The eccentric nut at the hotend carriage was adjusted with an open-end wrench to optimize the clearance between the wheels and the aluminum profile.



Step 5: The baseplate screws (red circles) at the top of the X- & Y-motor (29, 30) were removed. Afterward, the baseplates were adjusted, as shown in the picture and the screws were reinserted.

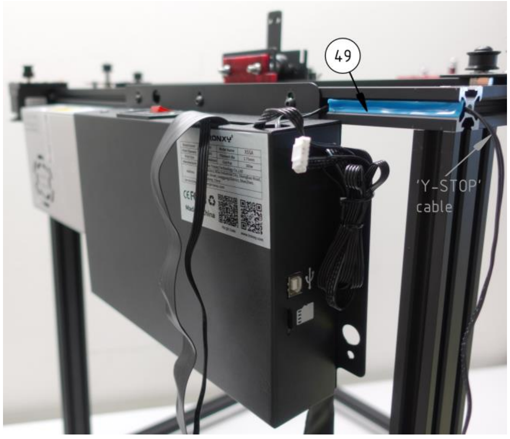


Step 6: The X- (29) and Y-motor (30) were mounted in the two back corners of the base frame using the T-nut system.

Step 7: The left pulley component (31) and the right pulley component (32) were fixed at the two front corners of the frame.

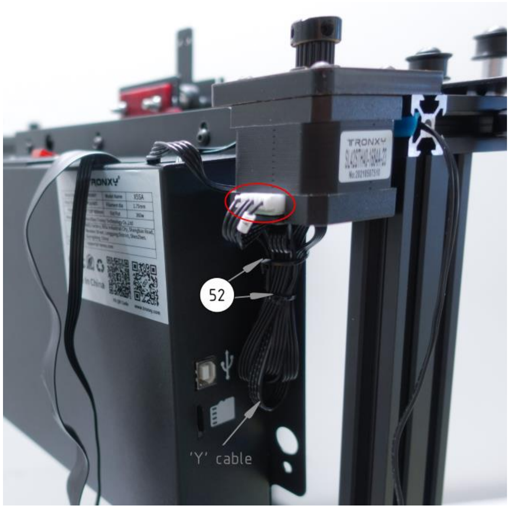
Step 8: The belt tensioner was connected to the aluminum profile at the front of the base frame.

Step 9: Using the T-nut system, the host box (41) was connected to the top aluminum profile on the back side of the base frame. The distance between the host box and the Y-motor must be as small as possible so that all relevant components can later be mounted to the same aluminum profile.



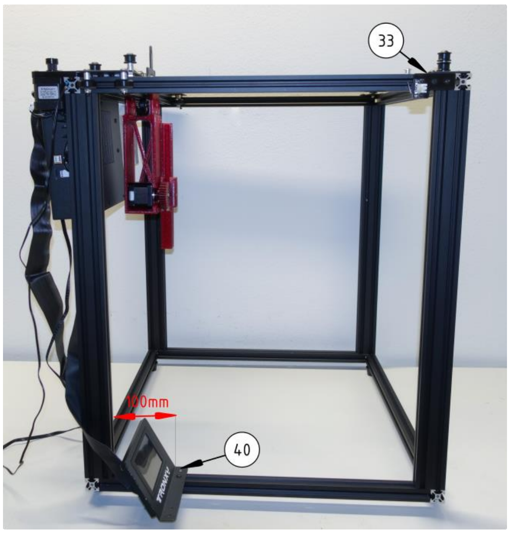
Step 10: The Y-motor was temporarily removed.

Step 11: The 'Y-STOP' cable was placed inside the groove of the aluminum profile and covered with a piece of aluminum profile seal (49).



Step 12: The Y-motor was reattached to the aluminum profile and connected to the 'Y-Motor' cable from the host box (red circle).

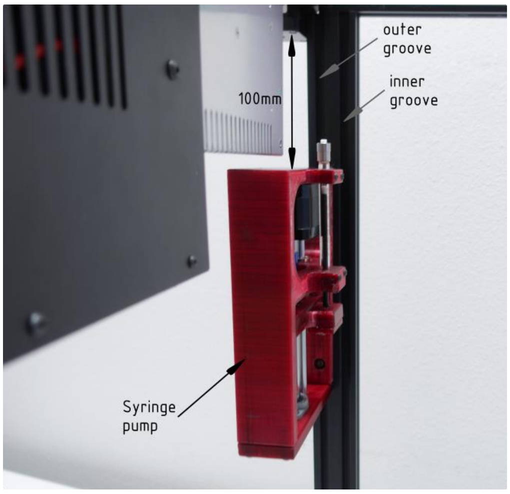
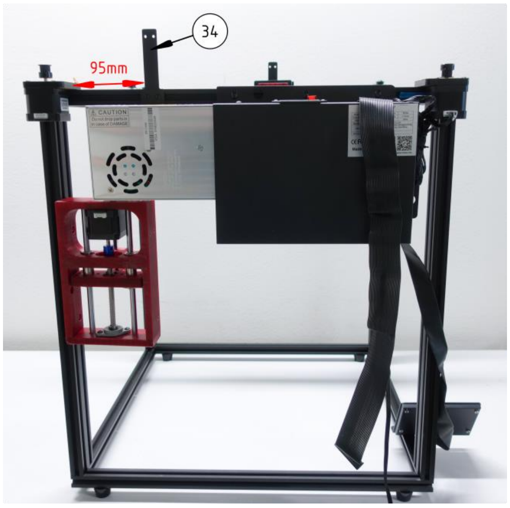
Step 13: The 'Y-Motor' cable was looped over itself a few times before it was cinched with two cable ties (52) and connected to the right edge of the host box.

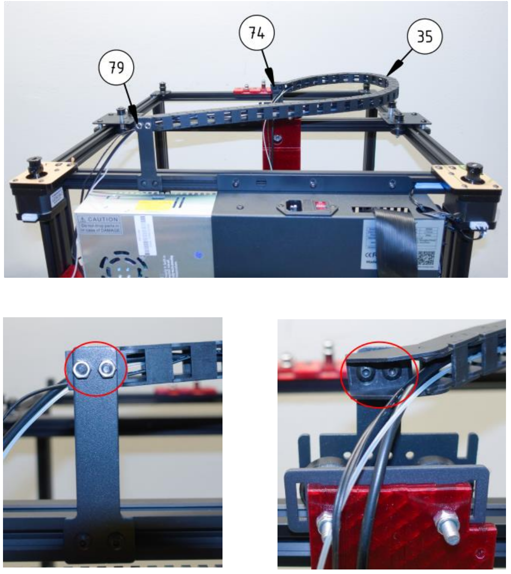
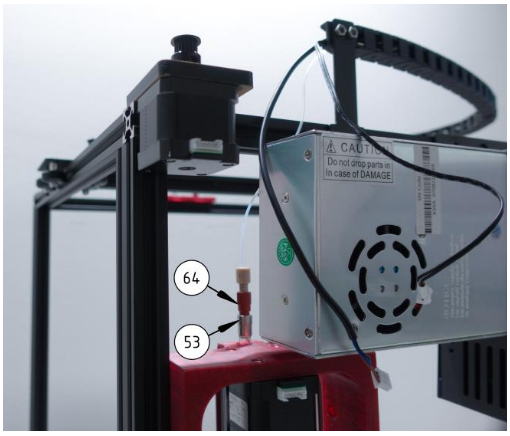
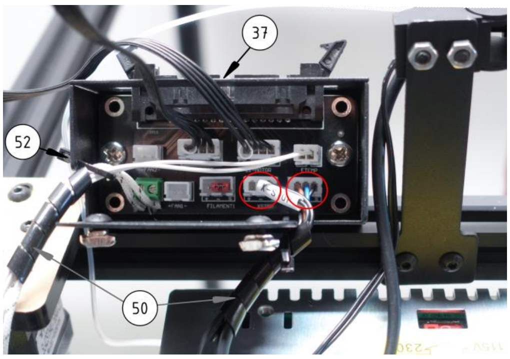


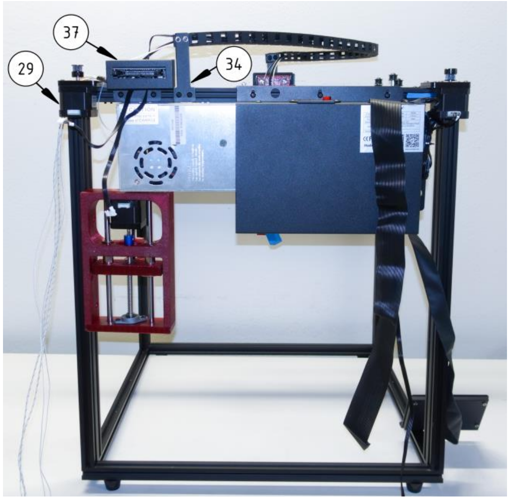
Step 14: Using the T-nut system, the display (40) was mounted to the bottom aluminum profile on the left side of the base frame.

Step 15: The Y-axis switch (33) was mounted at the right edge of the aluminum profile at the top.

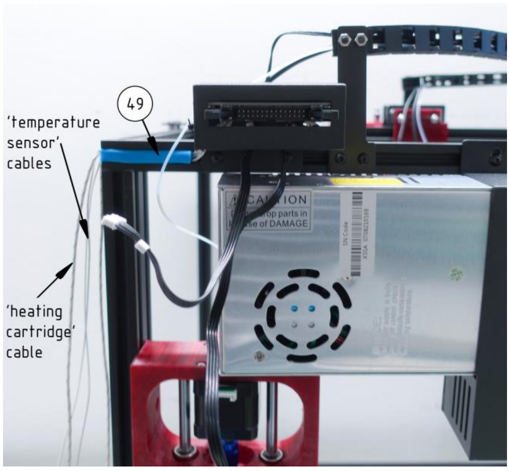
<table>
<tr><td>
</td><td>Step 16: The Y-axis Switch was connected to the 'YSTOP' cable from the host Box (red circle).

Step 17: The intermediate part of the cable was placed inside the grooves of the aluminum profiles on the top and the right side. The grooves were then covered with two pieces of aluminum profile seal (49).</td></tr>
<tr><td>
</td><td>Step 18: Using the T-nut system, the syringe pump was connected to the outer groove in the left aluminum profile on the back side of the base frame.</td></tr>
<tr><td>
</td><td>Step 19: The townline holder (34) was mounted to the top aluminum profile on the back of the base frame.</td></tr>
</table>

	**Step 20:** The cable drag chain (35) was placed on the top of the base frame so that the Z-axis sensor was located on the side of the hotend carriage. Afterward, the chain was connected to the townline holder and the hotend carriage (36) using two M4x8 screws (74) and two M4 nuts (79) each. Both times, the chain was mounted at a slight upward angle and the M4 nuts were tightened firmly to prevent the chain from getting caught on an Idler pulley during movement.
	**Step 21:** The adaptor (64) at the end of the PTFE-tubing was connected to the top of the syringe (53).
	**Step 22:** The cables originating from the Z-axis sensor and the X-axis switch were connected to "LEVEL" and "XSTOP" plugs (red circles) on the terminal block (37). The cables were then fixed with two pieces of cable spiral hose (50) and two cable ties (52).

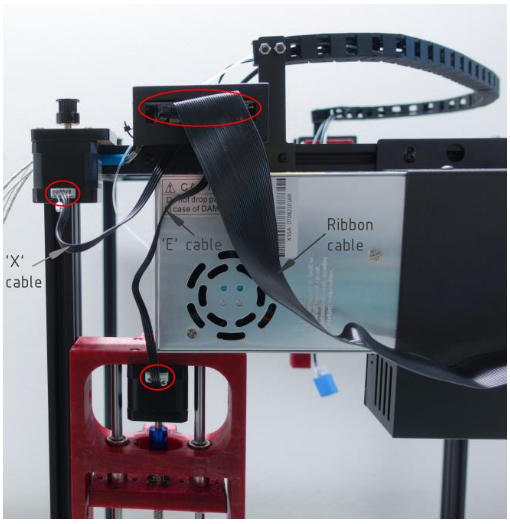


Step 23: The terminal block (37) was mounted to the aluminum profile at the top between the X-motor (29) and the townline holder (34).



Step 24: The X-motor was temporarily removed from the aluminum profile.

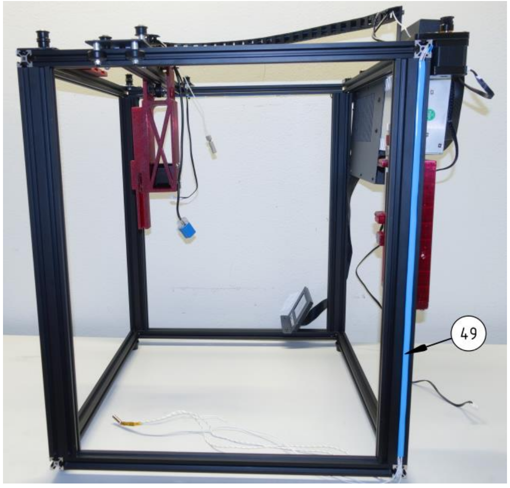
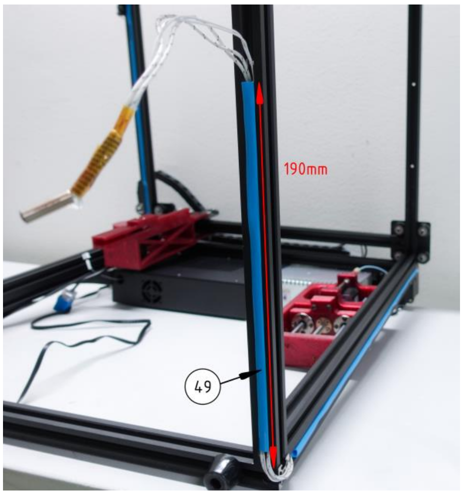
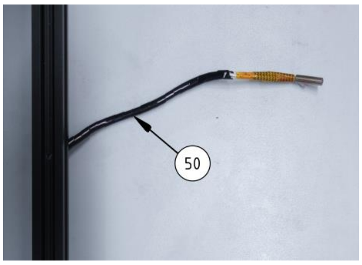
Step 25: The cables of the temperature sensor and the heating cartridge were placed inside the groove of the aluminum profile and covered with a piece of aluminum profile seal (49). Afterward, it was verified that the cables were not strained, as straining could loosen the connections to the plugs on the adaptor board.

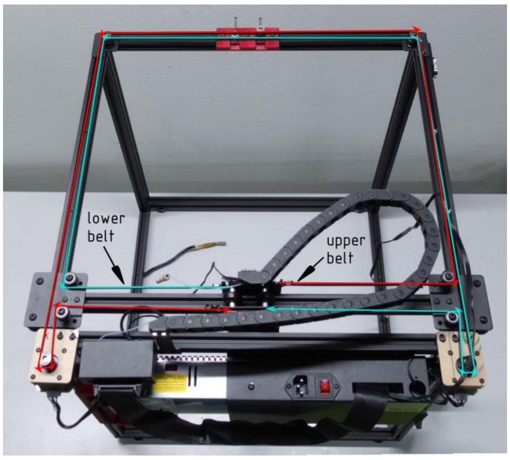


Step 26: The X-motor was reattached to the aluminum profile at the top and connected to the 'X' cable from the terminal block.

Step 27: The motor of the syringe pump was attached to the 'E' cable.

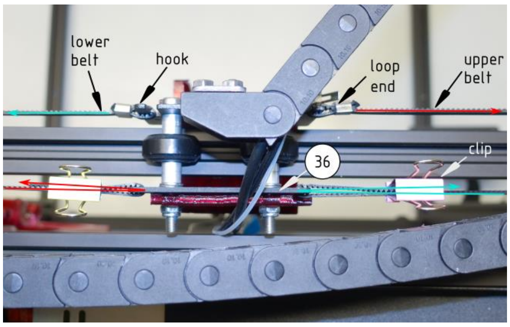
Step 28: The ribbon cable originating from the host box was connected to the terminal block.

	**Step 29:** The cables of the heating cartridge and the temperature sensor were placed inside the groove of the aluminum profile on the right and covered with a piece of aluminum profile seal (49).
	**Step 30:** The previous step was repeated with the aluminum profile groove at the bottom.
	**Step 31:** The part of the heating cartridge cable and the temperature sensor cable outside the aluminum profile was covered with a piece of cable spiral hose (50).

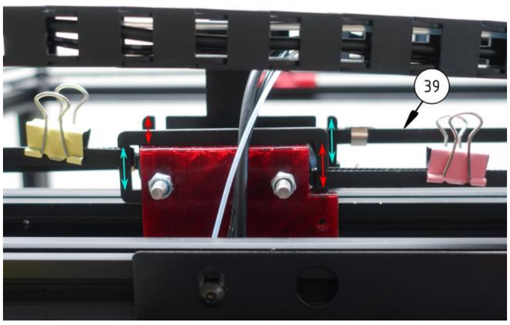


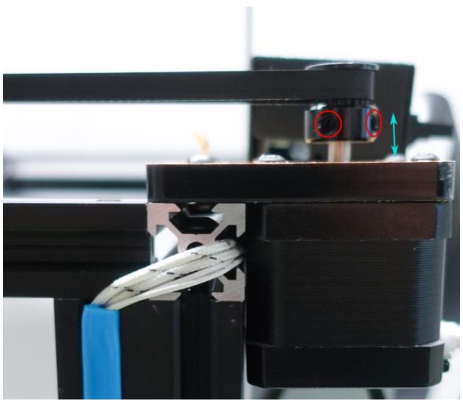
Step 32: While the back side of the base frame was facing the front, the lower tension belt (39) was added. First, the loop at one end of the belt was placed on the hook on the left side of the hotend carriage (36). After the belt was strapped around the lower Idler pulleys (as shown in the picture), its loose end was pulled through the loop on the right side of the hotend carriage and loosely fixed with a clip.

Step 33: The previous step was repeated with the other tension belt, which is strapped around the upper Idler pulleys.

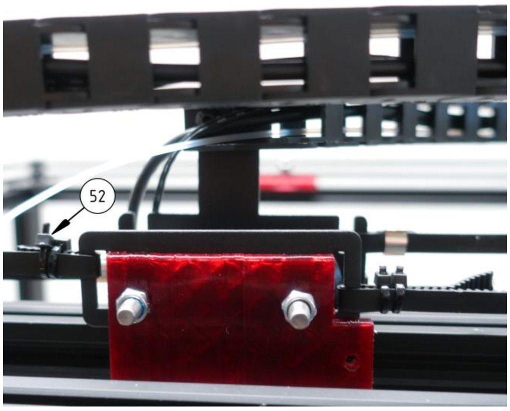
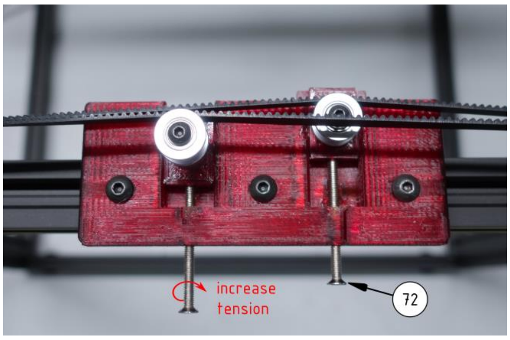
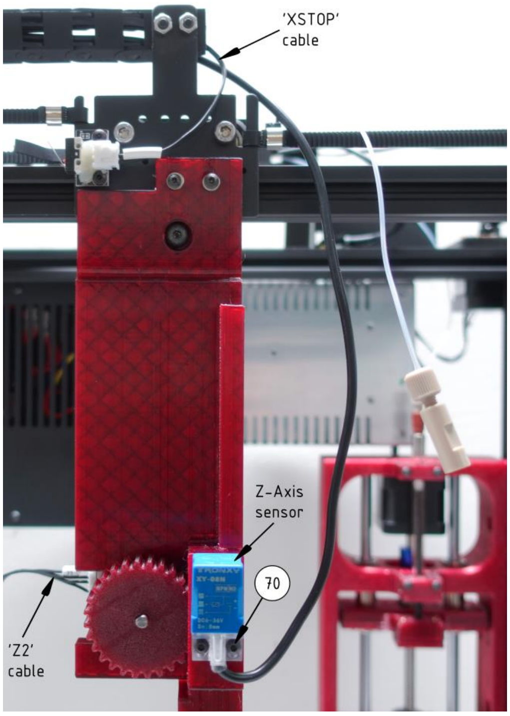


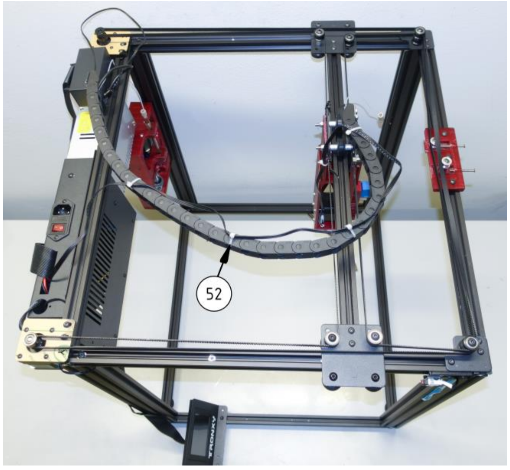
Step 34: The tension in the belt tensioner was decreased as far as possible. Furthermore, the return boards were pushed against the aluminum profile at the front. Then, the tension of each belt was increased as much as possible and the ends were fixed with two clips (from the Tronxy X5SA kit). Care must be taken to increase the tension of both belts equally, as otherwise, the shape of the base frame can be altered.



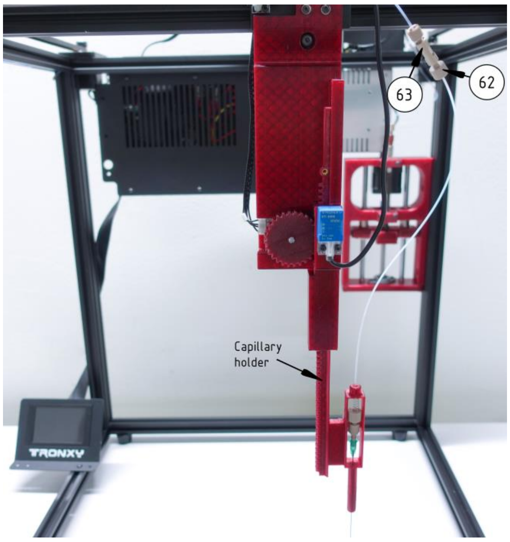


Step 35: The vertical position of the tension belts (39) at the hotend carriage and the idler pulleys on the X- and Y-motor was adjusted until the height of each tension belt was constant throughout the pulley system (aside from the belt tensioner).

	**Step 36:** The clips securing the tension in the two belts can be replaced with cable ties (52). **Step 37:** The tension belts were cut to the correct length and the ends were fixed with more cable ties.
	**Step 38:** The tension of the two belts was increased further by tightening the two M3x30 screws (72) of the chain tensioner. Care must be taken so that the increased tension does not distort the shape of the base frame.
	**Step 39:** The X-axis switch and the Z-Motor were connected to the "XSTOP" and "Z2" cables originating from the terminal block. **Step 40:** Two M3x16 screws (70) were used to connect the Z-axis sensor to the Z-axis mounting (1) of the hotend carriage. The position of the screws in the slots of the Z-axis sensor should be selected so that the topmost part of the Z-axis sensor is slightly above the topmost part of the guide rail.

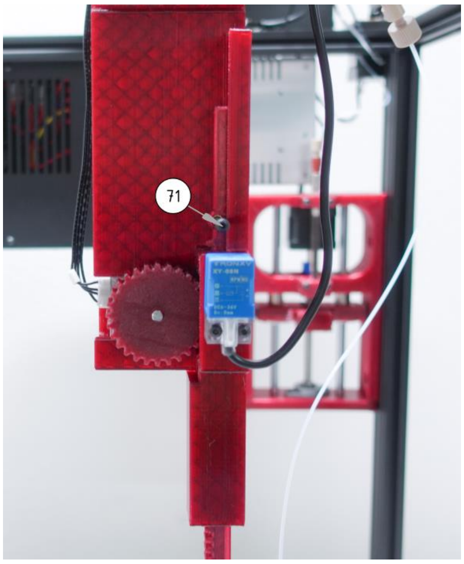


Step 42: Five cable ties (52) were used to connect the cable of the Z-axis motor to the Z-axis mounting (1) and the cable drag chain (35). Afterward, the hotend carriage was moved in all directions to ensure the "Z2" cable was not strained during these movements.

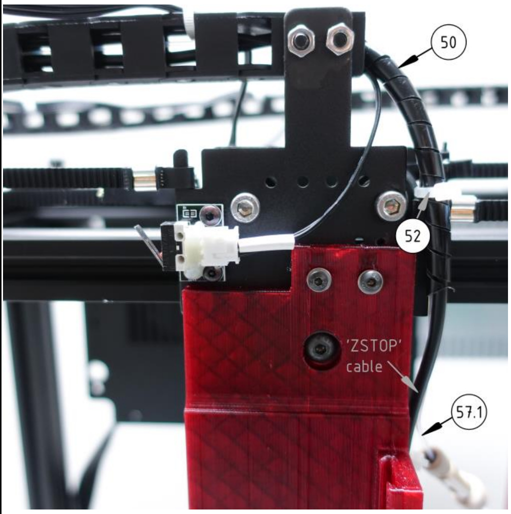
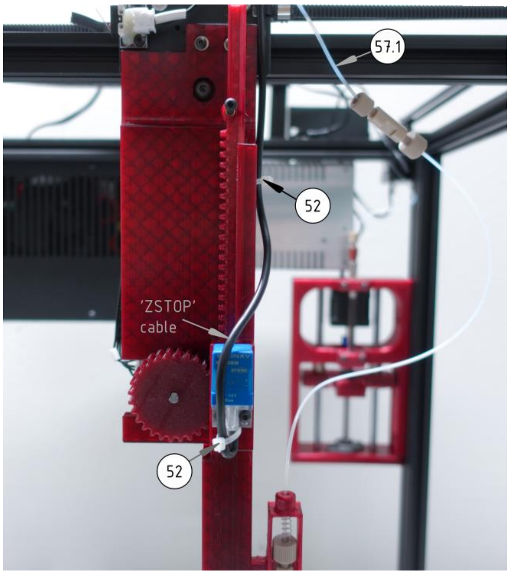


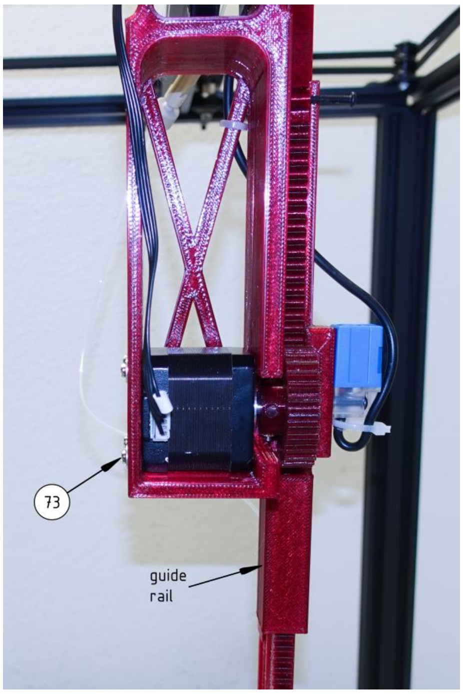
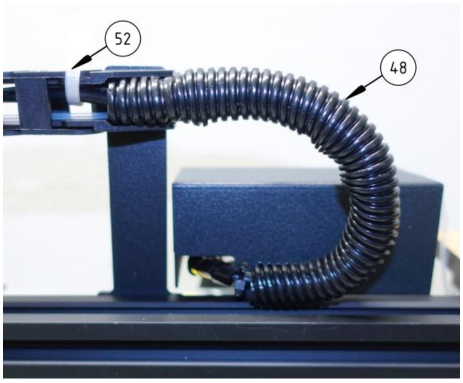
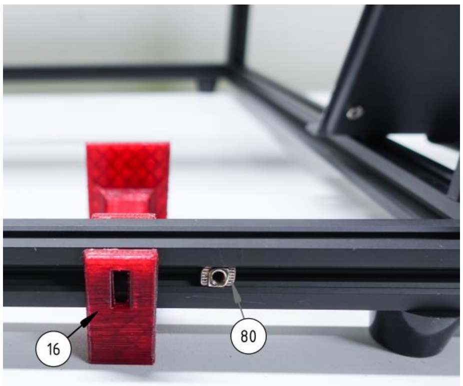
Step 43: The base frame was slightly tilted back so that the gear rack (3) could be inserted into the guide rail of the Z-axis mounting (1) from the bottom. If the gap between the gear wheel and the guide rail is too small to insert the gear rack, the M3x40 screws (73) at the back side of the Z-axis mounting have to be loosened further.

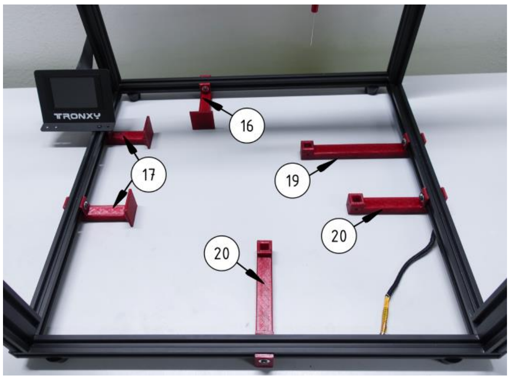
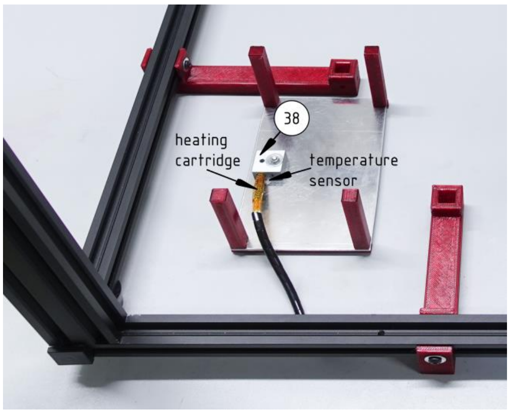
Step 44: The fitting (62.1) at the top of the capillary holder was connected to the adaptor (63) at the end of the cable drag chain.

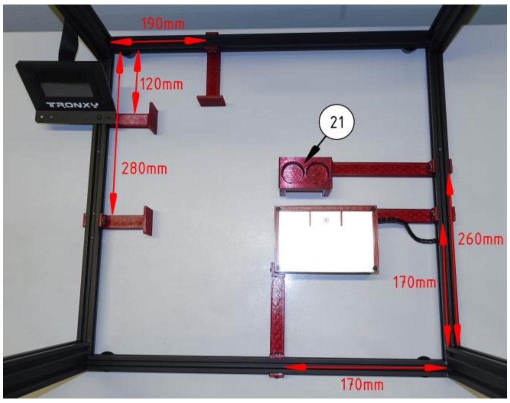
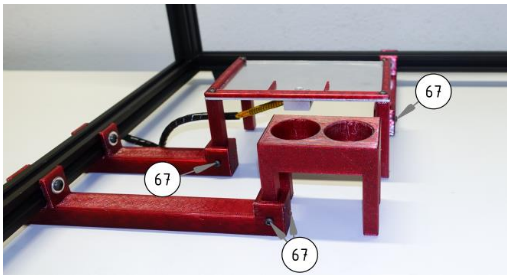


Step 45: The M3x25 screw (71) was connected to the gear rack.

	**Step 46:** The upper parts of the 'ZSTOP' cable and the PTFE-tubing (57.1) were covered with a piece of cable spiral hose (50) and fixed with a cable tie (52) to the hotend carriage.
	**Step 47:** Three cable ties (52) were used to fix the 'ZSTOP' cable and the PTFE-tubing (57.1). Afterward, the gear rack was moved several times up and down to verify that the cable and the tubing were not strained and could not be entrapped between the gear rack and the Z-axis mounting.

	**Step 48:** The positions of the M3x40 screws (73) on the back side of the Z-axis mounting were adjusted to optimize the clearance between the gear rack and the guide rail. After finding the correct positions, the screws were tightened fully.
	**Step 49:** The cables passing through the cable drag chain were looped over between the end of the chain and the terminal block. The loops were fixed with two cable ties (52). **Step 50:** The looped cables were covered with a piece of corrugated pipe (48) and the pipe was connected to the cable drag chain with a cable tie (52).
	**Step 51:** To mount the Y-axis positioner for the rack (16) to the aluminum profile at the back of the base frame, one M4 T-nut (80) was placed on the inside and one in the outside groove of the aluminum profile. **Step 52:** The aluminum frame was then slightly tilted so that the aluminum profile could be inserted into the positioner from the top.

	**Step 53:** The Y-axis positioner was moved sideways until its slot aligned with the M4 T-nut in the outside groove. **Step 54:** An M4x8 screw (74) and an M4 washer (81) were used to connect the positioner to the M4 T-Nut. The screw was not tightened fully so that the positioner could still be moved sideways. **Step 55:** The previous two steps were repeated to connect the other side of the positioner to the T-nut in the inside groove of the aluminum profile.
	**Step 56:** To mount the other Positioners (17-20) to the aluminum profiles at the bottom of the base frame, steps 51-55 were repeated five times.
	**Step 57:** The temperature sensor and the heating cartridge were placed in the corresponding holes in the heating block (38) at the bottom of the TLC holder. Their position was fixed with the grub screw inside the heating block.

	**Step 57:** The positioners (16-20) were moved to their final positions, shown in the picture. Although positioners 16 and 17 can be relocated in slightly different positions, care should be taken that positioner 19 is not moved further to the front. Otherwise, collisions with the cannula can occur during the homing process. **Step 58:** The TLC holder and the table (21) for the waste and the cleaning solution were inserted in the sockets of their corresponding positioners. **Step 59:** The TLC positioners were slightly adjusted so the TLC holder was oriented parallel to the base frame. **Step 60:** The screws of the positioners were tightened to fix their positions.
	**Step 61:** The two tables were fixed with four M3x6 screws (67) in their positions.

Calibration

Before the TLC robot can be used for the first time, the coordinates of all items must be determined and entered into the Excel file. Afterward, the g-code files required for the spotting process can be exported. The structure of the Excel file is explained in detail in the section "Generating the g-code for the TLC robot with the Excel file". After the calibration process is completed, a new calibration is only required if the positioners, ensuring that the items are always situated at the same position, are moved in the x- or y- direction, or if a new rack is introduced. Following the instructions in this user manual, the TLC robot can be calibrated, and the g-code files generated in less than one day.

Before starting the calibration process, all items necessary for running the spotting program must be positioned inside the base frame of the TLC robot, as shown in Figure S7. Two glass vials with an outer diameter of 3 cm or less must be placed inside the indentations of the table at the back. One glass vial is necessary to store the cleaning solution and one to collect waste. If desired, the TLC plates can be placed on the TLC holder and fixed with a clamp before calibration. However, as their positions must not be obtained during the calibration process, the calibration process can be completed without them. During the calibration, the racks must be aligned with the two positioners on the left and one on the back side of the base frame. Other items must be removed from the inside of the base frame to avoid collisions during calibration.

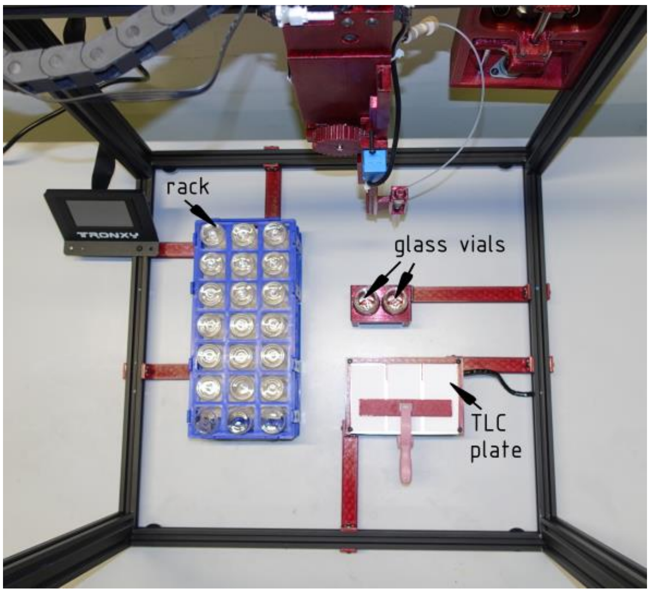


Figure S7: Setup of the TLC robot for the spotting process.

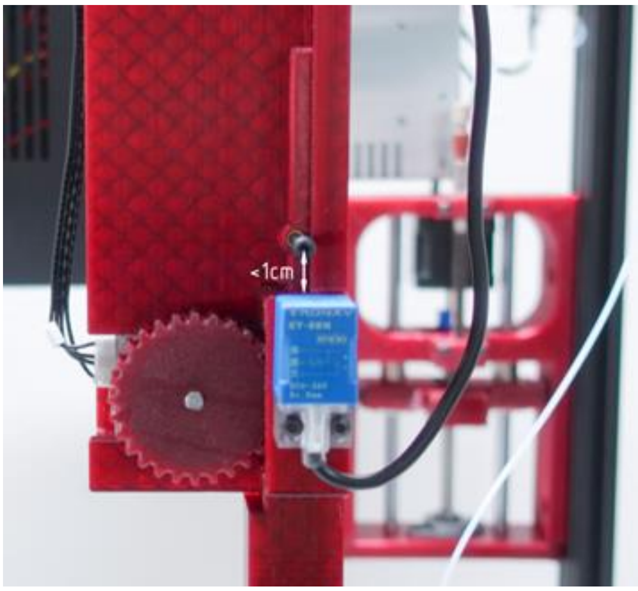


Figure S8: Initial position of the gear rack.

Furthermore, it is advised to initially move the gear rack downward until the distance between the screw in the gear rack and the Z-axis sensor is less than 1 cm (as shown in Figure S8). This position ensures that no collision can occur between the gear rack and the Z-axis mounting during the homing of the TLC robot.

For the calibration process, the Host box of the TLC robot must be plugged into a socket. Furthermore, a USB cable must connect the Host box to a computer on which a slicer software with a command line (e. g. Cura, Simplify3D) is installed. Afterward, the command line, the machine control panel of the slicer software or the machine control panel on the display of the TLC robot can be used to control the TLC robot.

First, the TLC robot has to be homed to determine its position in the three-dimensional space. The homing process can be initiated using the following g-code line. However, as the TLC robot ignores the remainder of a line after a semicolon, only the first part has to be entered into the command line of the slicer software.

```
G28   ; Homing
```

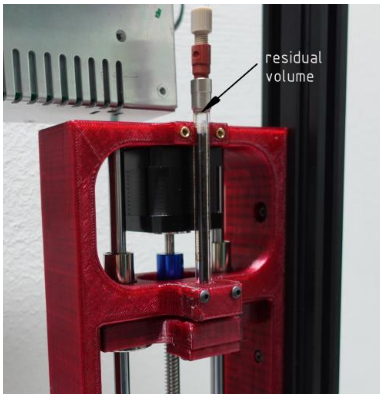
Alternatively, the homing button(s) on the machine control panel can be pressed to initiate the homing process. Note that some slicer software overwrites the homing procedure so that it might differ slightly from the homing process achieved by the "G28"-command.

As the Z-axis homing occurs between the table with the two glass vials and the TLC holder (at the coordinates x = 203.5, y = 175), collusions might occur if these components are not placed at the right spot. Therefore, the first homing process should be closely observed so that the TLC robot can be turned off before a collision takes place. Once the relevant positioners are readjusted and the printer is restarted, the homing process can be repeated.

After the homing process, the relevant parameters for calibrating a new setup can be measured and later entered into the Excel file. To simplify this process, the identifier of the corresponding entry field in the Excel file, e. g. A1, is written in brackets after each position. The "Generating the g-code for the TLC robot with the Excel file" section explains these identifiers in detail.

Calibrating the syringe

To calculate the right amount of E-units needed to collect a specific volume, three parameters must be known: the nominal volume of the syringe (A15), the residual volume in the syringe at the zero position of the syringe pump (A14) and the E-value needed to fill the syringe up to its nominal volume (A16).

<table>
<tr><td>
</td><td>Step 1: The middle part of the syringe pump is moved upward until it touches the top part and no further upward movement is possible.

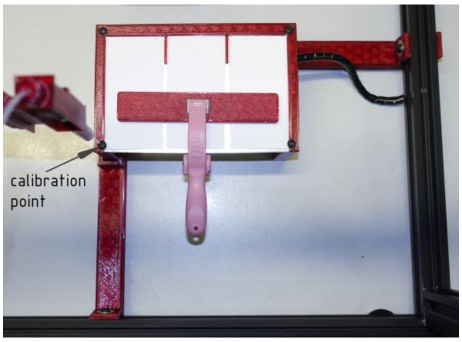
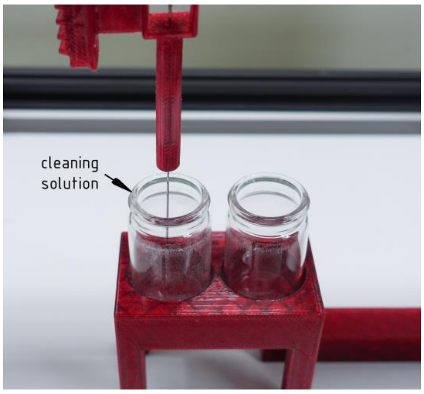
Step 2: The barrel clamp at the top of the syringe is removed. The residual volume in the syringe is identified and recorded (A14).

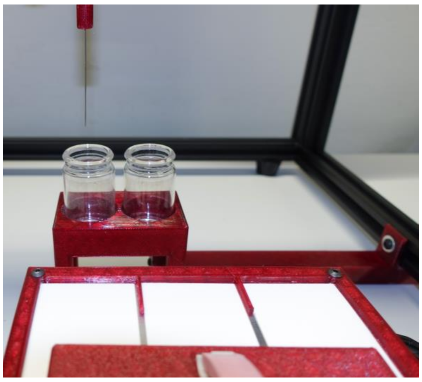
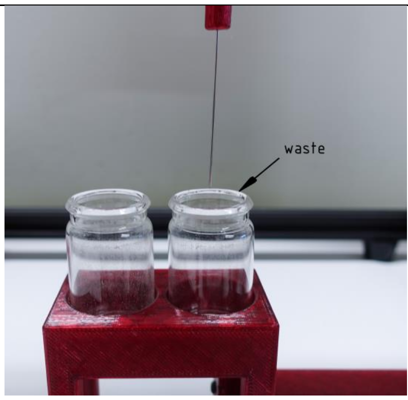
Step 3: The barrel clamp is returned to its initial position.</td></tr>
<tr><td><code>M302 ; Enable cold extrusion</code></td><td>Step 4: The g-code on the left is entered in the command line of the slicer software so that the syringe pump can be used without heating up the heating block.</td></tr>
<tr><td>
</td><td>Step 5: The E-value (A16) is necessary to move the middle part of the syringe pump from the previous position to the last item on the barrel. This scale must be determined. It can be obtained in two ways, option 1 and option 2, which will be explained in the following. While option 1 can be employed with most slicers (e. g. Cura, Simplify3D), option 2 can only be used if the slicer software has an output window (e. g. Simplify3D). However, as the second option is more comfortable and less time-consuming, it is recommended to use option 2 if possible.</td></tr>
<tr><td><code>G0 E-248 ; Move the middle part down</code></td><td>Option 1: The command on the left is entered into the command line of the slicer software. The E-value is incrementally increased or decreased to find the correct alignment.</td></tr>
</table>

`M114 ; Get current position`	**Option 2:** The buttons of the machine control panel on the display of the TLC robot or in the menu of the slicer software are used to determine the correct position. As the slicer software can swap the direction of a movement, each motion should be tried with small steps first. After obtaining the correct position, the command on the left is entered into the command line of the slicer software. The current E-value can be seen in the output window (among other parameters).

Calibrating a new set-up

The following procedure describes how all relevant coordinates of the TLC holder, the waste, and the cleaning solution can be determined.

	**Position 1:** The cannula is positioned slightly above the screw on the left front corner of the TLC holder. The x-, y- and z-coordinates of this position (A3) are noted.
	**Position 2:** The cannula is located inside the left glass vial at the appropriate height to collect the cleaning solution. The x-, y- and z-coordinates of this position (A2) are noted.

	**Position 3:** The cannula is moved upward to a height at which it can safely move laterally above the table with the two glass vials and the TLC holder. The z-coordinate (A5) is noted.
	**Position 4:** The cannula is positioned above the right glass vial at the appropriate height at which the syringe should be emptied into the waste. The x-, y- and z-coordinates of this position (A1) are noted.

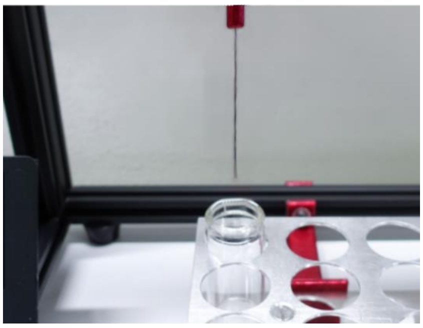
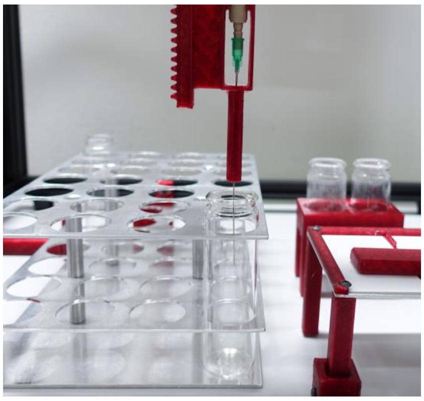
Positions 1-4 can be determined either by using the machine control panel for the movement and the command "M114" to obtain the coordinates or by entering variations of the following commands into the command line of the slicer software:

```
; Position 1
G0 Z5             ; Move the cannula up until it can move above all
                    relevant items.
G0 X177 Y60       ; Position the cannula above the front left screw of
                    the TLC holder, adjust the position incrementally.
G0 Z0.8           ; Lower the cannula to a position slightly above the
                    screw.
G0 X177 Y61       ; Readjust the position incrementally; if necessary,
                    move the cannula upward before doing so.

; Position 2
G0 Z5             ; Move the cannula up so that it can move above all
                    relevant items.
G0 X193 Y214      ; Position the cannula above the cleaning solution,
                    incrementally adjust the position.
G0 Z0             ; Lower the cannula to the height at which the
                    retraction should occur.
G0 X193 Y214.5    ; Readjust the position incrementally.


; Position 3
G0 Z3.2           ; Move the cannula up incrementally until it can move
                   above all relevant items.
```

```
; Position 4
G0 X226 Y214        ; Position the cannula above the cleaning solution,
                      and incrementally adjust the Position.
G0 Z0               ; Lower the cannula into the right glass vial.
G0 X226 Y214.5      ; Readjust the position incrementally.
G0 Z2.5             ; Move the cannula up to the position at which the
                      extrusion should occur.
```

Calibrating a new rack

To initialize a new rack, the number of samples in the x- and y-direction must be noted (R2). Additionally, the coordinates of the two vials at diagonally opposite ends of the rack must be determined (R4, R5). The process described in the following has to be repeated to calibrate different rack types.

	**Position 5:** The cannula is positioned inside the glass vials in the rear left corner of the rack at the height at which small volumes of each vial should be collected. The x-, y- and z-coordinates of the position (R3) are noted.
	**Position 6:** The cannula is moved upward to a height at which it can safely move laterally above the rack without streaking any vials. The z-coordinate (R5) is noted.
	**Position 7:** The cannula is positioned inside the right glass vial in the right front corner of the rack. The x- and y-coordinates of this position (R4) are noted.

Positions 5-7 can either be obtained by using the machine control panel and the command "M114" or by entering variations of the following commands into the command line:

```
; Position 5
G0 Z5              ; Move the cannula up until it can move above all
                     relevant items.
G0 X193 Y214       ; Position the cannula above the sample in the rear left
                     corner of the rack.
G0 Z0              ; Lower the cannula to the height at which the
                     retraction should occur.
G0 X193 Y214.5     ; Readjust the position incrementally.

; Position 6
G0 Z3.2            ; Move the cannula incrementally up until movement above
                     the rack is possible.

; Position 7
G0 X226 Y214       ; Position the cannula above the sample in the front
                     right corner of the rack.
G0 Z0              ; Lower the cannula into glass vial.
G0 X226 Y214.5     ; Readjust the position incrementally.
```

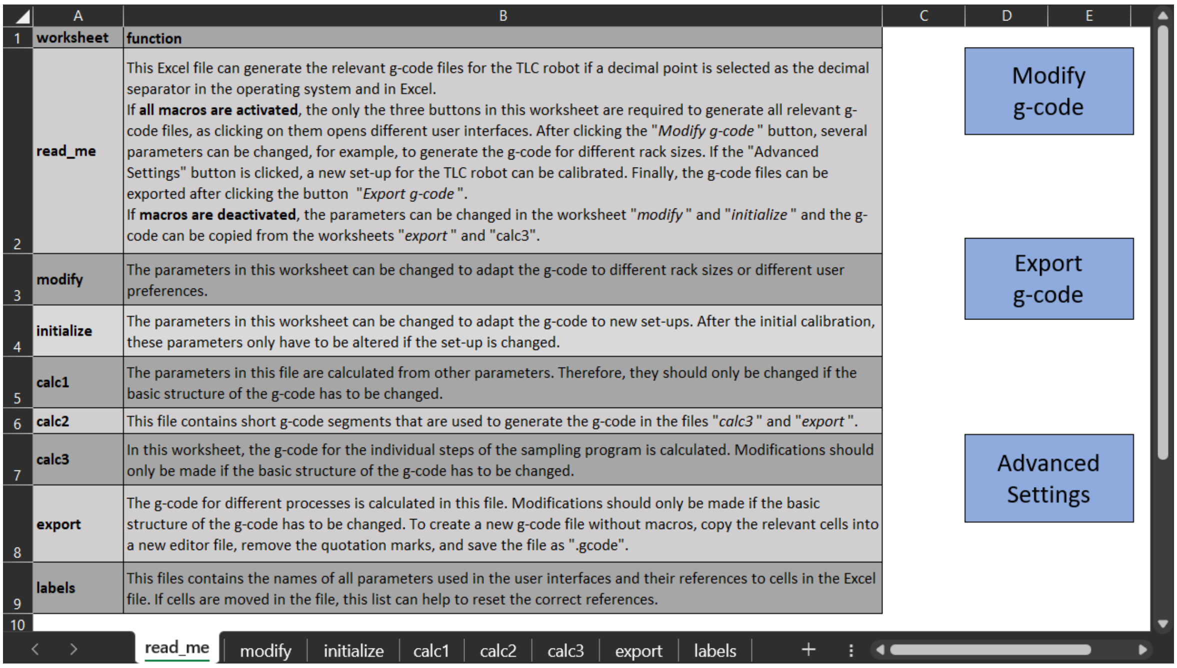
Generating the g-code with the Excel file

The necessary g-code for the spotting process can be generated with the provided Excel file. These g-code files has to be moved onto the SD card of the TLC robot. Once the SD card is inserted into the host box of the TLC robot, the different programs can be selected on the display of the TLC robot.

However, a version of Excel 2010 or a newer version is required to use our Excel file. Although the file can be used with or without macros, generating g-code files is more comfortable if macros are activated. Therefore, only this option will be explained in the following. Since the TLC robot can only interpret decimal numbers with the decimal separator "." correctly, this separator sign must be selected in Excel. For consistency reasons, a decimal point should also be used as the separator sign in the user interfaces generated by the macros. The best way to achieve both changes simultaneously is to modify the settings in the language menu of the operating system and keep these settings in Excel.

Furthermore, the maximum number of samples in a rack that the Excel file can correctly evaluate is 100. Since the average number of samples per rack is less than 30, this number should be large enough to generate the g-code for the most commonly used racks. However, if generating the g-code file for larger racks becomes necessary, the Excel file can be modified by adapting the cell references in the worksheet "calc3" and the user interface "export".

If all macros are activated in Excel and the four pictures of the different sampling orders are saved in the same folder as the Excel file, the three buttons in the worksheet "read_me" can generate all relevant g-code files. Clicking on one of the buttons, "Modify g-code", "Export g-code" or "Advanced Settings", which can be seen in Figure S9, opens user interfaces that allow the operator to modify and export g-code files.



worksheet	function
read_me	This Excel file can generate the relevant g-code files for the TLC robot if a decimal point is selected as the decimal separator in the operating system and in Excel. If **all macros are activated**, the only the three buttons in this worksheet are required to generate all relevant g-code files, as clicking on them opens different user interfaces. After clicking the "*Modify g-code*" button, several parameters can be changed, for example, to generate the g-code for different rack sizes. If the "Advanced Settings" button is clicked, a new set-up for the TLC robot can be calibrated. Finally, the g-code files can be exported after clicking the button "*Export g-code*". If **macros are deactivated**, the parameters can be changed in the worksheet "*modify*" and "*initialize*" and the g-code can be copied from the worksheets "*export*" and "calc3".
modify	The parameters in this worksheet can be changed to adapt the g-code to different rack sizes or different user preferences.
initialize	The parameters in this worksheet can be changed to adapt the g-code to new set-ups. After the initial calibration, these parameters only have to be altered if the set-up is changed.
calc1	The parameters in this file are calculated from other parameters. Therefore, they should only be changed if the basic structure of the g-code has to be changed.
calc2	This file contains short g-code segments that are used to generate the g-code in the files "*calc3*" and "*export*".
calc3	In this worksheet, the g-code for the individual steps of the sampling program is calculated. Modifications should only be made if the basic structure of the g-code has to be changed.
export	The g-code for different processes is calculated in this file. Modifications should only be made if the basic structure of the g-code has to be changed. To create a new g-code file without macros, copy the relevant cells into a new editor file, remove the quotation marks, and save the file as ".gcode".
labels	This files contains the names of all parameters used in the user interfaces and their references to cells in the Excel file. If cells are moved in the file, this list can help to reset the correct references.

Figure S9: Screenshot of the Worksheet "read_me". The three buttons on the right can be clicked to open different user interfaces.

The "Modifications" menu enables the user to adapt the g-code to individual preferences, e. g. changing the volume extruded on each spot of a TLC plate. On the other hand, the "Advanced settings" menu is only required for the calibration process, e. g. if the setup is changed or if a new rack has to be initialized. Furthermore, it is the place to make less frequent changes, such as modifying the velocities of different motions. Finally, the" Export" menu can generate and export different g-code files. The different options of these menus will be described in more detail in the following.

As the changeable parameters in the user interfaces are linked to the cell values at specific positions in the Excel file, it should be avoided to delete, insert or move any cells in the worksheets "modify", "initialize", "calc3" and "export". However, if such processes are required, it is necessary to change the references in the VBA menu accordingly to avoid wrong references. To update the references in the VBA menus quickly, in the worksheet "labels", the names of all parameters in the user interfaces are listed next to the current location of the cells in the Excel file they are referencing, as shown in Figure S10. However, the current worksheet of a cell can only be displayed with Excel 2021 or newer versions. Therefore, the column with the initial worksheet of the relevant cells was included.

	A	B	C	D	E	F
1		**type**	**name(s) in VBA**	**initial worksheet of cell**	**current worksheet**	**current row number**
2		ComboBox	rack_type	modify	modify	2
3		OptionButton	sampling_order	modify	modify	3
4		TextBox	sampling_number	modify	modify	6
5		TextBox	cleaning_number	modify	modify	2
6		TextBox	repeats	modify	modify	3
7		TextBox	cleaning_start	modify	modify	4
8		TextBox	cleaning_end	modify	modify	5
9		ComboBox	TLC_number	modify	modify	2
10		TextBox	extrusion_per_spot	modify	modify	2
11		TextBox	number_of_extrusions	modify	modify	3
12		TextBox	v_spotting	modify	modify	4
13	**modifications**	TextBox	pause	modify	modify	5
14		TextBox	temperature	modify	modify	4

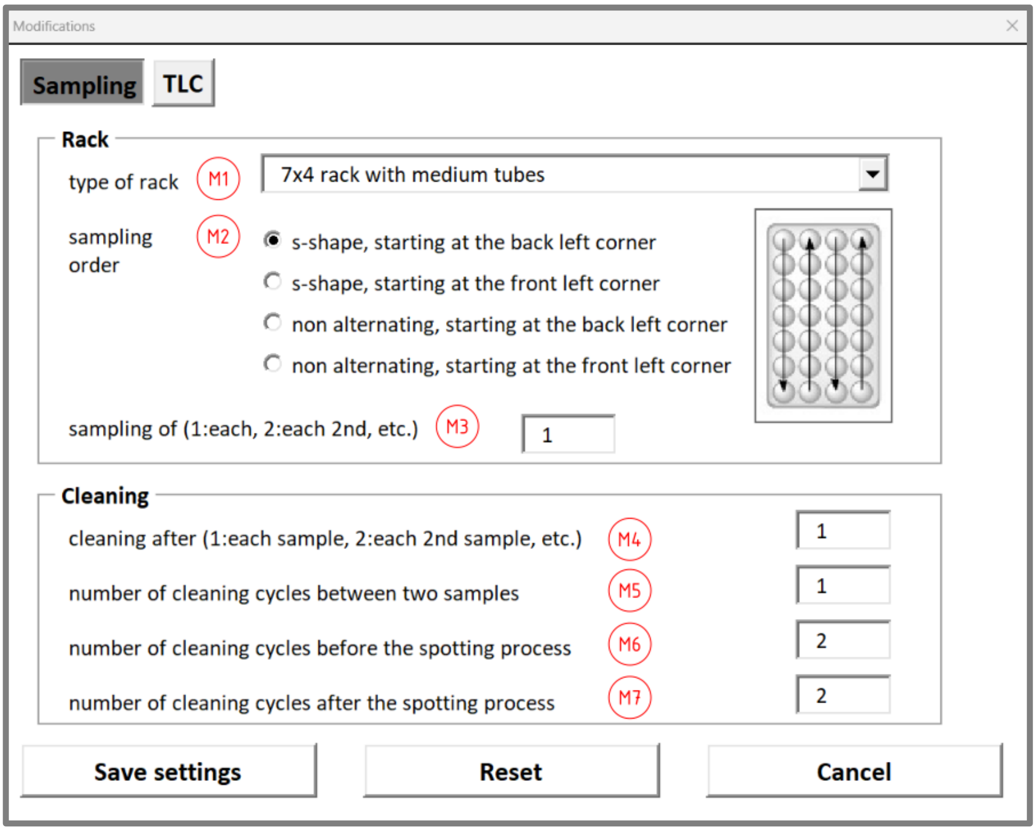
read_me | modify | initialize | calc1 | calc2 | calc3 | export | labels

Figure S10: Screenshot of the Worksheet "labels". All parameters that are used in the different user interfaces are listed in column "C" of this worksheet and the type of each parameter is explained in "B". The different parameters are sorted by the user interfaces they occur in ("A"). The cell in the Excel file that each parameter is referencing is explained in the columns "D-G".

The "Modifications" menu

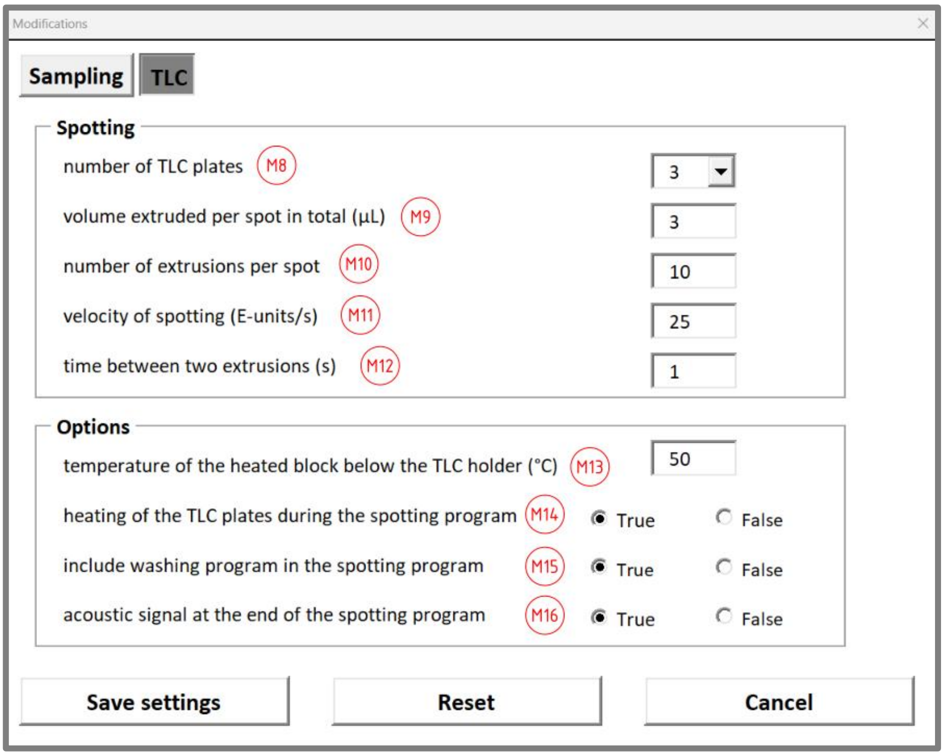
The "Modifications" menu allows the user to alter the g-code slightly. Different values can be modified in the two tabs, "Sampling" and "TLC", which will be explained in detail in the following. By clicking the "Save Settings" button, the modified values in the user interfaces are passed on to the Excel file. If the "Reset" button is selected, the initial values are shown in the menu and "Cancel" closes the menu without altering any settings in the Excel file.

Sampling



M1	The list contains all racks that have been initialized. Users can select the correct rack for their purpose. If the desired rack is not included in the list, it must first be initialized with the "Advanced Settings" menu.
M2	Choose the sampling order in which the different samples should be collected. The picture to the right illustrates the selected sampling order. Additionally, notice should be taken of the starting position, as the rack must be later oriented accordingly so that the sampling process starts with the first sample.
M3	Decide how many samples should be collected and spotted. If "1" is entered, each sample is collected. If the entry is "2", the collection process starts with the first sample. Afterward, each second sample is collected, e. g. the samples with the numbers 1, 3, 5, ... are gathered.
M4	Decide how many samples should be collected before a new cleaning cycle of the cannula is carried out. If "1" is entered, the cannula is cleaned after collecting and spotting each sample. If the entry is "2", then the cannula is only cleaned after each other sample, e. g. it takes place after the samples with numbers 2, 4, 6, ...
M5	Decide how many times the cannula should be cleaned during one cleaning process.
M6	Select how often the cannula should be cleaned before collecting the first sample.
M7	Decide how often the cannula should be cleaned after the completed spotting process.

TLC

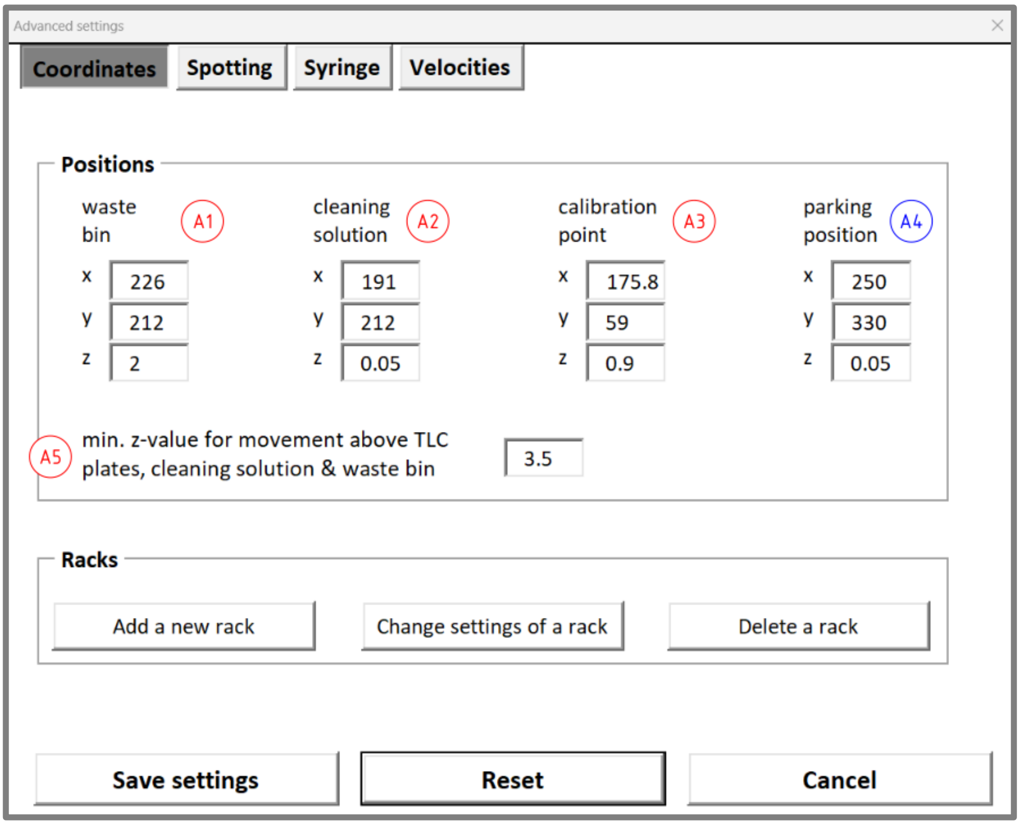


M8	The list contains the different numbers of TLC plate(s) that can be used during the spotting process. The operator can select the correct number from this list.
M9	Enter the volume (µL) that should be extruded per spot in total.
M10	Select the number of extrusions that should be made per spot. The extruded volume (M9) will be distributed equally among all extrusions.
M11	Enter the velocity at which the extrusion on the TLC plate(s) should occur.
M12	The TLC robot pauses above a spot before making a subsequent extrusion so that each spot can dry between two extrusions. The length of this pause can be altered here.
M13	If the option is selected (M14), the Heating block heats the aluminum plate with the TLC plates to speed up the spotting process and to obtain smaller spots. The temperature of the Heating block can be changed here. The temperature of the aluminum plate will typically be 5-10 °C lower than the temperature of the heating block. Furthermore, it will take approx. 1-2 minute(s) until the maximum temperature of the aluminum plate is reached.
M14	Decide whether or not the TLC places should be heated during the spotting process.
M15	Select whether or not the washing program should be included in the spotting program. If the TLC plates are heated during the spotting process, it is advisable to combine the washing and the spotting program, as approx. the time of the washing program necessary to heat the aluminum plate to the desired temperature.
M16	Choose whether or not an acoustic signal should signal the end of the spotting program.

The "Advanced settings" menu

The "Advanced settings" menu has four tabs, "Coordinates", "Spotting", "Syringe" and "Velocities", which enable the user to calibrate a new setup or to make changes that are required less frequently. While it is necessary to enter the values obtained during the calibration process (red indicators), other values (blue indicators) should only be altered after careful consideration, as, for example, the velocities were carefully optimized. The Excel file settings are changed accordingly by clicking on the "Save Settings" button. If "Reset" is clicked, the current values in the Excel file are displayed in the user interface and "Cancel" closes the menu without altering any settings in the Excel file.

Coordinates



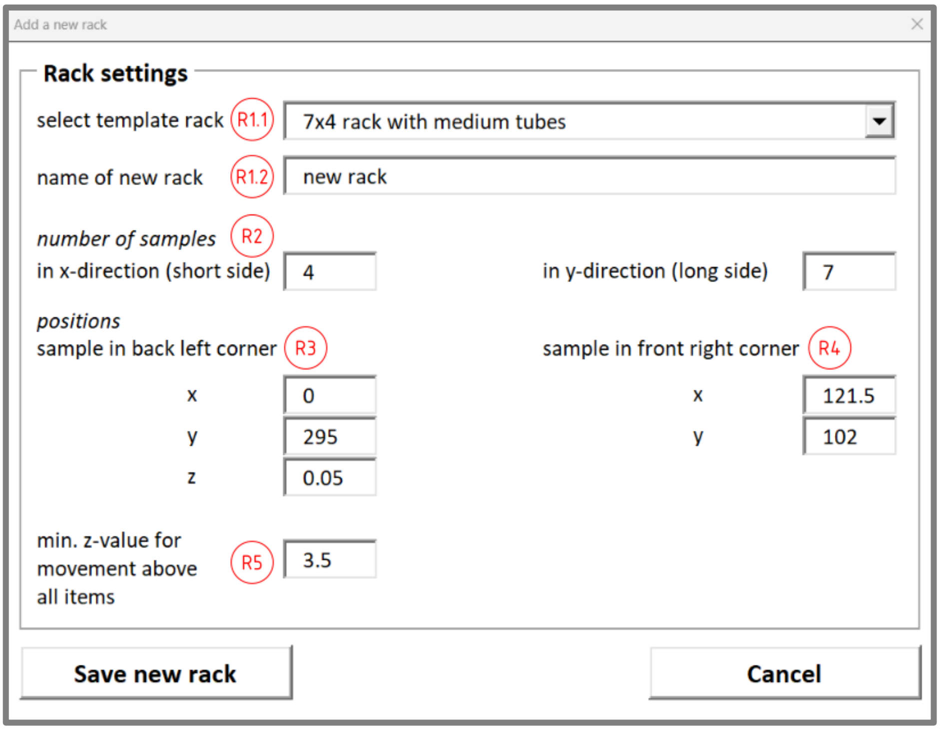
A1	Enter the x-, y- and z-coordinates of the position at which the cannula should be emptied in the waste.
A2	Choose the x-, y- and z-coordinates of the position at which the cannula should be filled with the cleaning solution.
A3	Enter the x-, y- and z-coordinates of the calibration position.

A4	Select the x-, y- and z-coordinates of the position at which the cannula is parked at the end of each program. The x- and y-coordinates should be selected carefully so that no collisions with items inside the base frame can occur. Furthermore, the z-value should only be altered slightly, as higher values might cause collisions between the gear rack and Z-axis mounting during the homing process.
A5	The z-value that is required for lateral movement above the TLC plates, the cleaning solution and the waste bin.

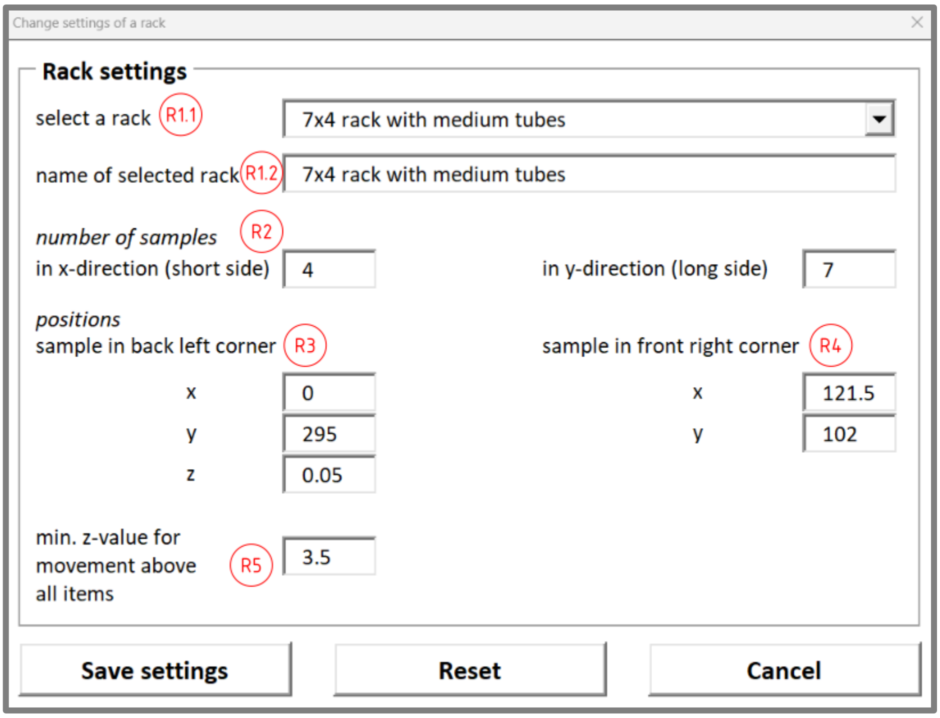
By selecting the buttons "Add a new rack", "Change settings of a rack" or "Delete a rack", new interfaces are opened that enable the user to modify entries in the rack list in the "Modifications" menu.

The three rack menus

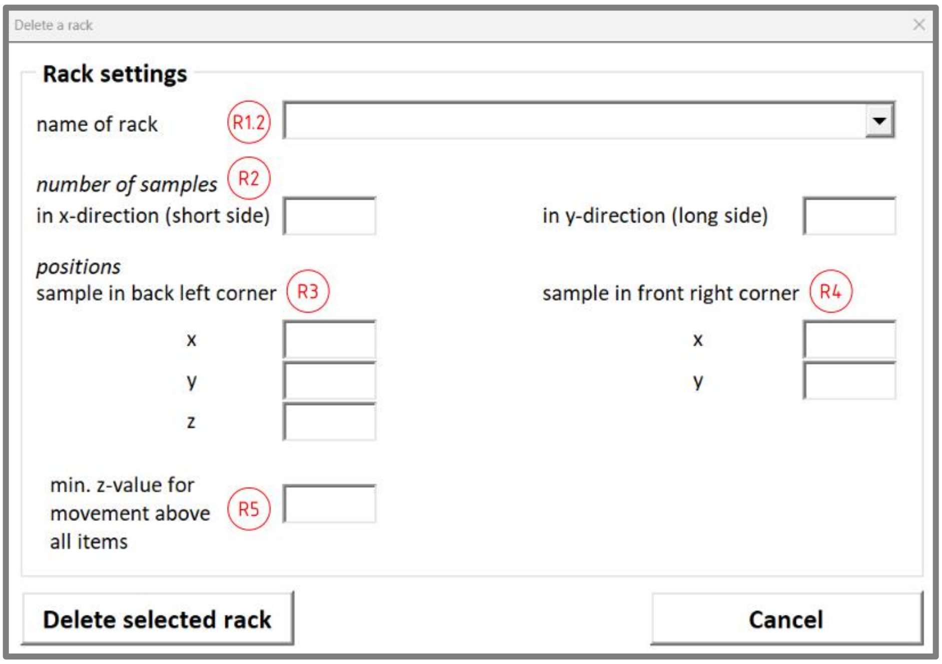
To add a new rack, choose a name (R1.2) and enter the positions obtained during the calibration process (R2-R5). Alternatively, select one rack from the rack list as a template (R1.2) and make the necessary modifications if the new rack differs only slightly from the already calibrated one. Click "Save new settings" to add the new rack to the rack list or "Cancel" to close the menu without altering any values in the Excel file.



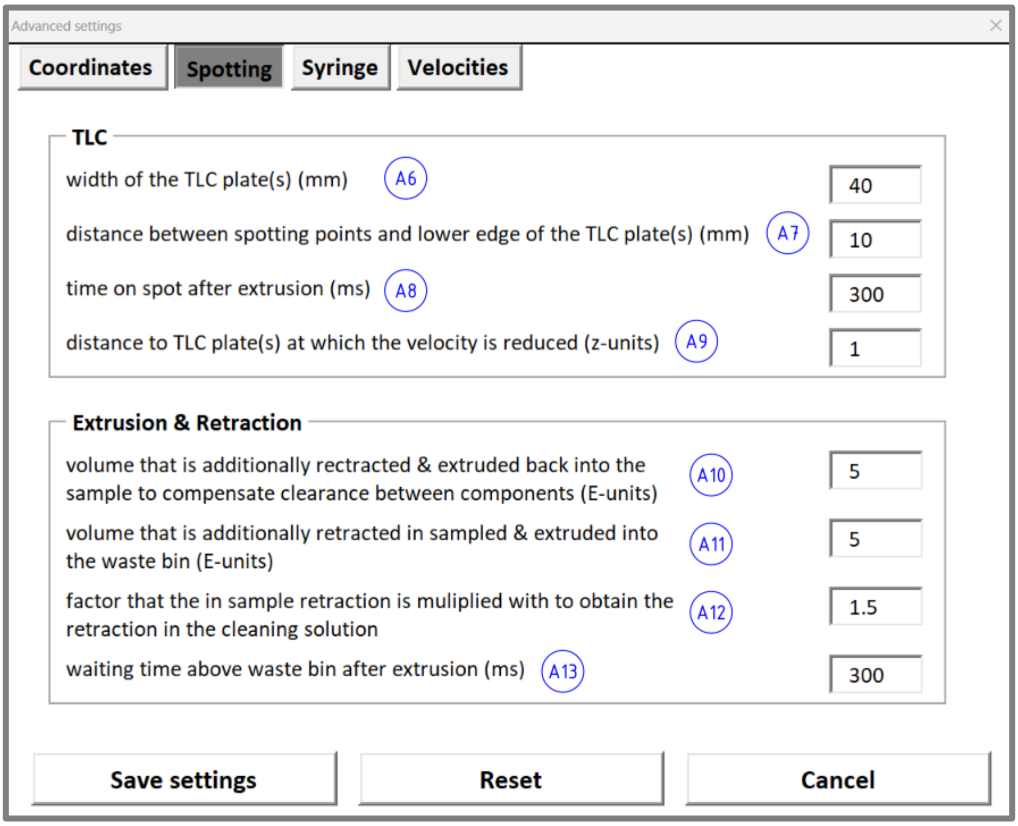
To change the settings of a rack, select the correct rack from the list of racks. Then modify the name (R1.2) or the settings (R2-R5) of the rack as desired and save them by clicking the "Save settings" button. Alternatively, the values can be reset to the current values in the Excel file or the menu can be closed without making any changes.



To delete a rack, select it from the rack list (R1.2). Subsequently, all the values of this rack will be displayed (R2-R5). Check that the correct rack is selected. Click "Delete selected rack" to remove the rack from the list in Excel or "Cancel" to abort the process.

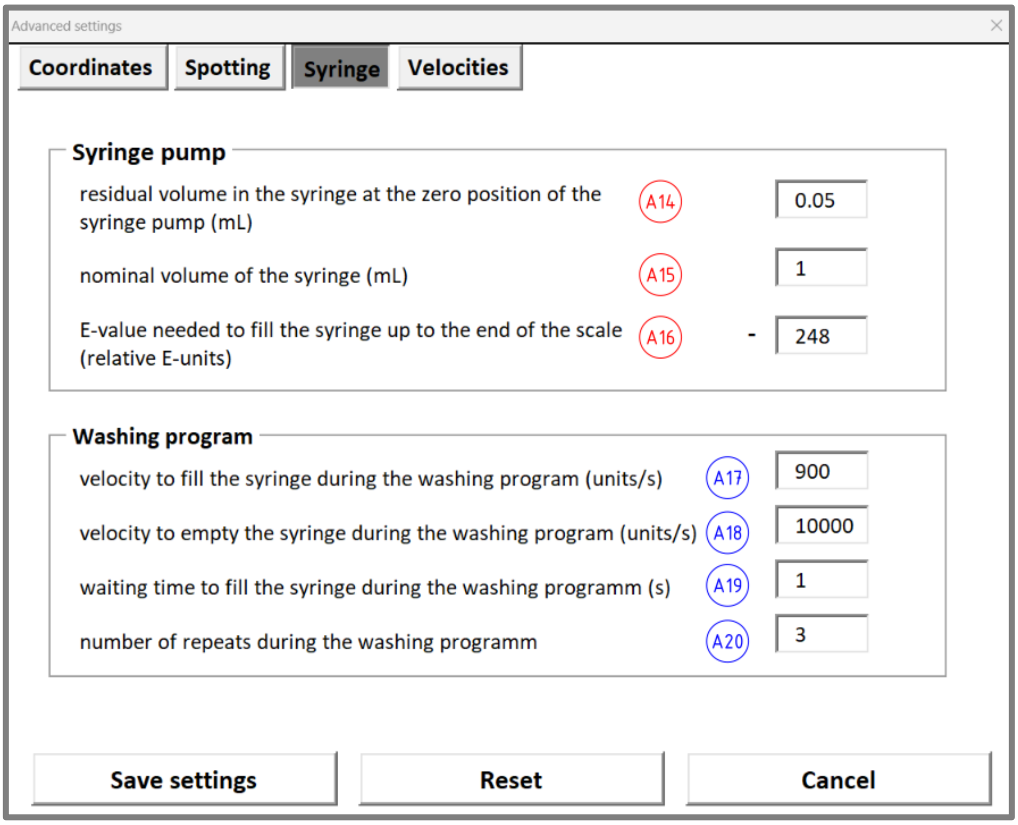


Spotting



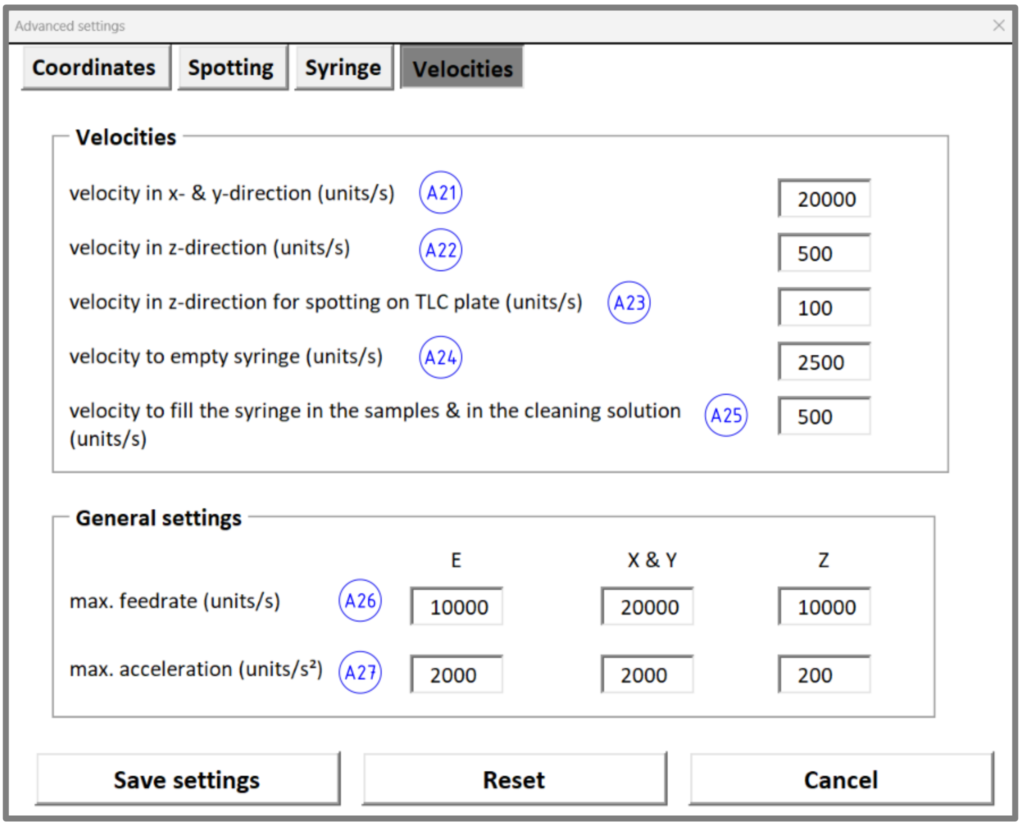
A6	The width of the TLC plates (mm) can be altered here if necessary.
A7	If necessary, adjust the distance between the spotting line and the lower edge of the TLC plates.
A8	Change the time the cannula stays on the TLC plate after completing each extrusion.
A9	The velocity in z-direction is reduced before the cannula is in contact with the TLC plate. The distance to the TLC plate at which the reduction occurs can be selected here.
A10	The clearance between the different components can influence the accuracy of the syringe pump when the mode is changed from extrusion to retraction. Therefore, a small volume is retracted and extruded directly into each glass vial to improve accuracy. The size of this volume can be adapted here.
A11	An additional volume of each sample is retracted to have some buffer during spotting on the TLC plates. The size of this additional volume can be changed here.
A12	During the cleaning cycles, the volume drawn into the cannula is larger than during the sample collection to ensure the cannula is always thoroughly cleaned. The ratio of cleaning solution volume to sample volume can be altered here.
A13	To avoid dripping from the cannula after the extraction, the cannula is paused for a short time above the waste bin. This time can be adapted here.

Syringe



A14	Enter the residual volume of the syringe at the zero position of the syringe pump when the middle part touches the top part and cannot move further up.
A15	Change, if necessary, the nominal volume of the syringe in the syringe pump.
A16	Enter the E-value necessary to fill the syringe up to its nominal volume.
A17	Change the velocity with which the syringe is filled during the washing program. However, the velocity should only be increased until gas bubbles start to form at the top of the syringe.
A18	Alter the velocity with which the syringe is filled during the washing program. Since a high velocity is required to remove air bubbles from the cannula, the velocity should only be slightly altered.
A19	During the washing program, the syringe pump briefly stops at its lowest point to fill the syringe completely. The length of the pause can be changed here.
A20	Select the number of times the syringe is filled and emptied during the washing program.

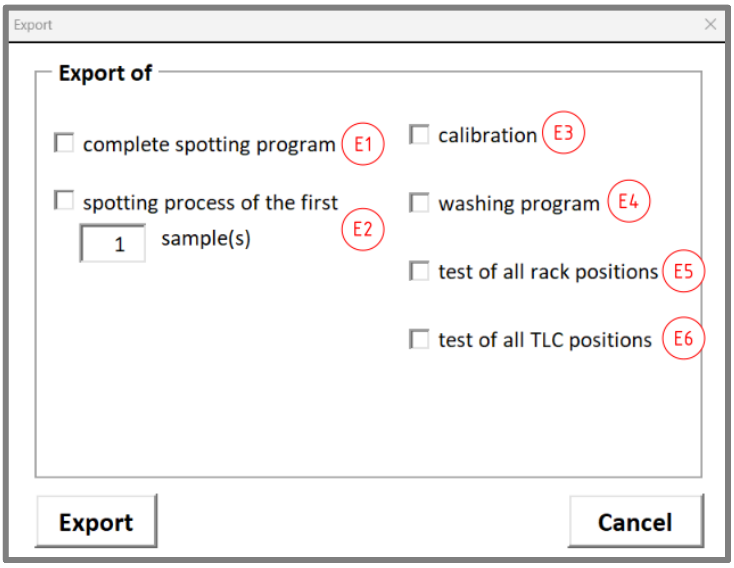
Velocities



A21	Change the velocity for movement in x- and y-direction. The value must be smaller than or equal to the max. velocity in x- and y-direction selected in A26.
A22	Enter the velocity for movement in z-direction. The entry has to be smaller than or equal to the max. velocity in z-direction selected in A26.
A23	Select the velocity with which the cannula is lowered onto the TLC plate(s). The velocity should be smaller than the velocity in A22.
A24	Change the velocity with which the solution is extruded onto the TLC plate(s).
A25	Adapt the velocity with which the syringe is filled during the spotting program.
A26	Change the max. velocity of the E-, X-, Y-, and Z-Motor.
A27	Alter the max. acceleration in the E-, X-, Y-, and Z-directions, if necessary, to enable faster movements.

The "Export" menu

After the settings have been adapted in the menus "Modifications" and "Advanced settings", the necessary g-code files can be exported using the "Export" menu. Select the desired programs by clicking on their checkbox and export their g-code using the "Export" button. The selected g-code files are then created in the same folder as the Excel file. If the "Cancel" button is clicked, the menu is left without exporting any g-code file. The following will explain in detail the files that can be generated using the "Export" menu.



E1	Saves the complete spotting program as "spotting.gcode".
E2	This option enables the user to create a run of the spotting program with fewer samples. The number of samples x can be entered and the new g-code file name is "spotting_x_sample(s).gcode". This program can, for example, be used for test runs or in cases when the last vial in a rack are empty.
E3	Generates the calibration program in which the cannula is moved to the calibration point (left front screw of the TLC holder) to allow readjustments of the cannula. The filename of the calibration program is "calibration.gcode".
E4	Export of a program ("washing.gcode") in which the syringe is filled with the cleaning solution and emptied several times to remove air from the system.
E5	Generates the file "rack_positions.gcode" that enables the user to test all selected rack positions without retracting any solution.
E6	The file "rack_positions.gcode" is generated, which allows operators to test all selected positions on the TLC plate(s) without spotting any solution.

Procedure prior to each run of the TLC robot

The procedure described in the following should always be carried out before each run of the TLC robot to avoid malfunctions and to achieve accurate and reliable results. For example, the syringe pump is not homed, so its position must be verified before a new run. Furthermore, it is always necessary to check the position of the cannula. Otherwise, problems can occur during homing if the previous run has been aborted.

<table>
<tr>
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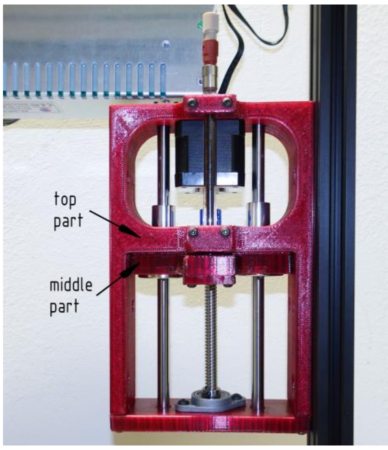

</td>
<td>

Step 1: Check the position of the syringe pump. If the middle part of the syringe pump does not touch the top part, move the z-axis above the waste container. Afterward, manually move the middle part to its zero position by turning the lead screw.

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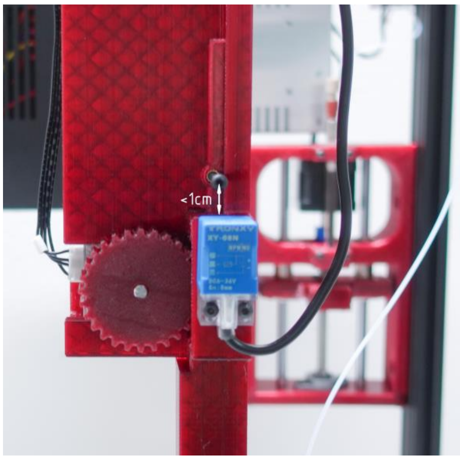
<tr>
<td>



</td>
<td>

Step 2: Check the position of the cannula in x- & y-direction. The cannula should either be slightly above the screw in the front left corner of the TLC holder (after the calibration process) or at the selected parking position (default value: right rear corner). If required, manually move the cannula to its parking position.

Step 3: Examine whether the distance between the screw in the gear rack and the Z-axis sensor is smaller than 1 cm. Lower the gear rack manually, if necessary.

Step 4: Restart the 3D printer if the syringe pump had initially not been at its zero position or if the z-axis had not been at its parking or calibration position.

</td>
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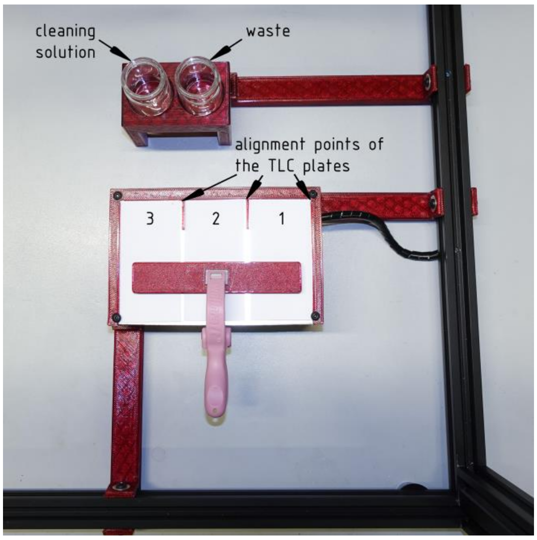
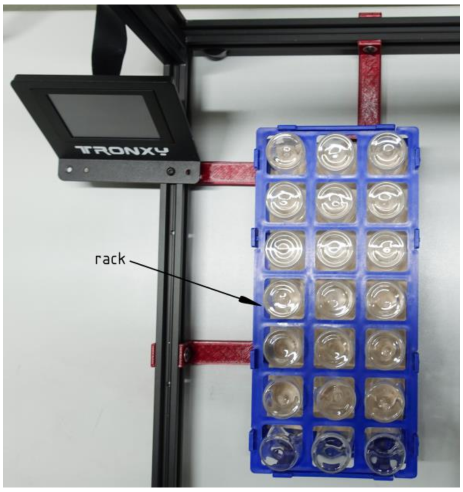
</table>

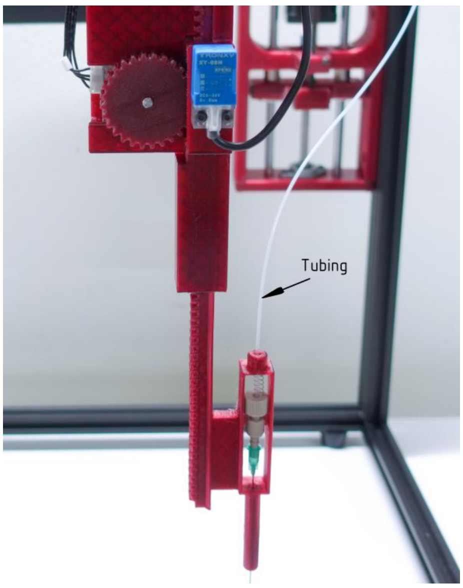


Step 5: Check whether two vials are placed on the table in the back. If necessary, refill or change the cleaning solution (e. g. isopropanol) or empty the waste.

Step 6: Place the desired number of TLC plates (max. size: 4x8 cm) at the positions for the first (1), second (2) and third (3) TLC plate and fix them with the clamp. Ensure that the right-hand side of each TLC plate is aligned with the TLC holder, as shown in the picture. This alignment is crucial when using TLC plates significantly smaller than 4 cm.



Step 7: Place a rack in the left rear corner of the TLC robot and align it with the three positioners. Verify that the rack is oriented correctly, so the collecting process starts with the first sample.



Step 8: Check the tubing for air bubbles. If air is present in the system, run the "washing program" and recheck the system afterward.

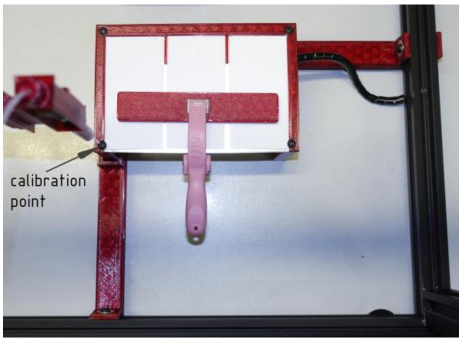
Step 9: Select the desired program on the display to start the spotting process.

Step 10: Monitor the collection and the spotting of the first sample(s). If any problems occur, stop the current run by turning off the power of the TLC robot. Possible solutions to the most frequent problems can be found in the section "Troubleshooting". Afterward, go through all procedure steps again before starting a new run of the TLC robot.

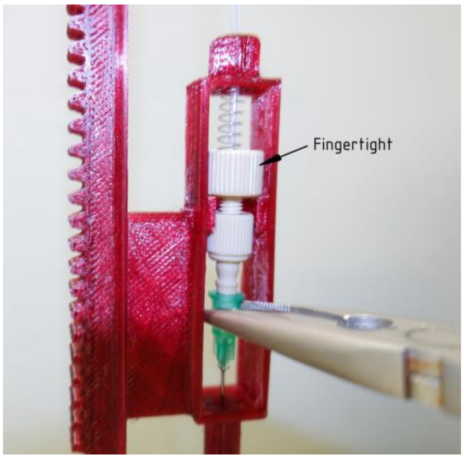
Rearranging and changing the cannula

Over time (especially if the cannula has collided with a misplaced component inside the frame of the TLC robot), the cannula can bend. As a straight cannula is required to achieve reliable positions, a bend in the cannula can influence the performance of the TLC robot. The following will describe two ways of dealing with such a bend.

If the bend is minor, it can be attempted to bend the cannula back to its original position.

	Use the calibration program to move the syringe above the screw in the left front corner of the TLC holder (calibration position) and bend it until it is positioned in the middle of the screw.

If the syringe is strongly bended or if the rearranging process has failed, it may be necessary to change the cannula.

	**Step 1:** The cannula is fixed with tweezers while the fingertight fitting (61.2) on top is simultaneously unscrewed.

	**Step 2:** The fingertight fitting and the adaptor are bent backward and the cannula is removed through the front opening of the capillary holder. **Step 3:** A new cannula is inserted through the front opening. Care should be taken not to bend the cannula during the insertion process. **Step 4:** The fingertight fitting and the adaptor are returned to their original position and tightly connected to the new cannula by fixing the cannula with tweezers and manually turning the fingertight fitting.

Troubleshooting

Problem 1

The TLC robot does not move to the correct positions.

Potential solutions

1. Check whether the cannula is bent or has a different length than usual. If necessary, replace the cannula.
2. Examine whether the tension of the belts has decreased. If necessary, use the belt tensioner to increase their tension again. If the tension cannot be increased enough by the belt tensioner, the tension of the belts must be altered directly at the hotend carriage. It is advised to decrease the tension of the belt tensioner beforehand so that it can later be used to adjust the tension.
3. Check if the g-code file contains "," as the separator signs. If so, replace them with decimal points.
4. Repeat the calibration process.

Problem 2

No fluid is spotted on the TLC plates.

Potential solutions

1. Check whether the cannula is lowered into the vials below the fluid level. If necessary, modify the z-value of the rack in the Excel file.
2. Examine whether any air bubbles are visible in the tubing. If necessary, run an extra washing program and recheck the system afterward. If the amount of air in the system stays the same, verify that all connections are tight.

Problem 3

The TLC robot does not perform the last few tasks of the g-code file or performs them incorrectly.

Potential solution

This problem usually occurs if the g-code file consists of only very few lines of code. Enter the following command a few times at the end of the file:

```
G4 S1       ; Pause for 1 s
```

Afterward, the TLC robot should perform the task as expected.

Problem 4

During the homing process, the cannula of the TLC robot does not move upward before homing the x- & y-axis.

Potential solution

This problem usually occurs if a run of the TLC robot has either been manually canceled or if the run has been prematurely aborted due to too few commands (see Problem 3). Move the TLC robot manually to the parking position and restart the robot by turning the power off and on.

Problem 5

The macros in the Excel file do not work.

Potential solution

1. Check the Excel version on the computer. The file can only be used with Excel 2010 or newer versions.
2. Verify that all macros are activated. If necessary, change the security settings.
3. As the macros do not work correctly on protected sheets, check whether any worksheets are protected.
4. If any cells have been deleted or moved in the Excel file, check if the references to the user interface values are still correct. Alternatively, reload the original version of the Excel file.

References

1. Tronxy X5SA hotend carriage: Adapted from Florovskiy, 2021: https://grabcad.com/library/tronxy-x5sa-hotend-carriage-1 (checked 05.06.2024).
2. X-axis switch: Adapted from Maxfield, 2021: https://grabcad.com/library/creality-limit-switch-z-stop-1 (checked 05.06.2024.
3. Tronxy X5SA Pro 330x330: Adapted from H. Schmidt, 2021: https://grabcad.com/library/tronxy-x5sa-pro-330x330-1 (checked 05.06.2024).
4. CoreXY Belt Tensioner Update v.2: Adapted from H. Schmidt, 2021: https://grabcad.com/library/corexy-belt-tensioner-update-v-2-1 (checked 05.06.2024).