

Supplement

Manufacturing monolithic glass columns and a compatible column holder produced by 3D printing

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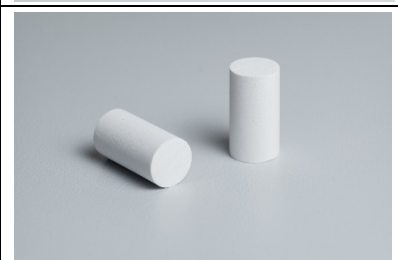


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Abstract: This instruction in the form of a picture story should facilitate preparing a monolithic column and its respective column holder. This column is intended to be attached to an FPLC or HPLC system to be used for analytical or semipreparative purposes.





Keywords: Affinity chromatography; affinity enrichment; bio-chromatography; biochemical interaction; regeneration, antibody purification; immunoglobulin; VitraPOR; glass filter; porous materials





Materials and instruments


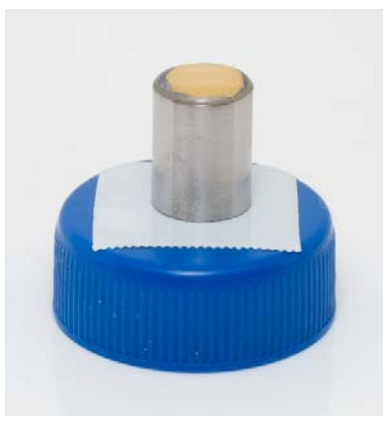


	<p>3D printer</p> <p>(Zortrax M200, software Z-SUITE: v2.9.0.0, Zortrax Z-ABS Filament Pure Black 1.75 mm, Layer thickness: 0.14 mm, Nozzle diameter: 0.4 mm, Quality: High Infill: 50 %, Fan Speed: Auto, Support 20°)</p>
	<p>Fluid Connector made of PEEK, titanium, or stainless steel</p> <p>(Engineering drawing: FluidConnector.pdf, PEEK: Technoplast v. Treskow GmbH, 56112 Lahnstein, Germany, Cat. No. 2520101500500, Turning machine DLZ „EMCO“)</p>

	<p>Sealing rings & blind plugs</p> <p>(Silicone plate: 1 mm, Modulor GmbH, Berlin, Germany, Art.-Nr. 0227329; Hollow punch, 10 mm and 12 mm; O ring: HUG Technik und Sicherheit GmbH, 84030 Ergolding, Germany, Art.-Nr.: 4301200200, NBR 70 Shore 12x2 mm); Blind plugs 1/16")</p>
	<p>Metal tube, preferable titanium grade 2</p> <p>(Engineering drawing: TitaniumTube.pdf, ID 10 mm, OD 12 mm, 15.2 mm height)</p>
	<p>Glass monolith with the desired porosity</p> <p>(ROBU Glasfilter-Geräte GmbH, Hattert, Germany. Por. 5 (P 1.6, Ultrafine, pore size 1-1.6 µm) or Por. 4 (P 16, Medium, 10-16 µm), respectively. The monoliths were prepared by custom order, diameter 8 mm, length 15 mm, front surfaces finely sawn.)</p>
	<p>Fixing screws and screw nuts</p> <p>(Engineering drawing: KnurledKnobM5.pdf, Cylinder head screws, hexagon socket, ISO 4762-M5 x 40 DIN 912 stainless steel V2A, hexagon nut, M5 stainless steel V2A ISO 4034)</p>
	<p>Silicone sealing</p> <p>(Silicon F liquid, WEICON GmbH & Co. KG, 48157 Münster, Germany, Art. No. 13200310)</p>

Preparation of the monolithic column

	<p>Prepare a small pedestal, e.g., a lid of a falcon tube.</p>
	<p>Glue a double-sided adhesive tape on the top of the pedestal.</p>
	<p>Press the glass monolith on the adhesive tape carefully.</p>
	<p>Put another piece of double-sided adhesive tape on top of the glass monolith and remove the protruding tape with scissors while not damaging the monolith.</p>

	<p>Take a small amount of silicone F and coat the glass monolith with the help of a small plastic spatula.</p>
	<p>Let the coated monolith rest for 24 h under a fume hood.</p> <p>On the next day, remove the hardened silicon remains near the bottom of the monolith.</p>
	<p>Clamp a 3 mL syringe without its plunger and a closed cannula attached to a stand. Fill the syringe with about 0.5 – 1.0 mL of silicone. Let it rest for approximately 5 minutes so the silicone can settle on the bottom of the syringe.</p>
	<p>Carefully put the metal tube around the monolith onto the adhesive tape. Make sure both the monolith and the cylindric tube sit concentrically and firmly on the adhesive tape and the gap between them is symmetric.</p>

	<p>Use the prepared syringe to fill the gap between the monolith and the metal tube with silicone. Due to the silicone's high viscosity, a relatively strong force is necessary to push the silicone out of the cannula into the gap. Ensure that the monolith is not moved to prevent contamination of the lower face.</p>
	<p>Make sure that there is no trapped air between the monolith and the column. The whole top surface of the column should be covered with silicone dome like a round "hat." Trapped air bubbles can move upwards and be replaced with down-flowing silicone.</p> <p>Let the silicone cure for 48 - 72 h under a fume hood.</p>
	<p>Carefully remove the silicone "hat" with a utility knife and peel off the adhesive tape.</p>
	<p>Cut the last pieces of cured silicone from the column and carefully remove the column from its pedestal.</p>



The column is now finished and can be installed in a suitable column holder or stored e.g. in an Eppendorf tube. The surface coating should be applied during the column installed in the column holder.

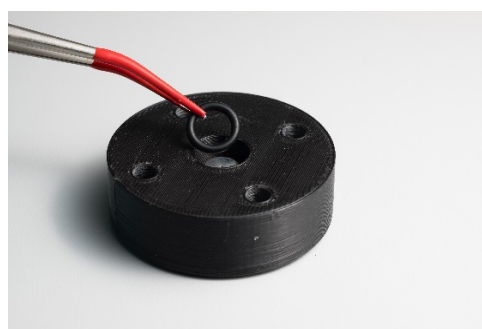
Installing the column in your 3D-printed column holder



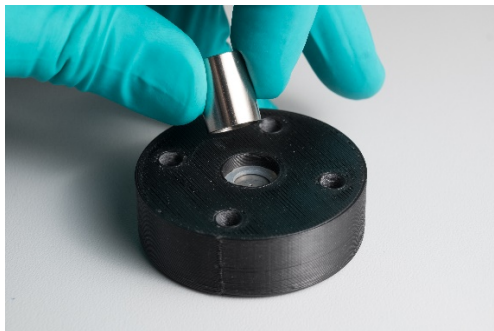
Prepare your 3D-printed main plates and insert suitable screw nuts in the bottom part.



Insert the fluid connectors in both main plates and push them in tightly.



Add one O-ring ontop of each fluid connector (may not be required for the PEEK connectors)

	<p>Place a flat sealing ring on top of each O-ring.</p>
	<p>Insert the monolithic column into the bottom main plate.</p>
	<p>Put the top main plate on top of the column and insert the four screws through the holes of the top plate into the holes of the bottom plate. Carefully tighten screws cross-wise hand-tight.</p>
	<p>The column is now ready-to-use and can be connected to any regular chromatography system or tubings with equivalent fittings (usually 1/16" tubing). Solvent compatibility may be limited by the silicone glue.</p>

Titanium Tube:

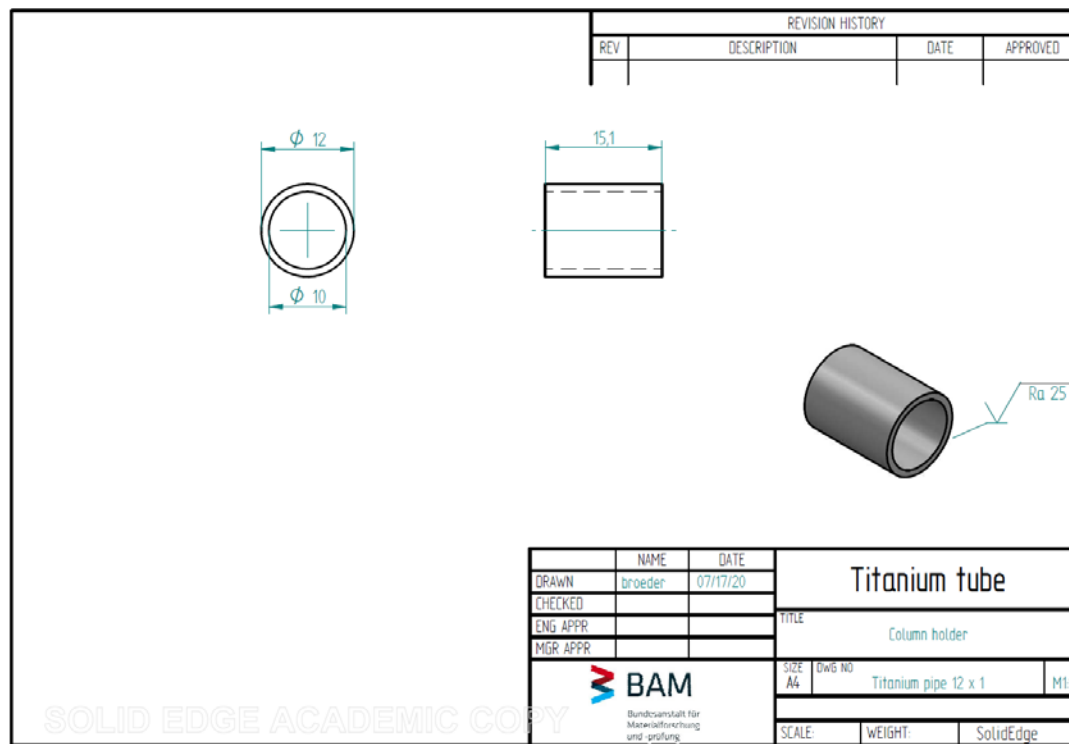


Plate 1:

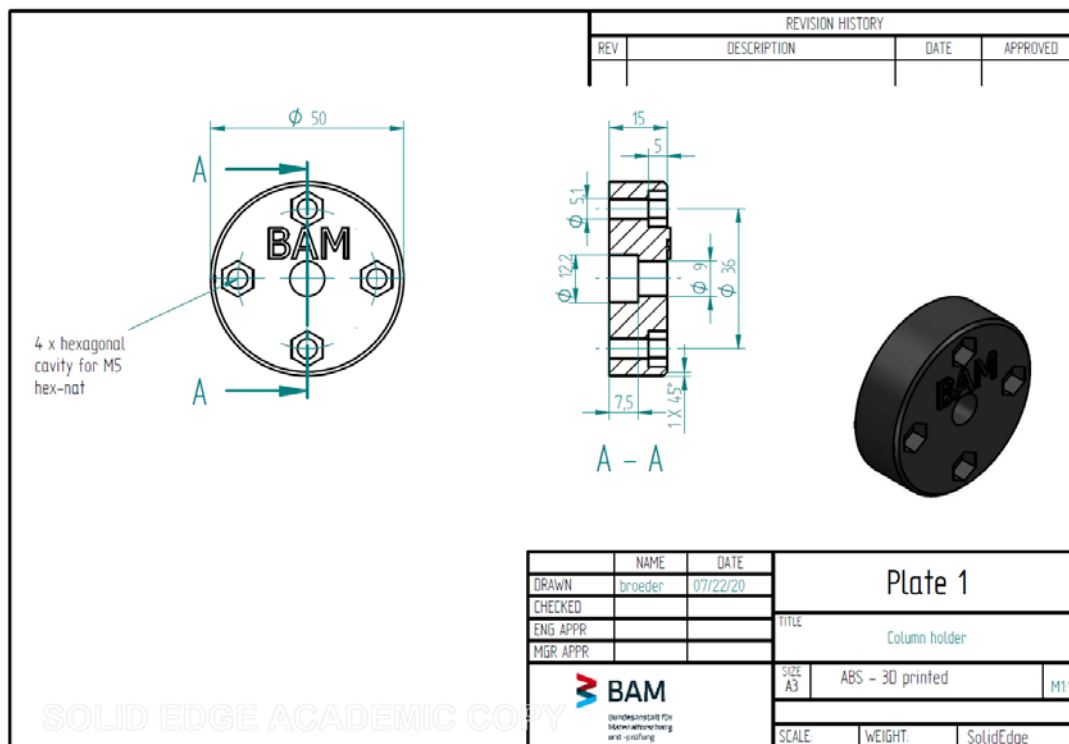
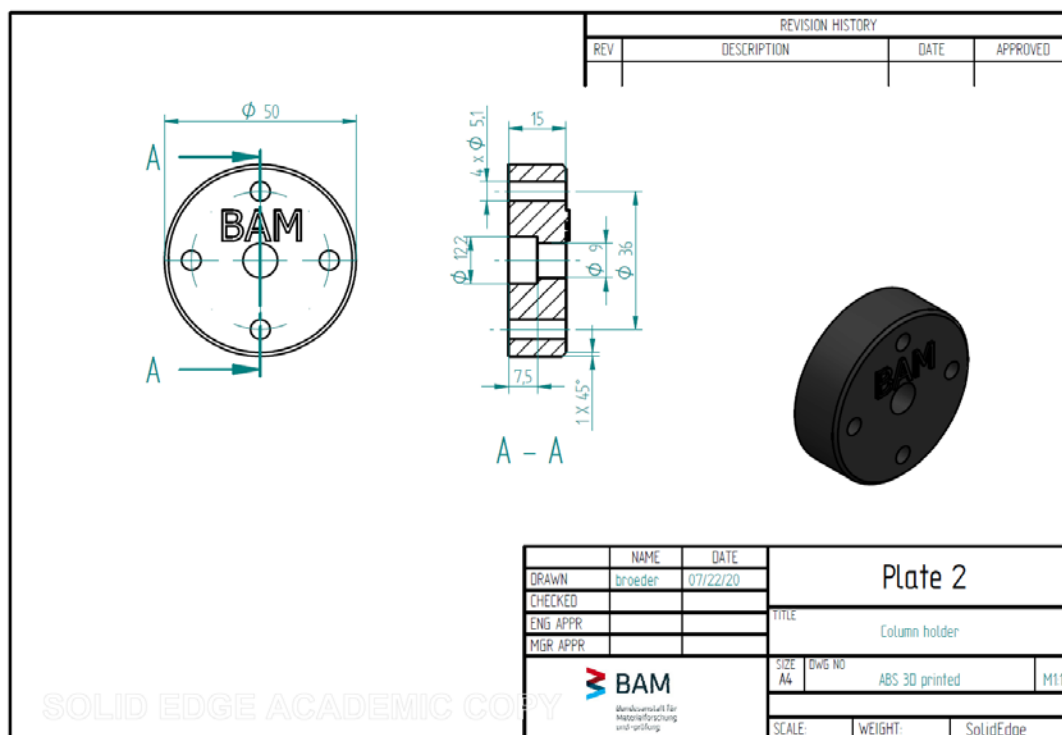
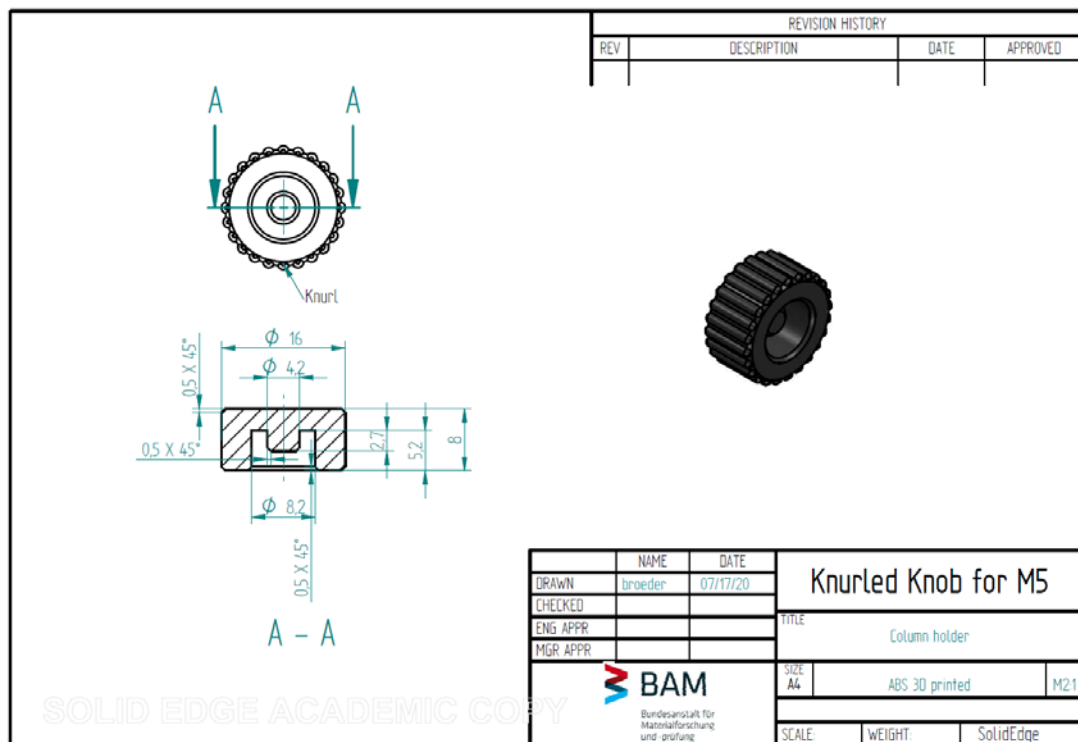


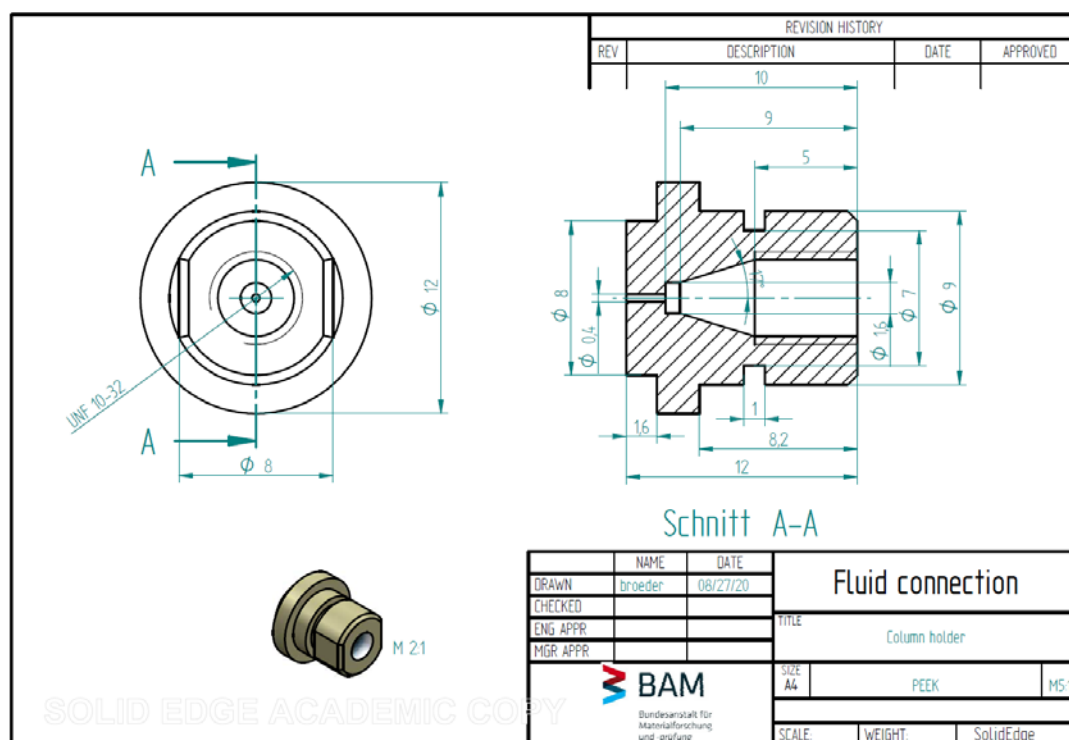
Plate 2:



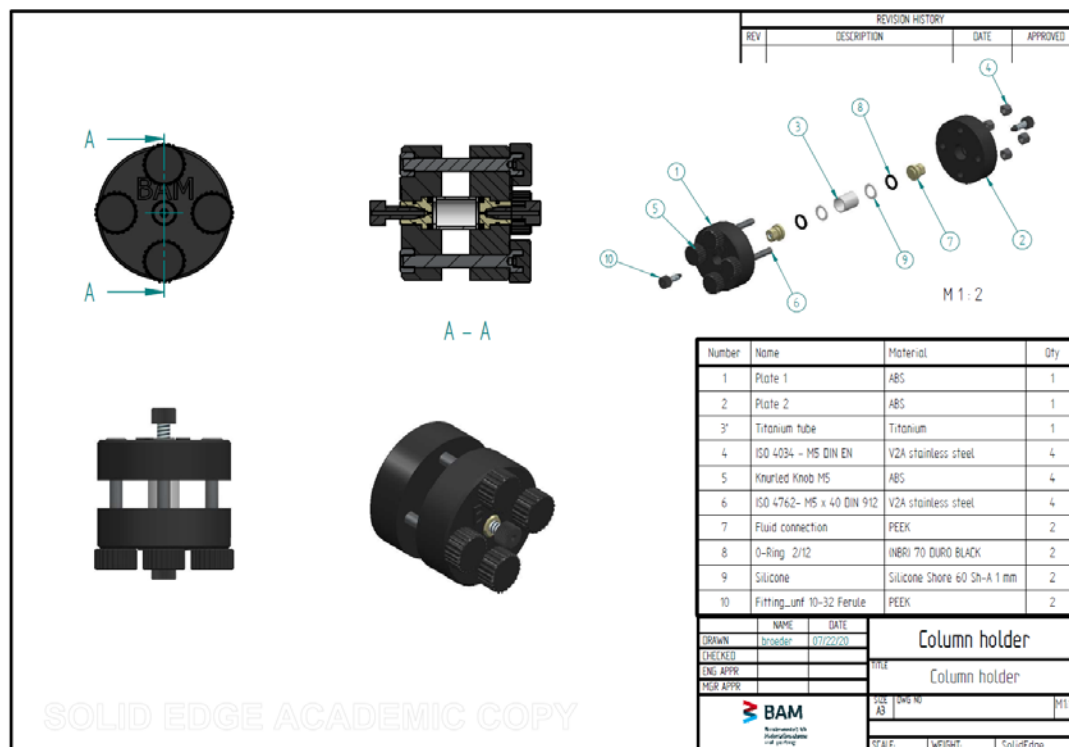
Knurled Knob:



Fluid Connector:



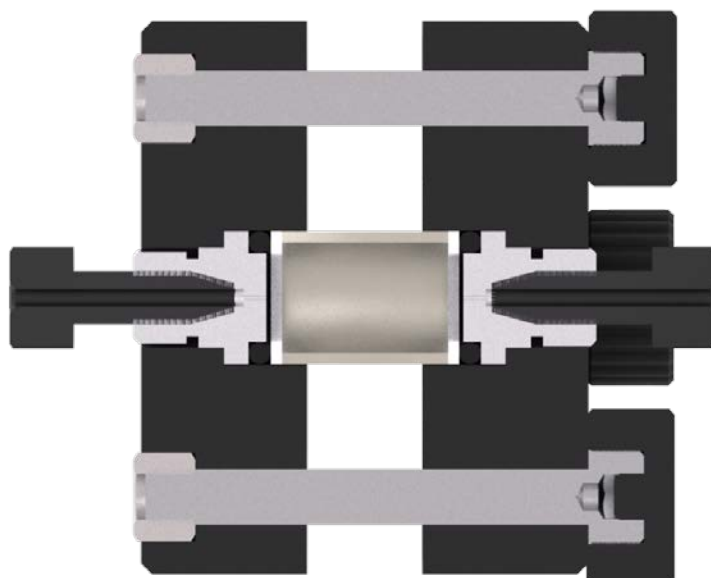
Materials List:



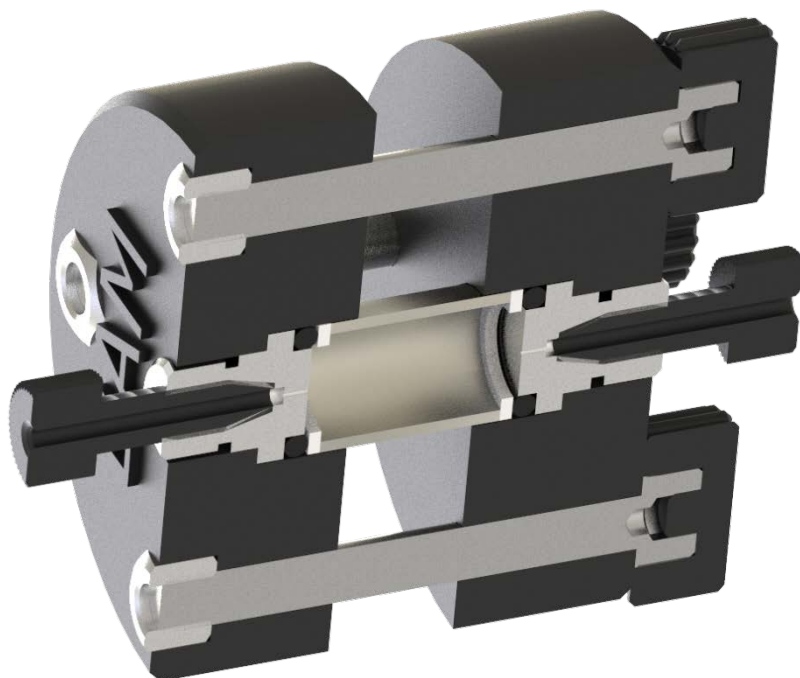
Exploded view:



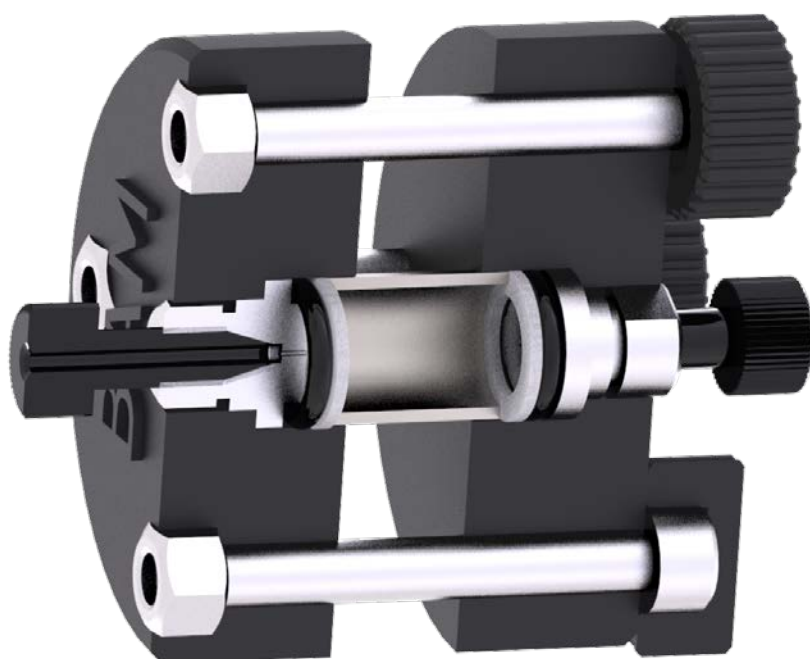
Sectional view 1:



Sectional view 2:



Sectional view 3:



Assembled view:

