Electronic Supplementary Material (ESI) for Lab on a Chip.

Research Article

Automation of Cell Culture Assays using a 3D-printed Servomotor-controlled Microfluidic Valve System

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Supporting Information

User Information for Operation of the 3D-printed Microfluidic Valve System

The protocols for servo opening and closure as well as pump steps of the 3D-printed microfluidic valve system are attached separately as .py and .xlsx files. The .py script files can be opened by integrated development environments such as the program Thonny. For operation of the "pumpsystem_LabOnAChip.py" file for the proof-of-concept experiments, the programming bibliographies stated at the beginning of the script must be installed. The .xsls files include the parameters used for each sequence of a specific protocol, such as servo number, servo position, pump volumes and more, while the "Commands.py" files include the main commands for the servos and the pumps. All files must be located in the same folder. When using different pumps or servos, the new commands must be imported into the main script. The script automatically determines the next sequence by reading the starting time of the .xlsx file. Thus, the sequences do not have to be sorted according to the "sequence start" parameter in the .xlsx file, but must follow the given matrix.

Table S.1: Mask parameters for automated image processing of cell confluence using IncuCyte 2021C software.

Parameter	Value	
Segmentation	0.5	
Hole Fill (µm²)	0,0000	
Adjust size (pixels)	-4	
Area (µm²)	min: 120	max: -
Eccentricity	min: -	max: -

Table S.2: Number of valve actuations including opening and closing until failure of the corresponding servomotors. Cases where no failure was observed until the end of the experiment were actuated 5608 times.

Treatment	Number of valve actuations until failure			
	Valve 1	Valve 2	Valve 3	Valve 4
No treatment	1561	no failure	no failure	no failure
Heat steam sterilized	no failure	no failure	4128	3000
Heat steam sterilized + incubation in ddH_2O (37 °C, 28 d)	4071	no failure	no failure	no failure

Table S.3: Parameters used for the microfluidic valve system during the proof-of-concept cell culture cytotoxity assay. (Seq. No: Number of a single sequence including valve opening, pumping and valve closure; Sequence start: Starting time as passed time since the start of the script; Servo No.: Number of the servomotor opened during the respective sequence, where servo 1-8 are connected to the well outlets and servo number 9 to the waste outlet for rinsing steps; Flow rate_Pump1/2: Flow rates of the channels with (Pump2) and without (Pump1) camptothecin)

Seq. No.	Sequence start (s)	Servo No.	Flow rate_Pump1 ($\mu L \cdot min^{-1}$)	Flow rate_Pump2 ($\mu L \cdot min^{-1}$)
1	1	9	500	0
2	131	8	500	0
3	153	9	0	125
4	164	9	492.2	7.8
5	294	7	492.2	7.8
6	316	9	484.3	15.7
7	446	6	484.3	15.7
8	468	9	468.7	31.3
9	598	5	468,.7	31.3
10	620	9	437.5	62.5
11	750	4	437.5	62.5
12	772	9	375	125
13	842	3	375	125
14	864	9	250	250
15	934	2	250	250
16	956	9	0	500
17	1026	1	0	500
18	1048	9	500	0

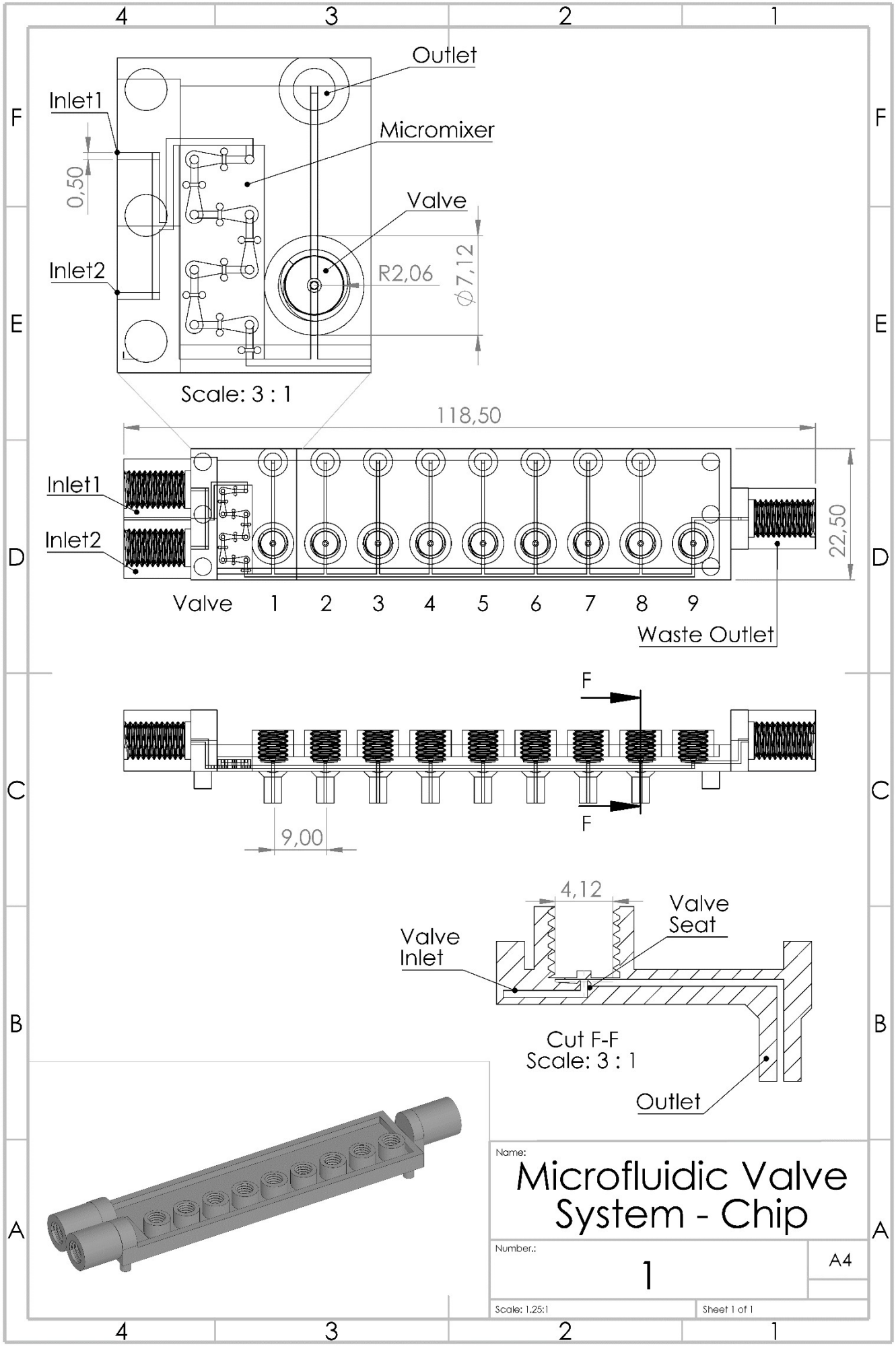
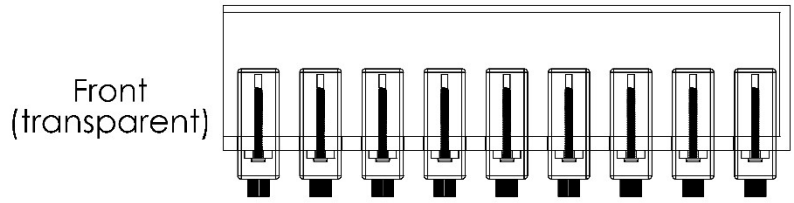
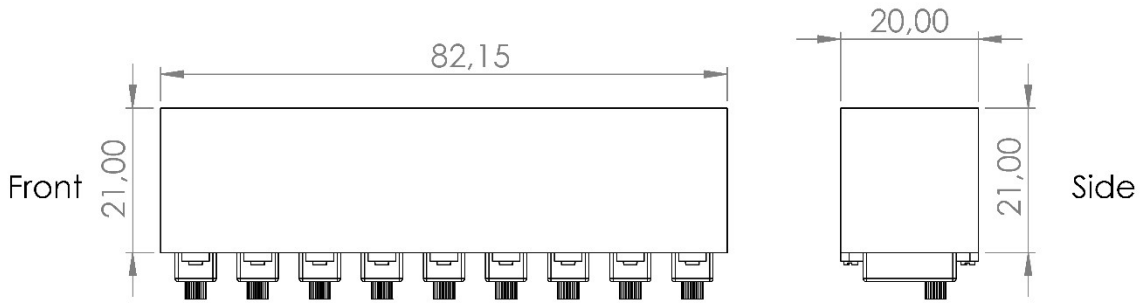
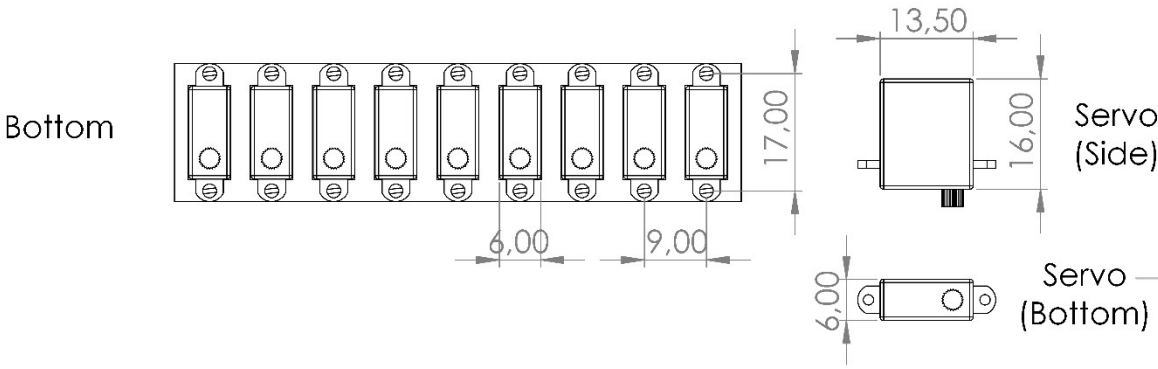
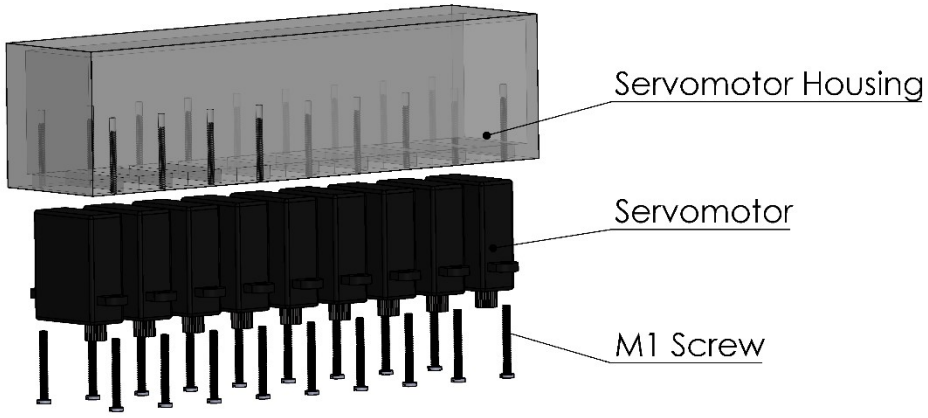


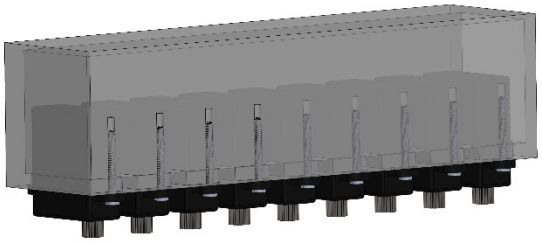
Figure S.1: Technical drawings of the 3D-printed microfluidic valve system.











Name:

Microfluidic Valve System - Housing

Number.:

2

A4

Scale: 1.25:1

Sheet 1 of 1