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

Supplementary Information

1 Evaluation of metering performance

A slightly modified microfluidic chip was designed and fabricated to determine the reproducibility of the metering structures. The microfluidic layout of the metering sectors on the disk mirrors the digestion sectors' layout, except for some alterations, to be able to precisely determine the amount of liquid metered by each of the four metering structures, M1-M4. Metering performance for sample and UT-buffer can be evaluated with the metering structures M1 & M2 in combination with the evaluation channels C1 & C2. As can be seen in Figure 1 the upstream fluidic of the metering structures is identical to the layout in the digestion sector. Instead of a dilution chamber, two measurement channels C1 & C2 are connected to the metering structures. This design allows the metered fluid to fill the channel, and observation of the meniscus with a stroboscopic setup makes it possible to determine the metered volume with high accuracy.

The same approach was used to evaluate the amount of metered dilution (M3), trypsin and acetic acid. The metering structure M4 was used for both trypsin and acetic acid metering.

For the determination of the metering accuracy, each reagent was metered in its respective metering structure on three different disks. After metering, the liquid was centrifuged into evaluation channels and the height of the meniscus in the channel was monitored with a stroboscopic setup at 30 Hz. At this high rotational frequency, capillary forces acting on the meniscus become negligible compared to the centrifugal forces. Thus, the resulting meniscus of the liquid column in the microfluidic channels is flat, which facilitates exact determination of the liquid volume. For final quantitation of the metered volume, the stroboscopic images were imported into Solidworks and aligned with the CAD structure of the evaluation channels. The channel geometry was then trimmed at the position of the meniscus and the remaining volume was measured. To minimize the influence of variations during the production process on the obtained volumes, the dimensions of all metering structures were measured at three different positions in the PDM cast with a Confovis DUO Vario microscope *(confovis GmbH, Germany)*. Measurement results are presented in Table 1 and were used as the dimensions of the CAD structures the stroboscopic images were aligned to. The disks were manufactured by thermoforming coextruded COC 6013/8007 foils with a thickness of 200µm. As the glass transition temperature of the 6013 layer (135°C) is 25°C higher than the highest process temperature during the thermoforming process, the contour accuracy of the foil disks was assumed to be high and reproducible.

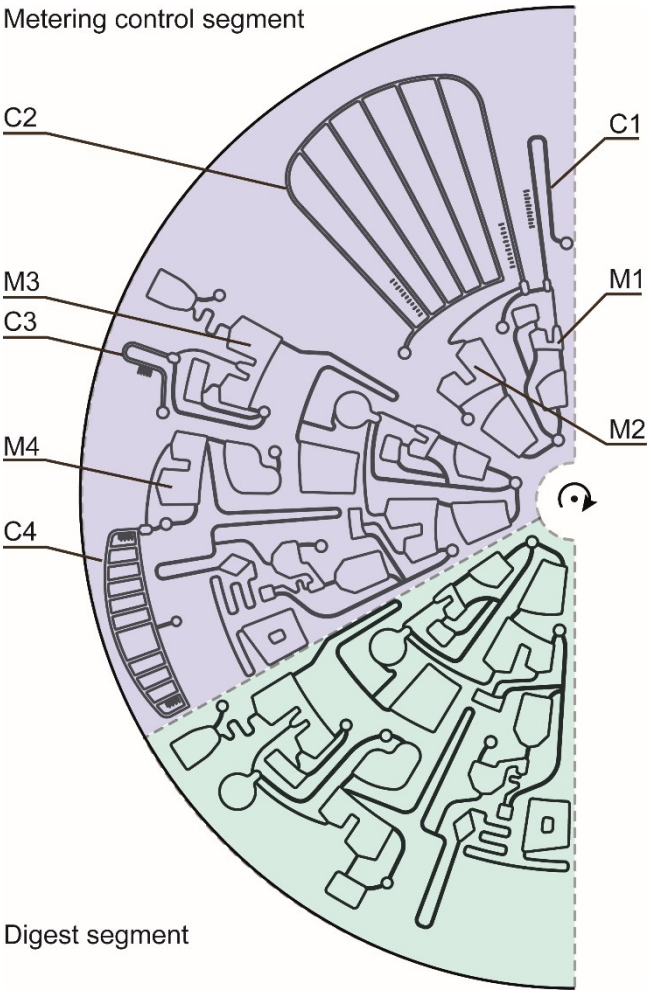

Figure 1: One half of the microfluidic disk showing the principal layout of one sector for the digestion of serum as well as the metering control segment which allows to determine how precisely reagents and sample are metered in the different structures.

Table 1: Dimensions of the metering structures in the PDMS cast

Structure	Width	Height
C1	279.1 µm ± 5.5 µm	208.6 µm ± 0.45 µm
C2	403.6 µm ± 3.3 µm	316.0 µm ± 0.6 µm
C3	403.4µm ± 1.3 µm	311.3 µm ± 2.4 µm
C4	466.8 µm ± 5.2 µm	369.3 µm ± 0.4µm

Table 2: LC-MS/MS parameters

Reversed phase liquid chromatography (RPLC)	
Instrument	Ultimate 3000 RSLC (Thermo Scientific)
Trap column	Acclaim PepMap100 (Thermo Scientific) 75 µm inner diameter, packed with 3 µm C18 particles
Analytical column	Accucore 150-C18 (Thermo Fischer Scientific) 25 cm x 75 µm, 2.6 µm C18 particles, 150 Å pore size
Flow rate	300 nl/min
Column oven temperature	50°C
Buffer system	Binary buffer system consisting of 0.1% acetic acid in HPLC-grade water (buffer A) and 100% ACN in 0.1% acetic acid (buffer B)
Gradient	Gradient of buffer B: 2 min 2% to 5%, 8 min 5%, 120 min 5% to 25%, 5 min 20 to 40%, 2 min 40% to 90%, 5 min 90%, 3 min 90% to 2%, 10 min 2%
Mass spectrometry (MS)	
Instrument	Q Exactive HF (Thermo Scientific)
Operation mode	Data-dependent
Electrospray	Nanospray Flex Ion Source
Full MS	
MS scan resolution	60,000
AGC target	3e6
Maximum ion injection time for the MS scan	20 ms
Scan range	333 to 1650 m/z
Spectra data type	Profile
dd-MS2	
Resolution	15,000
MS/MS AGC target	1e5
Maximum ion injecting time for the MS/MS scans	25 ms
Spectra data type	Profile
Selectiob for MS/MS	15 most abundant isotope patterns with charge ≥2 from the survey scan
Isolation window	1.4 m/z
Dissociation mode	HCD
Normalized collision energy	27.5%
Dynamic exclusion	30 s
Charge exclusion	Unassigned, 1, >6

Table 3: Frequency and temperature protocol

Step number	Description	Duration [s]	Frequency [Hz]	Acceleration [Hz/s]	Temperature [°C]
1	Metering and transfer	65	75	30	-
2	Relax pressure in timer pneumatics	120	25	1	-
3	Lower shakemode frequency	0.1	7	30	-
4	Upper shakemode frequency	0.3	15	30	-
5	Repeat steps 4 &5 80 times				
6	Denaturation	300	25	30	-
7	Build up pressure in pneumatic chamber	45	60	30	-
8	Prime siphon	0.5	20	30	-
9	Transfer of dilution	5	40	30	-
10	Metering and transfer of dilution	60	60	30	-
11	Lower shakemode frequency	0,1	2	30	-
12	Upper shakemode frequency	0,1	15	30	-
13	Repeat steps 11 & 12 12 times				
14	Reload timer	45	60	30	-
15	Repeat steps 11 & 12 12 times				
16	Relax pressure in timer pneumatics without priming the adjacent siphon	150	25	30	-
17	Digestion	61200	30	30	37
18	Decrease temperature	10	30	30	22
19	Build up pressure in timer pneumatic	3	60	30	-
20	Prime siphon of timer structure	75	12	30	-
21	Metering and transfer of acetic acid	50	60	30	-
22	Upper shakemode frequency	0,1	15	30	-
23	Lower shakemode frequency	0,1	-15	30	-
24	Repeat steps 22 & 23 30 times				
25	Centrifugation	5	30	30	-