Supplementary Material (ESI) for Lab on a Chip

Separation of Parasites from Human Blood using Deterministic Lateral Displacement

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-Electronic Supporting Information-

1. Device parameters

Our device was designed to have 13 sections each consisting of one discrete critical diameter, D_c, according to Eq. 1, see Fig. E1a. Array parameters are defined in Fig. E1b. The diameter of the posts, D_{post}, is 20µm throughout the device. The gap between the posts, $d=\lambda-D_{post}$, is 12µm and is also constant throughout the device. λ is the centre-to-centre spacing of the posts. The critical diameter in the device is varied by varying $\Delta\lambda$, the amount each row is laterally shifted with regard to the previous row. Table E1. shows $\Delta\lambda_n$ for each of the n=1,…,13 sections and the $D_{c,n}$ that this gives rise to. $N=\lambda/\Delta\lambda_n$ is the periodicity of the array. The small deviations from whole and half micrometer values for $D_{c,n}$ are the result of technical constraints of the mask generating process, forcing us to fit our designs to a 0.2 µm manufacturing grid.

$$D_{c,N} = 1.4 \cdot d \cdot N_N^{-0.40} \qquad (1)$$

Table E1. The device was designed to have 13 sections containing discrete critical diameters. All sections have equal D_{post} and λ but $\Delta\lambda$ is varied. Each different $\Delta\lambda_n$ gives a specific N_n and therefore, according to Eq. 1, a specific $D_{c,n}$.

Section Number n	Row Shift $\Delta\lambda_n$ [µm]	Period N_n	Critical Diameter $D_{c,n}$ [µm]	Lateral displacement x [µm]
1	0.80	40.00	2.86	160.0
2	1.20	26.67	3.47	156.0
3	1.60	20.00	3.99	160.0
4	2.00	16.00	4.44	160.0
5	2.60	12.28	5.04	156.0
6	3.20	10.00	5.56	160.0
7	3.80	8.42	6.04	152.0
8	4.40	7.27	6.48	154.0
9	5.20	6.15	7.02	156.0
10	6.00	5.33	7.52	150.0
11	6.80	4.71	7.99	136.0
12	7.80	4.10	8.53	156.0
13	8.80	3.64	9.04	132.0

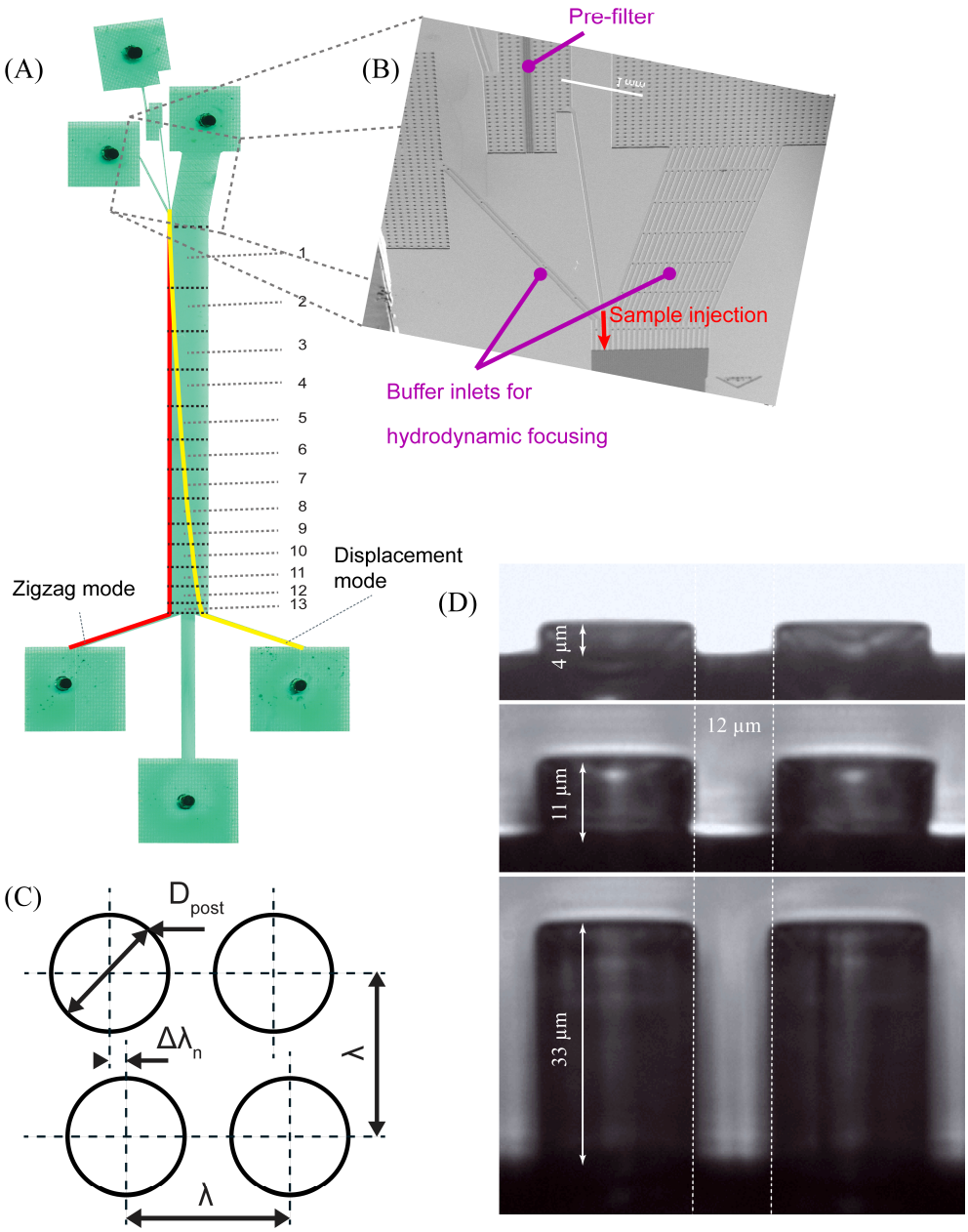


Fig. E1. Device parameters. (A) Our device contains 13 sections, each with one critical size. This image was taken by filling the device with food colouring and scanning on a flat bed scanner. Each section has a $\Delta\lambda$ chosen to give critical diameters from ~3µm to ~9µm in steps of ~0.5µm, see table E1. (B) Electron micrograph of the device. Sample is focused by a sheath flow of buffer. The pre-filter removes large particles (diameter>12µm) that would otherwise clog the separation array. (C) Definitions of device parameters. (D) Devices were fabricated at 3 different depths. These cross sections show the profiles of the pillars in each of the three devices.

2. Size measurement of *T. cyclops*

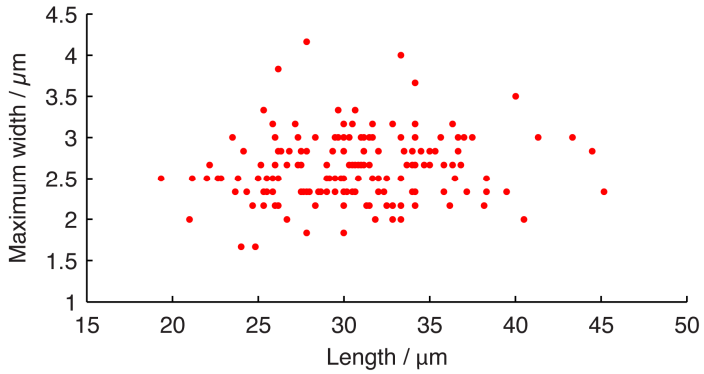


Figure E2. Scatter plot showing the size variation of the cultured *T. cyclops*. 160 trypanosomes were measured with a mean length of 30.6±4.6µm and a mean width of 2.6±0.4µm. This corresponds to values found in the literature and also shows its close relation to the pathogenic *T. brucei*.

3. Handling of *T. cyclops*

Freezing

- Harvest cells by centrifuging log-phase culture (i.e. 5-7 days since last split) at 1000 g for 10 min
- Aspirate medium and resuspend cells in Cunningham's medium containing 20% Fetal Calf Serum (FCS) which has been preheated to 28°C. The final volume should be chosen to reach a concentration of ~$7 \cdot 10^7$ cells/ml
- Slowly add 10% Dimethyl sulfoxide (DMSO) while stirring
- Transfer cells to autoclaved cryotube, wrap with parafilm
- Encapsulate tube with foam rubber to protect cells from freezing too rapidly, place package in a Styrofoam box in freezer at -80°C

Reactivation

- Heat Cunningham's medium with 20% FCS to 28°C
- Take out the tube from freezer and immerse immediately in 28°C warm water, shake tube while still immersed in water
- Rinse outside of tube with tap water followed by rinsing with ethanol
- Transfer cell suspension to autoclaved 50 ml Falcon tube. Add 10 ml medium at a rate of 1 ml/minute drop by drop. The remaining 40ml can be added faster

Culture splitting (every 14 days)

- Autoclave culture flasks
- Sterilize laminar flow hood and gloves with 70% EtOH, allow to air dry for 30s
- Add 20% FCS to the Cunningham's medium, heat to 28°C
- Fill each culture flask with 19ml
- Use sterile pipettes to transfer 1ml of trypanosome suspension to each culture flask

- Collect another 1ml of suspension to perform cell counting
- Store culture flasks in a sterile environment at 28°C

4. Sample preparation

Drawing blood samples

- Prick finger with Haemedic Haemolance® low flow
- Fill a pipette tip with 190µl autoMACS™ running buffer
- Aspirate one large drop of blood (~10µl)
- Pipette down and up in an Eppendorf tube to homogenize the liquids
- Add around 20µl into the sample inlet reservoir of the device

***T. cyclops* samples**

- Use log-phase growth trypanosomes, 5-7 days since last split
- Fill a 1.5ml eppendorf tube with 1ml of trypanosome culture
- Wash sample twice at 1000g for 1min to exchange medium to autoMACS™
- Pipette 20ul of trypanosomes into the sample inlet reservoir
- Use autoMACS™ in the other inlets

Simultaneous analysis of RBCs and *T. cyclops*

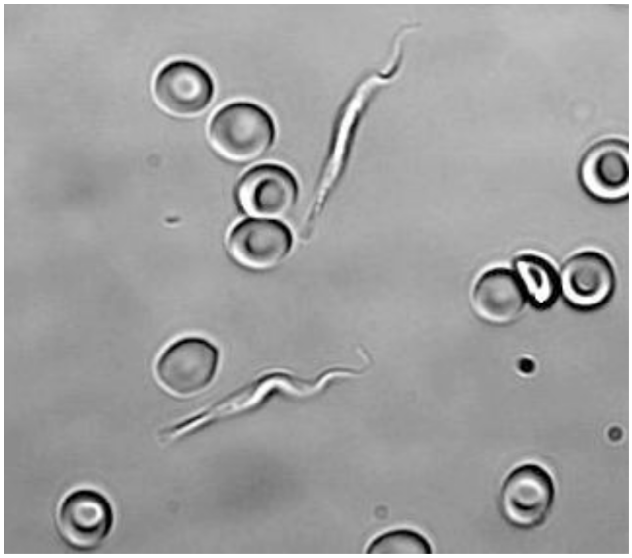
- Pool a 1ml trypanosome sample at 1000g for 1 min, wash twice and resuspend in 100ul autoMACS™
- Aspirate 160µl of autoMACS™ in a 200µl pipette tip
- Prick finger with Haemedic Haemolance® low flow
- Aspirate 40µl of blood with the filled pipette tip into the Eppendorf tube
- Wash blood sample three times with autoMACS™ at 1000g for 1 min, resuspend pellet with 20µl of the trypanosome sample
- Add the samples to the inlet reservoir

Staining of *T. cyclops*

- Dilute the DAPI stock solution to 50µg/ml
- Fill an eppendorf tube with 1ml trypanosome sample
- Add 10µl of the DAPI solution to the trypanosomes, pipett up and down to mix, cover with aluminium foil and let rest for 45 minutes
- Stop staining by washing three times in autoMACS™

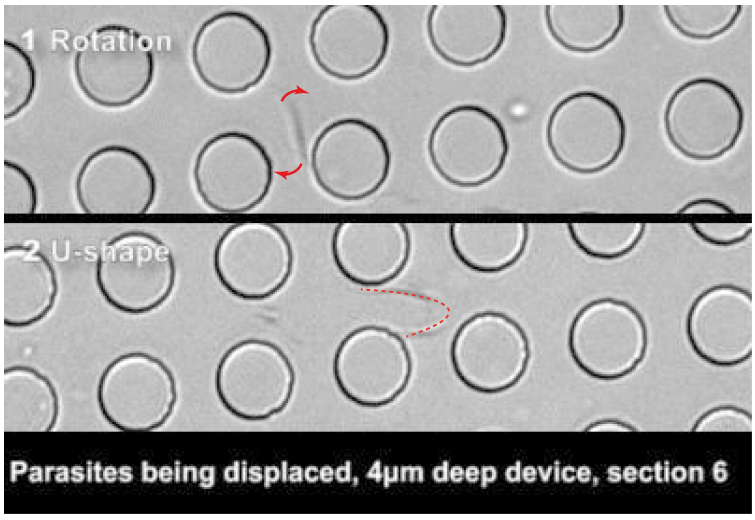
5. Motion of parasites through the separation device

In this movie *T. cyclops* and RBCs can be seen. The RBCs are laying flat on a glass slide and are therefore seen disc side onwards. The diameter across the disc is ~7.5µm. The parasite can be seen swimming with the characteristic undulations of its flagella.



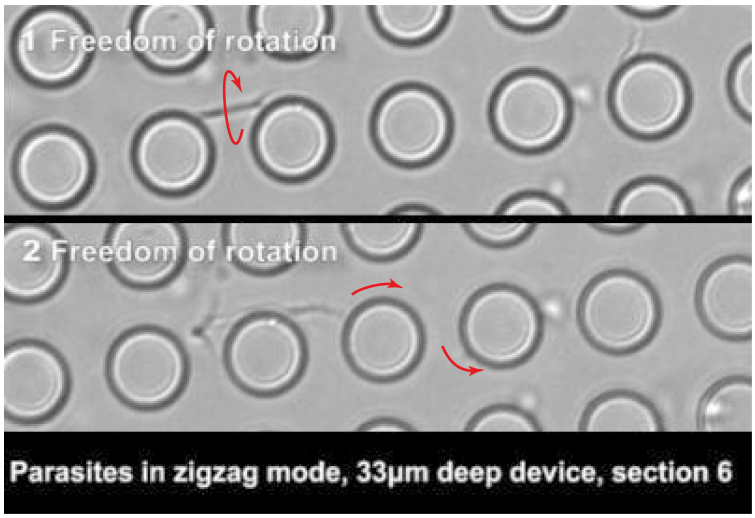
Movie 1

The following movies are representative of the behaviour of parasites in devices of different depths. All movies are at half actual speed.
Movie 2 shows how the parasites are being displaced in section 6 of the device, where the critical diameter is 5.5 µm. Rotation in the plane of the device (frame 1) and being trapped in a u-shape (frame 2) are two common modes, that we have observed, that greatly increase the effective size of the parasites.



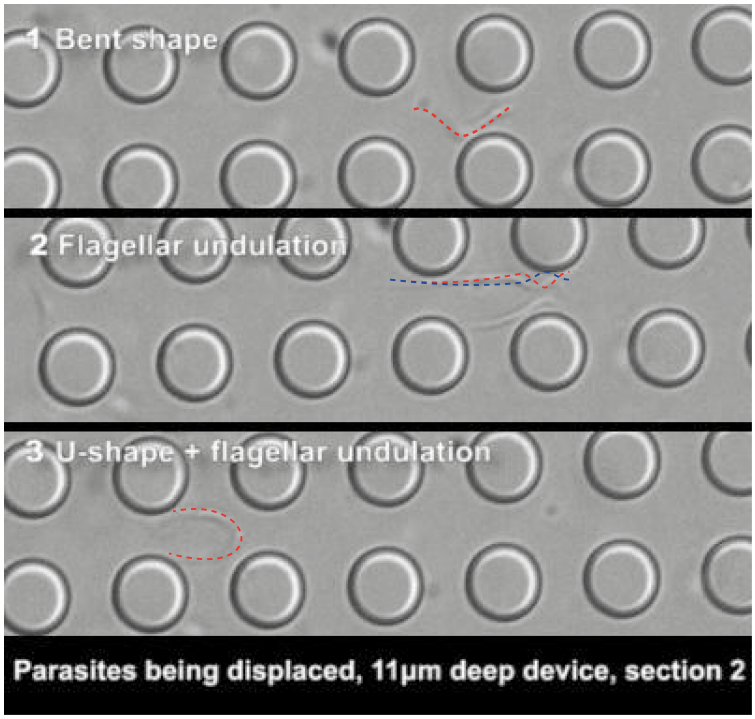
Movie 2

Movie 3 shows how freedom of rotation about their long axis in a deep device (33µm) allows parasites to adopt an orientation that gives them a smaller effective size. In section 6 of the device at this depth all parasites are moving in the zigzag mode.



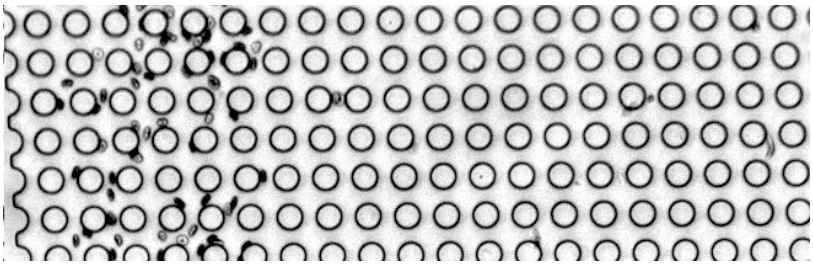
Movie 3

Movie 4 shows examples of parasite being displaced in section 2 of the 11µm deep device. Frame 1 shows how the bent shape of a parasite gives it a large effective size if it is unable to rotate around its long axis. Frame 2 shows the larger effective size due to rapid flagellar motion and Frame 3 a combination of flagellar motion and the u-shape configuration.



Movie 4

In movie 5 RBCs can be seen following the flow in the zigzag mode straight down on the left of the field of view. As this device is 11µm deep the RBCs can be seen rotating freely as they pass the posts. A parasite can be seen moving in the displacement mode on the right of the field of view. This parasite has been separated from the RBCs. Before it leaves the field of view the parasite can be seen performing a characteristic flip, as seen above in movie 2, frame 1 and it remains in the displacement mode.

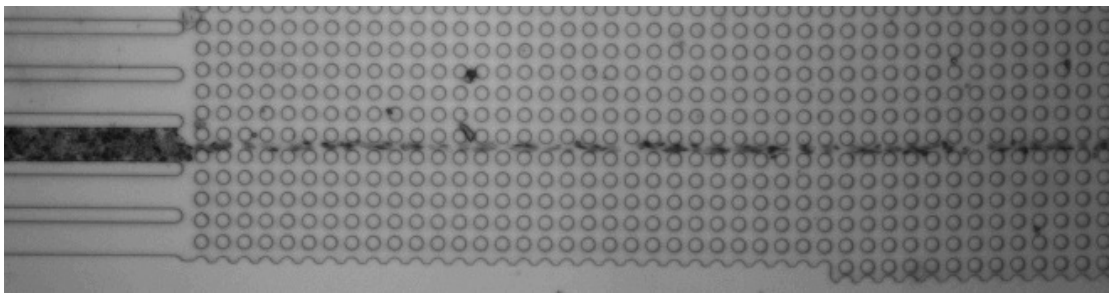


Movie 5

6. Do devices become clogged with blood cells or blood serum?

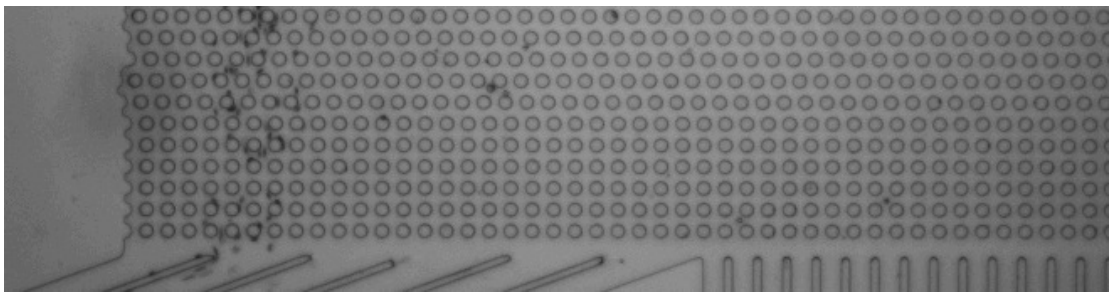
The PDMS surfaces in our devices are modified with PLL(20)-g[3.5]-PEG(2) (SuSoS AG, Dübendorf, Switzerland) as described in the materials and methods section of the paper and 1.75mg/ml Na_2EDTA is used as an anti coagulant. In order to test the effectiveness of these two treatments whole blood was injected into a device (11µm in depth) and the flow of blood through the device was monitored for 75 minutes. The following movies show whole blood at the entrance and exit of the separation array. Figure E3 shows the distribution of the RBCs at the end of the device over a 75-minute period.

Movie 6 shows whole blood + 1.75mg/ml Na_2EDTA 30 minutes after first being injected into our device. The stream is being focused by sheath flow to approximately 10µm in width.



Movie 6

Movie 7 shows the same device under the same conditions as in movie 6 but taken at the outlet. The stream of blood cells has broadened to approximately 100μm but the RBCs are not being displaced.



Movie 7

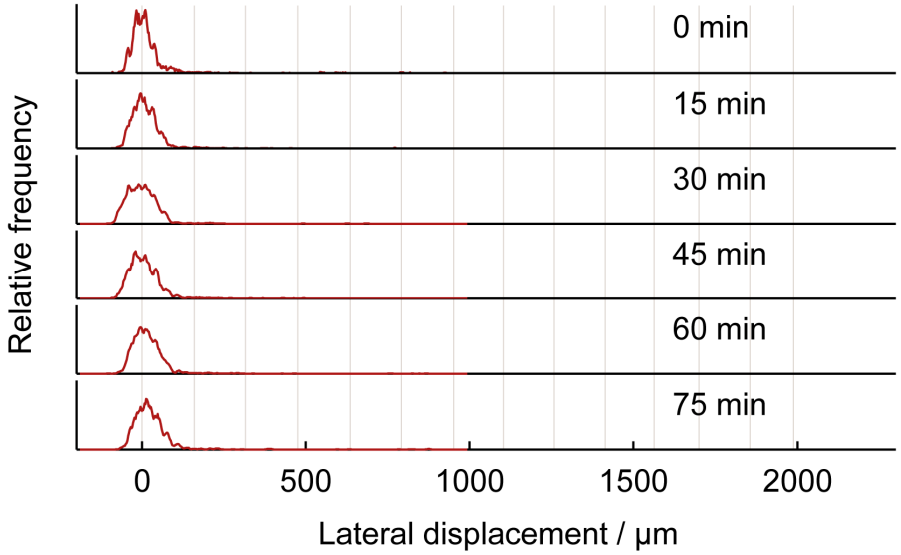


Fig. E3. The distribution of RBCs at the end of the device is shown as a function of time. Distributions are measured by tracking particles in films similar to movie 7. No change in the distribution can be seen after 75 minutes of running the device.