SUPPLEMENTARY INFORMATION

A portable droplet microfluidic device for cortisol measurements using a competitive heterogeneous assay

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Supplementary experimental

Pump fabrication

All components of the pump are shown in Fig. 3. The pump chassis, rotorhead, pumpline support bed and motor attachment plate were all 3D-printed. Each was first modelled using CAD software (SolidWorks, Dassault Systèmes) and then printed in “VeroClear” material using an Objet500 Connex3 polyjet printer (Stanford Marsh Ltd). A DC motor (499:1 Metal Gearmotor 25Dx58L mm LP 6V) was attached to the chassis via M3 plastic screws. The motor was used to drive the rotorhead (12 mm in diameter including raised features). Santoprene™ tubing of 0.51 mm inner diameter was used as the pumplines. The rotorhead diameter used was 12 mm to allow for pumping of the 8 phases of pulsations for fluorous oil and aqueous solutions. A silicon lubricant (Rocol Silicone Grease SAPPHIRE Aqua-Sil) was used to decrease the torque experienced in the pump and to increase pumpline lifetime. The complete pump assembly has a dimension of 43 mm×33mm×10mm.

Microfluidic chip fabrication

The droplet generation chip was fabricated by standard PDMS casting procedures. First a mould was designed using CAD software (SolidWorks) and 3D printed in “VeroClear” material using an Objet500 Connex3 polyjet printer (Stanford Marsh Ltd). The printed mould was dried overnight at 70 °C, to remove any unreacted monomer or solvent. Liquid PDMS (Sylgard 184, Farnell Onecall) was poured into the mould and oven cured at 70 °C overnight. The chip was peeled from the mould, cut to shape and then a layer of half-cured PDMS was used to seal the channel structure. PTFE tubing (Adtech Polymer Engineering Ltd.) connections were used as inlets and outlets and sealed using PDMS on a hotplate (Fisher scientific) at 105 °C for approximately 5 minutes. After fabrication, the microfluidic channels were surface functionalised by manually flowing a small volume of Aquapel, followed by flushing with air and drying in the oven at 70°C for ten minutes in order to render the channels hydrophobic and ensure the oil phase preferentially wet the channel.

Magnetic trap fabrication

The magnetic trap used for these assays comprises of two ferrite cores (3mm diameter, 25mm length) (RS Components) and windings of copper wire and tubing in place. The core tips are aligned and fit tightly around the PTFE tubing (0.3mm ID and 0.5mm OD) (Adtech Ltd.). Each electromagnetic tweezer set was constructed by wrapping 200 windings of enamelled copper wire of 0.35 mm thickness around each ferrite rod. The length of the coil varied between 15-16 mm depending on the manual winding. The tips of the ferrite core were tapered to increase the magnetic field density and provide sufficient force for extraction of the magnetic beads. The magnetic trap is controlled using a feedback mechanism and when energised the trap is capable of instantly attracting magnetic beads for carrying out the various steps of the assays.

Selection of ferrite core shape and quantification of magnetic bead capture

In order determine the optimal design of the ferrite core tip and the effectiveness of the electromagnet, a series of experiments were performed with sharp and blunt ferrite core tips. The sharp and blunt tip tweezer pairs were subject to currents ranging from 0.1 A to 0.5 A for different flow rates (5 µl/min, 10 µl/min and 20 µl/min) of the droplet sequence. Fig. S3 illustrates the capture of magnetic beads by the two types of tweezers for the mentioned currents and flowrates for different bead concentrations. The bead stock solution used in the experiment was prepared using 50 µl of bead in 500 µl of Phosphate Buffered Saline (PBS) with 1 % Bovine Serum Albumin (BSA) and 1 % Tween20. FC40 carrier was used without any surfactant. Droplets were generated by manual aspiration using a syringe pump (Harvard Apparatus PHD 2000), using the withdrawal mode set at the aforementioned flow rates.

Increasing the current delivered to the coil or the bead concentration increases the probability of bead capture (bead capture represented by red points) as there is an increased magnetomotive force which allows the magnetic beads to break the droplet interface. Increased total flow rate decreases probability of capture for both setups due to stronger hydrodynamic forces and reduced time for beads to sediment in the droplet and form a pellet. We would expect better performance from the sharp tips due to a more focused field. However, it can be seen that the blunt tips provide more reliable capture (especially at higher bead quantities). The lower capture probability and inconsistencies for the sharp tips could be due to two hypothesised reasons: oversaturation of the tips resulting in field density leakage, and misalignment of the tips. This is also likely the cause of some anomalous data present in Fig. S3 c with 0.2A current delivered to the tweezers not being sufficient for capture, most likely due to misalignment. The blunt tip tweezers easily fit tightly around the PTFE tubing and the larger surface area of the tips makes them easier to align for high field density in the centre of the channel. The larger surface area of the tips may also in fact produce a greater field density due to reduced leakage. High field density is vital for good extraction of the magnetic beads. Therefore, the blunt tips were chosen as the most suitable configuration for the assay based on the experimental results.

Automated magnetic trap circuit

The automated magnetic trap circuit was designed using DipTrace and exported in Gerber format for the PCB to be manufactured. AVRStudio was used to develop the software and to control an Atmel ISP chip programmer. Arduino IDE was used to develop and program the connection software running on the Adafruit Featherboard 32u4. The graphical user interface (GUI) was also developed using Embarcadero C++ Builder. The GUI is used to view, define and upload different parameters of the tweezers such as the coil current and light intensity thresholds required to reliably trigger the powering of the tweezers. The GUI lets the user either manually power the tweezers or set the unit into fully automatic mode. A snapshot of the GUI can be seen in Fig. S4.

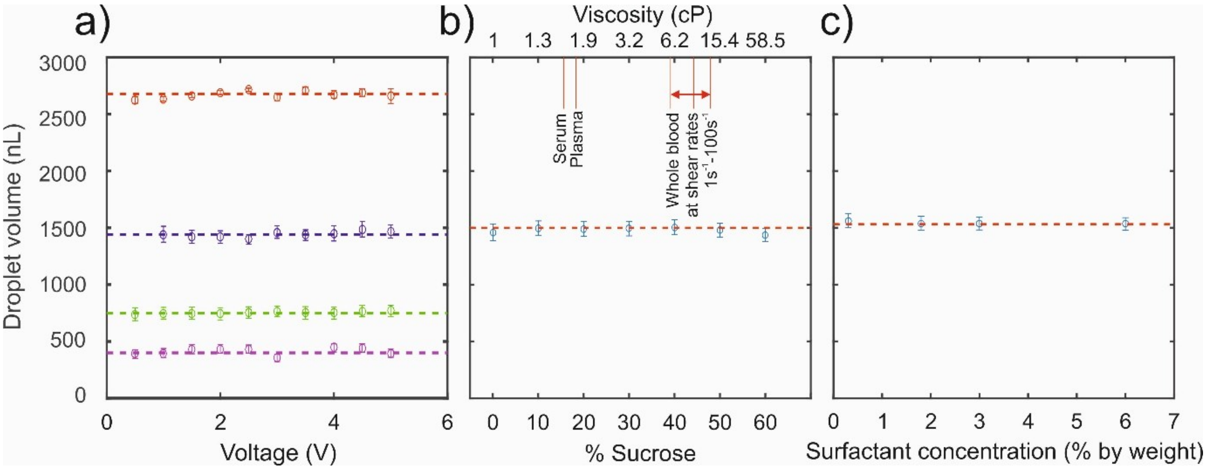

Figure S1: Characterisation of miniaturised micro peristaltic pump. Droplet generation dynamics for rotorheads with different feature spacing. a) Droplet volume corresponding to each feature spacing remains constant irrespective of motor speed (controlled by voltage supplied to the motor). Error bars show the standard deviation in droplet volume (consistently <1%). Droplet volume increases with feature spacing. b) Droplet volume vs viscosity (controlled by sucrose concentration in the aqueous phase). c) Droplet volume vs interfacial tension (controlled by concentration of PFPE-PEG surfactant in the FC40 carrier fluid).

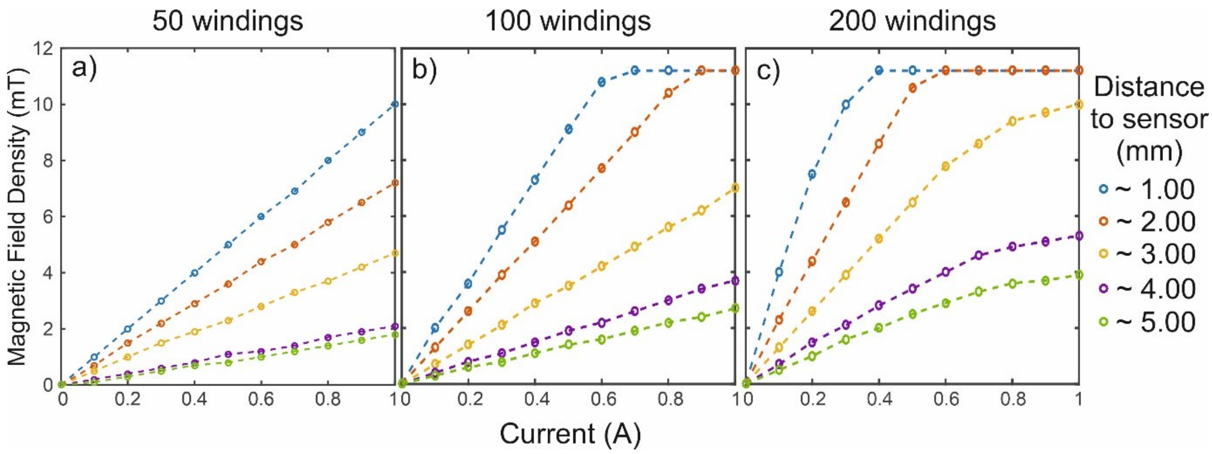

Figure S2: Characterisation of field strength of a single electromagnet tweezer. a) Magnetic field density (mT) vs. Current (A) for tweezer with 50 windings. b) for tweezer with 100 windings. c) for tweezer with 200 windings at varying distance to hall effect sensor. With increasing current, magnetic field density increases proportionally in similar projections for the three different coil windings. 200 windings was decided as the most optimized number of windings for the size restrictions and applications.

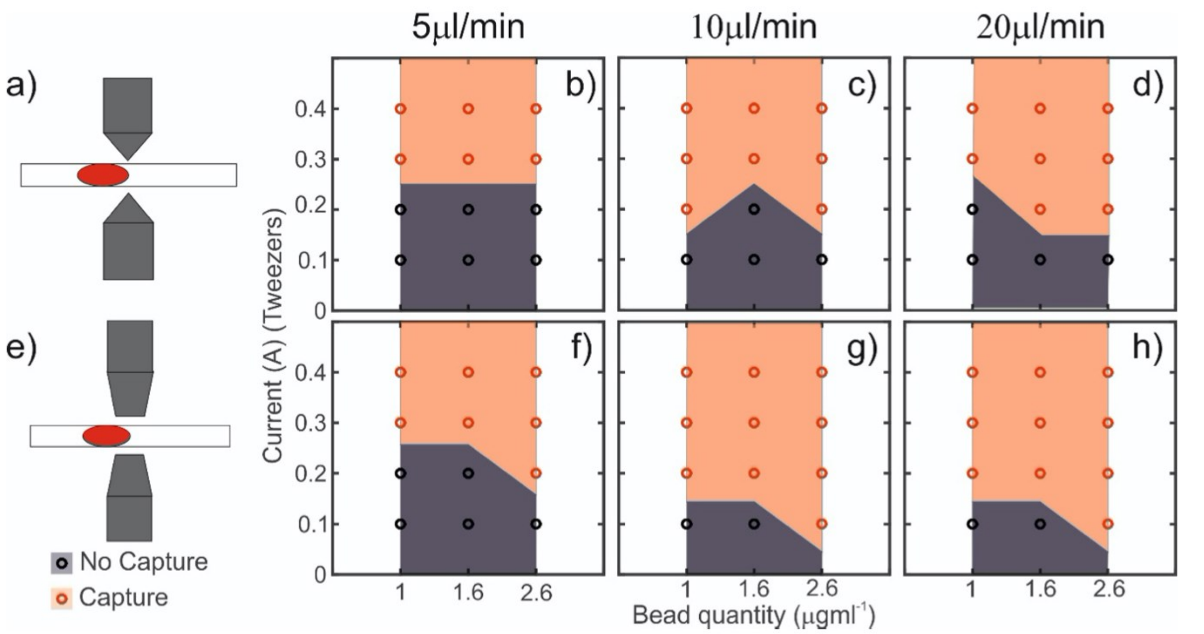

Figure S3 Capture of magnetic beads in the basic trap for a range of different currents (0.1 A to 0.7 A), flow rates (5, 10 and 20 µl/min) and different bead quantities (1-2.6 µg/ml) for sharp (a-d) and blunt (e-h) tip tweezers.

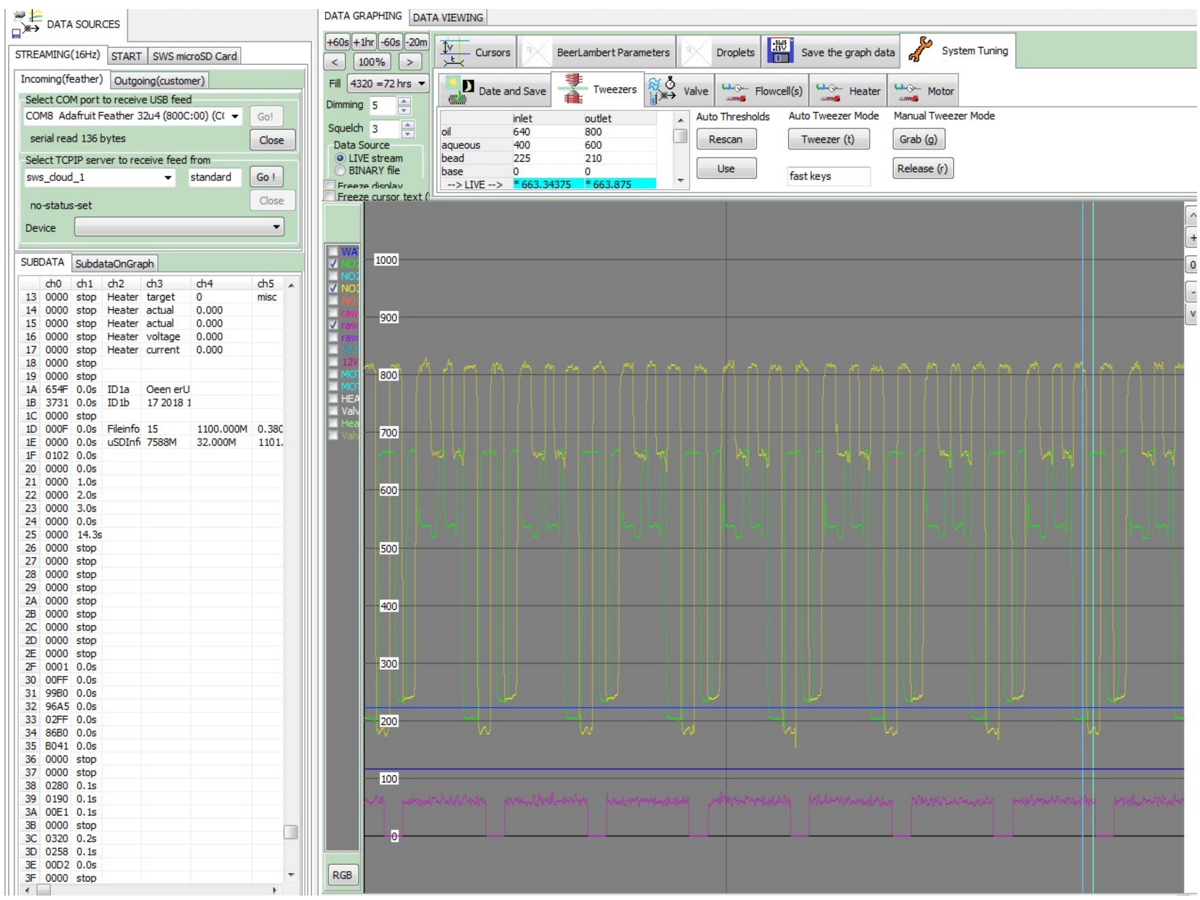

Figure S4: Screenshot of the GUI reporting automatic magnetic tweezer activity over time. Tweezer parameter control shown at the top, USB connection and debug readings are shown on the left side. The data traces from photodetector 1 (PD 1), PD 2 and coil current can be seen in the middle which are yellow, green and purple *respectively. The data traces demonstrate 8 complete droplet trains passing through the light gates and the successful automatic switching (purple line) of the electromagnetic tweezers.*

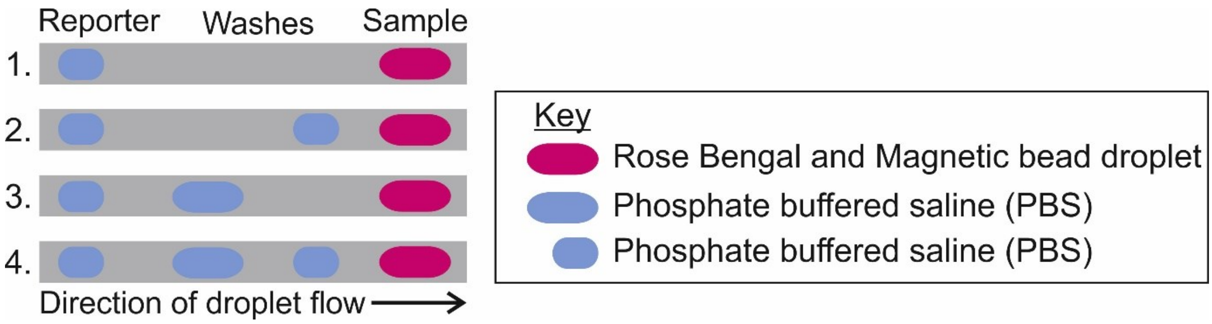

Figure S5: Different droplet sequences generated for testing washing efficiency of magnetic tweezers. In train 1 (row1) the beads were simply captured, removed from the dye droplet (the rightmost one) and re-dispersed, without washing, in the last PBS droplet (the leftmost one) which represents the indicator droplet. For trains 2&3 (row 2 & 3) the beads were washed in a single droplet of 550 nL and 1060 nL, respectively. In train 4 (row 4), two wash droplets totalling 1610 nL volume were used.