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Closed-loop feedback control for microfluidic systems through automated capacitive fluid height sensing†

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Precise fluid height sensing in open-channel microfluidics has long been a desirable feature for a wide range of applications. However, performing accurate measurements of the fluid level in small-scale reservoirs (<1 mL) has proven to be an elusive goal, especially if direct fluid-sensor contact needs to be avoided. In particular, gravity-driven systems used in several microfluidic applications to establish pressure gradients and impose flow remain open-loop and largely unmonitored due to these sensing limitations. Here we present an optimized self-shielded coplanar capacitive sensor design and automated control system to provide submillimeter fluid-height resolution (~ 250 μm) and control of small-scale open reservoirs without the need for direct fluid contact. Results from testing and validation of our optimized sensor and system also suggest that accurate fluid height information can be used to robustly characterize, calibrate and dynamically control a range of microfluidic systems with complex pumping mechanisms, even in cell culture conditions. Capacitive sensing technology provides a scalable and cost-effective way to enable continuous monitoring and closed-loop feedback control of fluid volumes in small-scale gravity-dominated wells in a variety of microfluidic applications.

I. Introduction

Devices for precise fluid handling at the micro- and meso-scale, with characteristic lengths (λ) < 10 cm, are becoming increasingly common across a variety of fields, including biological, pharmaceutical and medical research.¹ Most microfluidic systems require at least one pump to produce flow, as well as open- or closed-channels or reservoirs to supply, store and collect fluid. Such devices can be categorized in terms of their primary pumping action as mechanical, chemical, surface-, force-field and mixed systems.¹⁻³ Examples of mechanical pumping at these scales include syringe-pumps, microelectromechanical (MEM) pumps,⁴ magnetically actuated pumps,^{5,6} vacuum or pressure-driven pumps, peristaltic pumps and centrifugal pumps.^{1,7,8} Chemically-induced flow can be seen in osmotic and effervescence pumps, as well as systems using electrochemical reactions.^{1,9-11} Surface-effect pumps include all devices making use of spontaneous capillary forces and substrate wicking to passively induce fluid

flow.¹² Further, force-field pumps are usually defined as non-mechanical including electro-osmotic,^{3,13} electrowetting,¹⁴ poroelastic,¹⁵ electrodynamic, thermocapillary and gravity-driven pumps.^{1,3,16,17}

Common metrics for performance comparison in microfluidic applications include: operational fluid volumes, flow rates, directionality, persistence time, flow pattern control, fluid capacity, recirculation, optical accessibility, power requirements, heat transfer, and cost of implementation.¹ Furthermore, criteria such as imposing minimal mechanical, chemical and electrical stress during cell or particle handling, as well as providing material/surface stability against fouling or analyte absorption may also be important to consider.¹ Understanding the expected performance and inherent limitations associated with each fluid handling and pumping scheme is essential. For example, mechanical pumps connected to open wells or channels do not usually deliver robust steady-state flows for long periods of time as they are bounded by a specific input volume (*e.g.* syringe pumps), rely on pulsatile flow (*e.g.* peristaltic, pneumatic and MEM-based pumps),^{11,12,18–21} are transient in nature (*e.g.* gravity-driven,²² capillary-driven¹² and centrifugal pumps²³), or are prone to performance variation (*e.g.* fabrication errors, use-induced stress). Thus, it remains desirable to identify novel approaches to provide robust, scalable and cost-effective monitoring and control of both pumping performance and fluid volume allocation in microfluidic devices.

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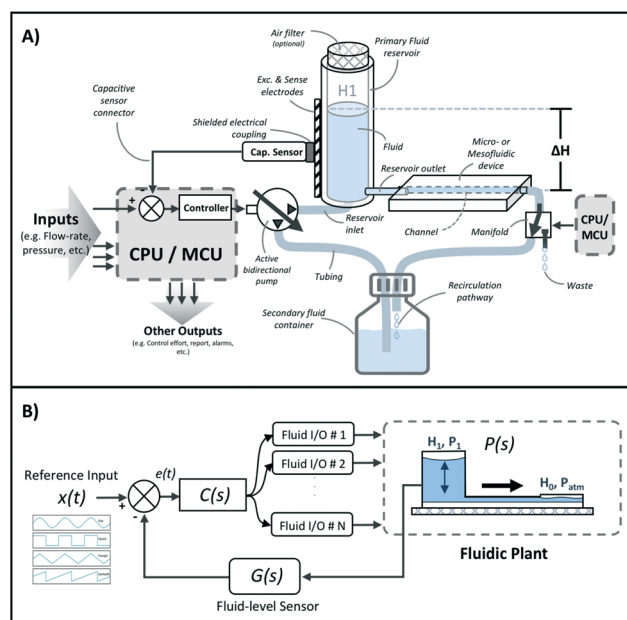
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Traditional fluid-level sensing technologies such as mechanical floaters, displacers, bubblers, magnetic level gauges, magnetostrictive level transmitters and ultrasonic distance sensors are all currently inadequate or unreliable at the scale of most micro- and mesofluidic devices.²⁹ More recent options such as in-line flow sensors and differential pressure transducers have become increasingly smaller and sensitive over the past years, and are now used in several microfluidic applications.^{30–32} Nonetheless, these and other available sensing techniques are limited in that they require some degree of fluid contact, which may be undesirable in many sterile biological applications. Non-contact fluid-level sensing technologies; on the other hand, have only become popular in a limited number of large-scale industrial applications including monitoring and handling of large volumes of hazardous fluids. From direct visualization of fluid height (*e.g.* using optical monitoring through translucent windows), to time-of-flight (TOF) measurements (*e.g.* ultrasonic, laser and radar transmitters), and capacitive fluid sensing;³³ non-contact fluid-level sensors are now an increasingly common strategy to control macrofluidic systems where relevant height changes happen in the order of tens of centimeters. From these non-contact options, capacitive fluid sensing is perhaps the most attractive due to scalability and low cost. In the case of microfluidic applications, several groups have previously demonstrated the use of capacitive sensing for binary detection of droplets³⁴ and droplet volume quantification.^{17,35} Furthermore, other research groups have used this technology to develop contact-based in-line pressure sen-

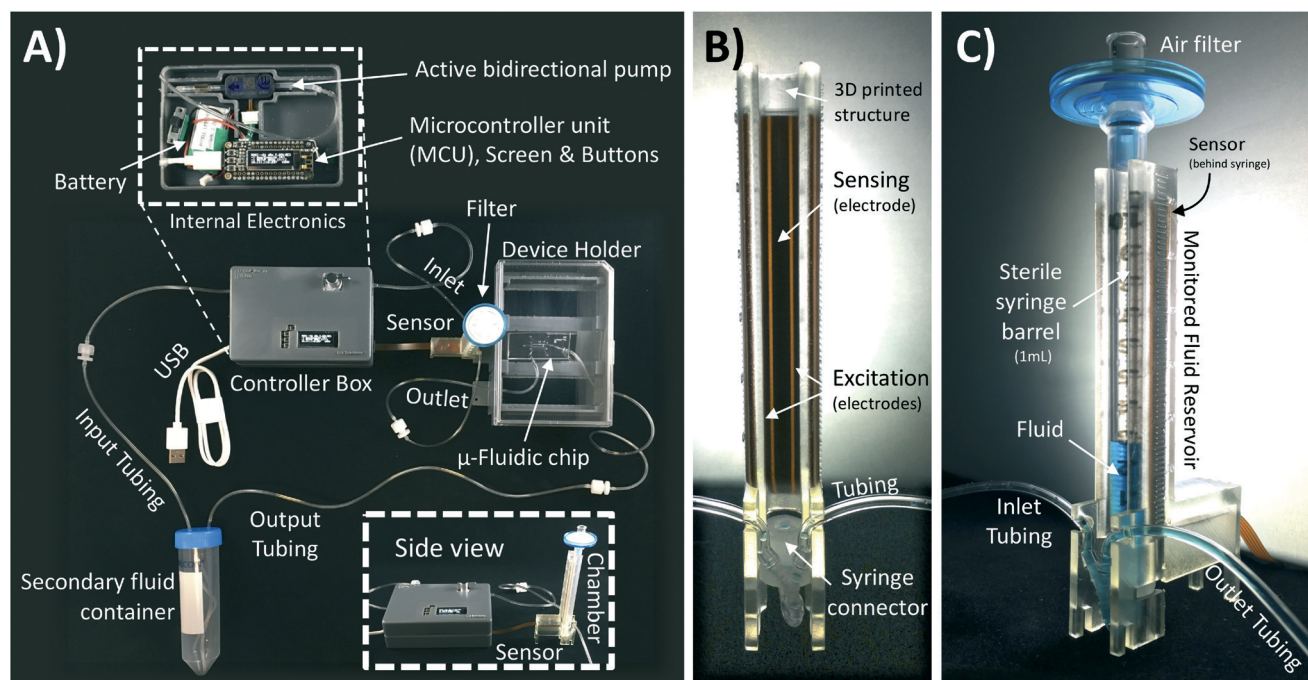
A schematic of the assembled testing setup developed to demonstrate the capabilities of our capacitive fluid-level sensor can be seen in Fig. 1A. The elements depicted in this system include a capacitive-fluid sensor, a hydrostatic chamber, a microcontroller unit, a bidirectional pump, a microfluidic device and a recirculation container, as well as tubing and connectors. In this testing setup, the fluid level in the primary fluid reservoir is set to the desired position (H1) to act as a hydrostatic chamber, which drives gravity-driven flow through a meso- and/or microfluidic chip. This chip is located inside a device holder to be connected to the fluidic



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II.a.1 Hydrostatic fluid chamber. Fig. 2C shows the assembled hydrostatic chamber with capacitive sensing used in the feedback-controlled gravity-driven setup. In this assembly, a

II.a.2 Capacitive fluid-level sensor for microfluidics. Our optimized capacitive fluid-level sensor for microfluidic applications consists of self-shielded coplanar electrodes connected to an AD7746 24 bit Σ - Δ capacitance-to-digital converter in differential mode (Analog Devices, Norwood, USA). Fig. 3A illustrates the layout of this sensor in conjunction with the monitored fluid reservoir. In our design, two pairs of excitation



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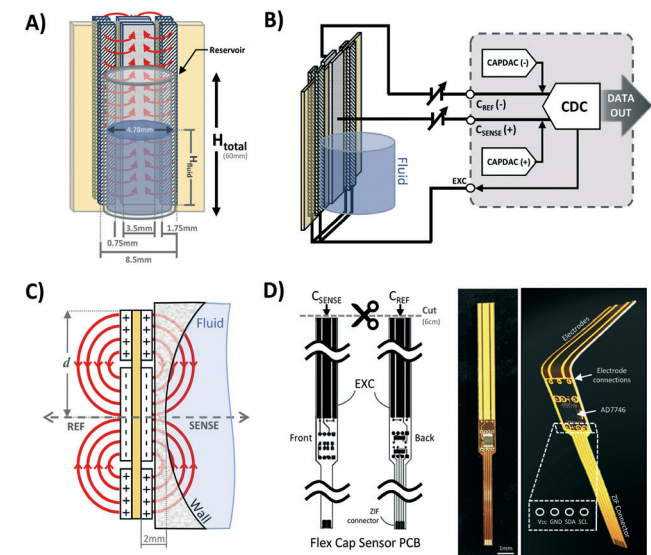


Fig. 3 Design of the capacitive fluid-level sensor for microfluidic applications. A) Planes filled with diagonal patterns are excitation electrodes, while planes with dotted patterns are sensing and reference electrodes. The yellow plane is a middle polyimide dielectric layer, while the blue cylinder represents the monitored fluid reservoir in experimental setup. B) Shows the sensing, reference and excitation electrodes connected to their respective terminals on the CDC chip. C) Cross-sectional detail of charge distribution and fringing capacitance fields (red arrows) among sensing, reference and excitation electrodes. The relative position of the fluid reservoir wall and the sensing electrode is also shown. D) Flex-PCB layout and images of fabricated sensor. CDC = capacitance-to-digital converter; CAPDAC = programmable on-chip digital-to-capacitance converter; GND = ground supply; PCB = printed circuit board; SDA/SCL = I²C communication pins; Vcc = power supply.

electrodes (diagonal pattern in Fig. 3A and B) are positioned around two sensing electrodes (dotted pattern) to measure fringing capacitance in the direction of the fluid as shown in Fig. 3C. We refer to this electrode design as excitation-sensing-excitation/inter-digitated arrangement (ESE-ID).

Two symmetrical gaps separate the excitation electrodes from the central sensing electrodes. The gap length is a parameter that affects the penetration depth of the most sensitive fringing pathways in other types of capacitive sensors using coplanar electrode arrangements.³⁸ Thus, L_{gap} can be iteratively adjusted to achieve optimal sensing in other applications with different fluid-sensor wall thicknesses. For our hydrostatic chamber prototype in Fig. 3A, $L_{\text{gap}} = 0.75$ mm was heuristically determined (from several design iterations) as sufficiently small to allow for capacitive sensing using our electrode geometry.

The AD7746 chip is connected to the front sensing electrode *via* the $C_{\text{sense}}(+)$ terminal, while the back sensing electrode is connected to the reference terminal $C_{\text{ref}}(-)$ to allow for differential measurements. The excitation electrodes of both sensing and reference planes are connected to the same excitation (EXC) terminal. The differential mode of the AD7746 chip was selected to maximize the robustness of capacitance measurements, while the addition of a symmetri-

cal reference arrangement at the back of the sensing layout was designed to maximize signal-to-noise ratio. This effect can be explained by referring to the expected fringing capacitance pathways on the presented mirrored electrode design (Fig. 3A), which shows a self-shielding effect within the sensor.

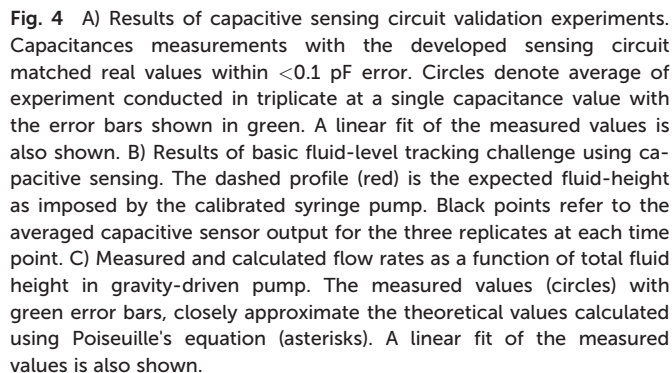
In commonly used capacitive sensing instruments, the region separating the electrodes from a target fluid is usually made of materials with low electrical permittivity (ϵ) such as plastic, glass or air.³⁹ This renders the capacitance due to plastic structures and air small as long as $\epsilon_{\text{Air}} < \epsilon_{\text{Plastic}} \ll \epsilon_{\text{Fluid}}$ is maintained. Thus, in our system the capacitance attributable to the region next to the fluid can be approximated to the total capacitance detected at the $C_{\text{sense}(+)}$ terminal. Furthermore, in previously described coplanar sensor designs,³⁹ the reference electrodes are usually situated in the same plane as the main sensing electrode (next to a region constantly filled with fluid). This traditional configuration simplifies the sensor compensation for different kinds of fluids and temperature changes, but it also brings several limitations in terms of footprint, minimum detectable fluid volume and achievable signal-to-noise ratio.

Our sensor prototype is implemented using a 3-layer flexible printed circuit board (Flex-PCB) with total thickness of 0.2 mm. Electrodes are defined as 0.5 oz copper layers with 17.5 μm thickness, while a 55 μm polyimide film was used as dielectric. A layer of dielectric film lies between the mirrored electrodes, as well as at the top and bottom of the sensor to protect the conductive material from corrosion (Fig. 3D). Connection traces between sensing circuit and electrodes use 0.127 mm traces. The electrodes are made with an original length $L_{\text{total}} = 20$ cm, and then are cut at the top end to fit the 6 cm fluid reservoir. This design allows for use of the sensor in longer hydrostatic chambers with minimum modification of the sensor design. The separation of the mirrored reference electrode from the main sensing plane was achieved using a 75 μm adhesive-polyimide-adhesive dielectric layer. Since increasing the distance between the central electrode and the fluid is expected to reduce sensitivity of the capacitive sensor, we aimed to reduce this separation considering the minimal resin thickness achievable with our 3D printing equipment without producing significant wall defects (~ 0.45 mm). This structural separation, in addition to the sensor's outer polyimide dielectric layer (55 μm), and the 1 mL syringe's wall thickness added to a minimum electrode-fluid separation for our testing setup of approximately 2 mm (shown in Fig. 3C). As with other capacitive sensing systems, the materials separating the electrodes from the fluid (*e.g.* syringe wall) are not expected to significant effects on the sensor's performance as long as the electrical permittivity of these materials are at least one order of magnitude less than that of the used fluid ($\epsilon_{\text{Plastic}} \ll \epsilon_{\text{Fluid}}$). Many other potential sources of variability affecting the performance of our capacitive sensor, such as electrode positioning, temperature changes, defects in structural materials and properties of the handled fluid, can all be automatically accounted for

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II.a.7 Structural and other components. Structural elements such as the sensor/chamber holder, controller box and microfluidic device holder were fabricated from stereolithographic (SLA) resin using a Form 2 3D printer (Formlabs, Somerville, USA). The hydrostatic chamber and sensor holder are made from clear resin to allow for direct optical visualization of the monitored fluid front for validation purposes. The ESE-ID flexible capacitive sensor was positioned at the desired distance from the fluidic chamber using a guiding slot within the 3D printed structure, and was permanently fixed in this position using PDMS. The design and fabrication files of these structural components are also included in the ESI.†

II.b.3 Gravity-driven flow rate validation. To validate flow rates established using a given hydrostatic pressure (*i.e.* fluid



height), we connected our pump to a 50 cm long silicone tube with inner diameter of 0.79 mm (1/32") and measured the mass of 1× PBS displaced over a one minute duration ($n = 3$) using a laboratory grade scale. The Poiseuille equation was used to calculate the expected flow rate given a hydrostatic pressure difference $\Delta P = 8 \mu LQ/(\pi R^4)$, where ΔP is the hydrostatic pressure difference, L is the length of the tubing, μ is the dynamic viscosity of PBS which is assumed to be close to that of water (8.9×10^{-4} Pa s at 25 °C), Q is the volumetric flow rate and R is the inner radius of the tubing. Additionally, the hydrostatic pressure was calculated as $\Delta P = \rho gh$, where ρ is the density of PBS which is approximately that of water (1 kg m^{-3}), g is gravity and h is the total fluid height above the outlet feeding to the collection tubes used for weight measurement. In order to achieve high flow rates, we

added an additional 53 mm offset to the fluid column by placing the collection tubes below the gravity driven pump outlet. The obtained flow-rate measurements (in triplicate) were compared to expected values using Poiseuille's equation as shown in Fig. 4C.

II.b.4 Characterization of fluid-type dependency. As described earlier, the gain of our capacitive sensor design is expected to change depending on the charge distribution resulting from the conductivity and the electrical permittivity of the target fluid. Therefore, changes in charged solute concentration can affect these readings significantly. To characterize such effect, we conducted a titration experiment in triplicates using 1 $\times$, 0.5 $\times$, 0.25 $\times$, 0.125 $\times$ and 0.0625 $\times$ PBS, with deionized (DI) water and a steel inserts of known lengths as controls. Two inserts were made by cutting 10 and 30 mm sections from a tight-tolerance multipurpose O1 tool steel rod with 0.1750" diameter (McMaster Carr, Robbinsville, USA). The associated capacitance value from all fluid conditions and materials was recorded at two fixed fluid heights: lower bound ($H_0 = 10$ mm) and upper bound ($H_1 = 30$ mm) as shown in Fig. 5A. To confirm experimental results, we also computed the electric displacement flux density (D) around relevant target fluids (*i.e.* 1 $\times$ PBS solution, DI-water), *via* finite element analysis (FEA) using the AC conduction field solver of ANSYS Maxwell software (ANSYS, Inc., Canonsburg, USA) as shown in Fig. 5D and E. In these simulation, the two middle electrodes were fixed to zero voltage, and the four electrodes on the side were excited with a sinusoidal voltage of 5 V amplitude at an excitation frequency $f_{\text{EXC}} = 32$ kHz. These conditions are analogous to the settings used by the AD7746 24 bit $\Sigma\Delta$ capacitance-to-digital converter in the developed sensor.

II.b.5 Flow-rate tracking and calibration of external pneumatic micropumps. Since accurate contactless assessment of flow in open-channel microfluidics is a valuable potential application for the present technology, we conducted a series of proof-of-concept experiments to demonstrate flow-rate extraction and open-loop pumping calibration based on continuous fluid-level monitoring. The same previously described fluid-height tracking methodology (at 37 °C and 95% humidity) was used, except that in these experiments, the calibrated syringe pump was programmed to impose specific flow-rate profiles (instead of volumetric changes) ranging from 100 nL min⁻¹ to 1 mL min⁻¹. These conditions included constant, ramp and intermittent flow-rates in input and output mode. Fluid-height changes over time were measured in triplicates and compared to theoretical flow estimates using the pre-programmed flow-rate parameters imposed by the calibrated syringe pump. Extraction of flow-rate was approximated by generating a linear fit of fluid volume change over time every 1000 samples ($t = 10$ s). Experiments were limited to a maximum input/output volume ($V_{\text{max}} = 0.5$ mL) and a maximum experimental period ($T < 400$ s). The results are shown in Fig. S3 of the ESI.†

In a subsequent experiment, this height-based flow-rate tracking methodology was used to characterize and calibrate

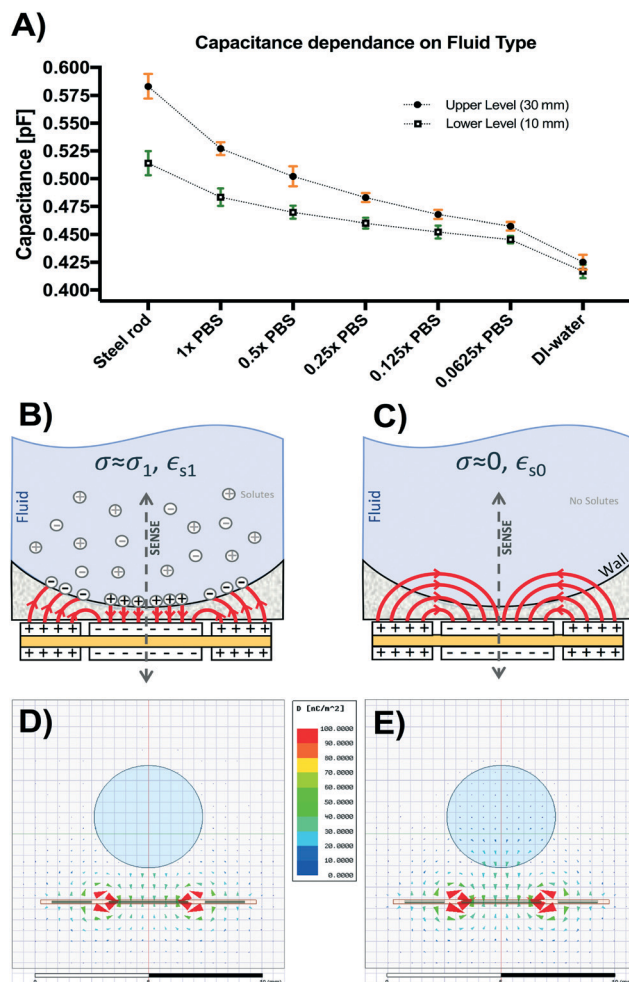


Fig. 5 A) Results on dependence of capacitive sensing on fluid type. Use of fluids with high concentration of solutes forming free ions lead to higher capacitance measurements and ranges. Error bars representing standard deviation of upper measurements ($h = 30$ mm) are shown in orange, while error bars of the lower measurements ($h = 10$ mm) are shown in green. B) Capacitive fringing model for electrolytic fluids with free ions. C) Capacitive fringing model for non-conductive fluids with high dielectric constant. Fringes at the fluid-wall interface exhibit a change in angle towards the symmetry axis (not shown) depending on properties of wall material and fluid. D) Finite element analysis (FEA) of the electric displacement flux density for $1\times$ PBS target fluid. E) FEA simulation of electric flux density for DI-water as target. In both D) and C) the blue circle is the target liquid, and the outer box is plastic material ($\epsilon_r = 2.7$, $\sigma = 0$ S m^{-1}) following the same dimensions as the physical prototype chamber. Outside the plastic box region there is air. The thin orange contour surrounding the sensor electrodes is a polyamide film ($\epsilon_r = 4.3$, $\sigma = 0$ S m^{-1}). The color of the vectors represents the magnitude of the flux density D (nC m^{-2}) shown in the middle color bar. The size of the arrows is proportional to the magnitude of the flux density to facilitate interpretation.

two presumably identical open-loop pneumatic diaphragm micropumps. These micropumps were set to supply and extract fluid from the same monitored reservoir at $1 \mu\text{L s}^{-1}$. These pumps were fabricated in acrylic using a CNC mill and a thin polyurethane membrane according to a previously reported protocol.⁴³ Both pumps were actuated for 40 min at 1 Hz (stroke volume = $1 \mu\text{L}$) under 37°C and 95% humidity

37 °C, 5% CO₂ and 95% humidity). Presence of flow through the microfluidic device was confirmed by observing dripping fluid within the secondary recirculation container. This experiment was conducted in triplicate and the fluid height was also verified using video recordings of the fluid front to assess drift as shown in Fig. 7A.

After verifying adequate closed-loop performance for a constant fluid-level set-point, a dynamic fluid-level target experiment was conducted under similar experimental conditions. However, in this case the target was pre-programmed to be a dynamic fluid-level profile stored in non-volatile memory of the MCU. This profile was a 40 min sequence including constant, sine, triangular, saw tooth and step waveforms (two periods each). This experiment was also conducted in triplicate within an incubator (37 °C at 95% humidity) using video recordings to verify location of the fluid front. Results are shown in Fig. 7A.

II.b.7 Cell culture experiments. In order to show biocompatibility of the setup, a 24 hour cell culture experiment was performed using iPSC-derived vascular endothelial cells as an example cell type. In this test, an IBIDI μ -Slide VI 0.4 channel slide (IBIDI, Martinsried, Germany) was coated with human fibronectin (Life Technologies, Woburn, USA) at a concentration of $30 \mu\text{g mL}^{-1}$ for 1 hour at room temperature. Induced Pluripotent Stem Cell (iPSC) derived endothelial cells (CDI, Madison, WI) were seeded in all the channels, allowed to adhere for 3 hours then excess cells were washed away using two successive media changes. Results are shown in Fig. 8. The device was cultured for 24 hours in the supplier-recommended media under standard incubator conditions.

II.b.6 Validation of closed-loop control of gravity driven pump. The performance, robustness and dynamic range of the proposed closed-loop feedback control system was characterized using the entire monitored setup as illustrated schematically in Fig. 1A. A single-channel microfluidic device made in a PDMS (length = 50 mm, width = 1 mm and height = 0.2 mm) was connected to the outlet of the hydrostatic chamber with capacitive sensing. After the chip was connected to the setup, sensor calibration and flow testing was performed to assess the emptying time constant of the hydrostatic chamber given the fluidic resistance from the connected microfluidic chip. After this, a closed-loop feedback control mode was activated to automatically control fluid input/output from the secondary piezoelectric pumps to maintain a target height ($\Delta H = 30$ mm). For this experiment, the hydrostatic chamber was filled with $1\times$ PBS, and was monitored over a period of 48 hours inside an incubator (at

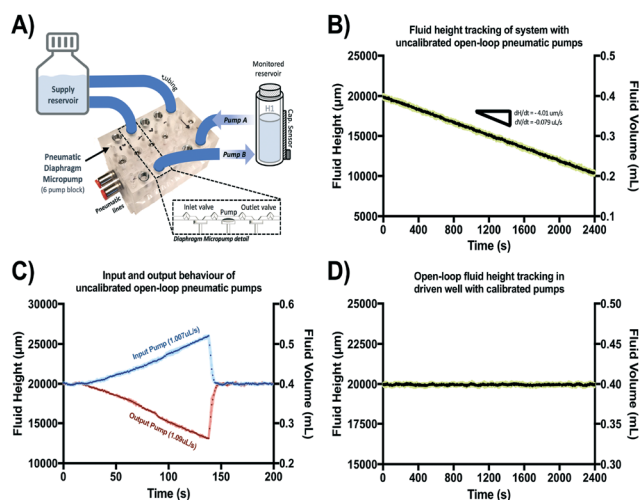


Fig. 6 A) Testing block with six parallel pneumatic diaphragm micropumps. Two pumps were used to drive fluid in and out the monitored fluid reservoir. B) Fluid-height change over time (40 min) as measured by the capacitive sensor in the system driven by unbalanced input/output micropumps. C) Overlay of isolated experimental input and output behavior of each pneumatic diaphragm micropump. Blue profile denotes fluid-height over time produced by pump A (input) alone, while red profile shows the same for pump B (output). D) Fluid-height change over time (40 min) in the same system after micropump calibration using extracted flow-rate from capacitive sensing readout. Black points denote average for the three replicates at each time point. Standard deviation is shown in light green.

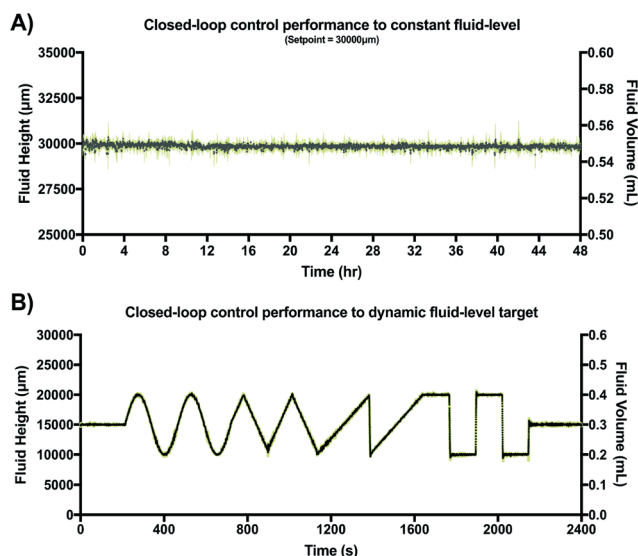


Fig. 7 A) Results of closed-loop feedback control for a constant set point ($\Delta H = 30$ mm). Black points refer to the averaged capacitive sensor output for the three replicates at each time point. Error bars are shown in green. B) Results of closed-loop feedback control for a dynamic set point using constant, sine, triangular, saw tooth and step waveforms. Black points refer to the averaged capacitive sensor output for the three replicates at each time point. Error bars representing standard deviation are shown in green.

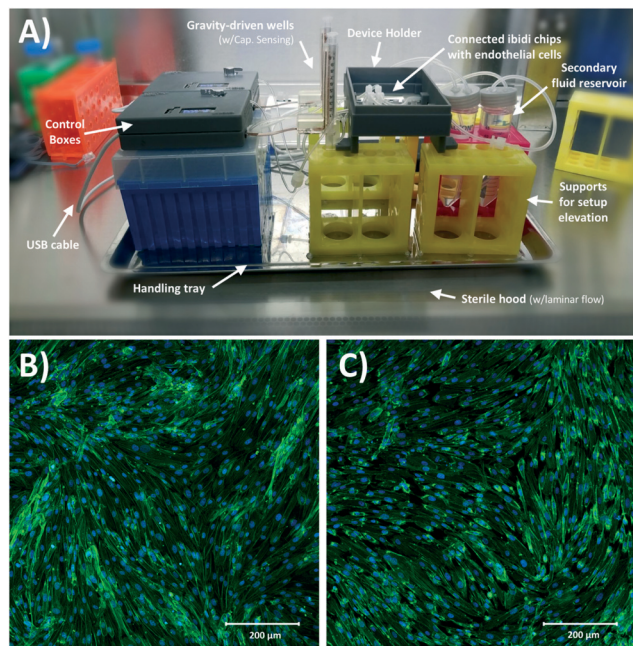


Fig. 8 A) Closed-loop gravity-driven setup used for cell-culture experiment. B) Confocal microscopy of endothelial cells cultured under static conditions as control. C) Confocal microscopy of endothelial cells cultured under 4 cm of closed-loop gravity-driven flow. Both cultures present with no observable differences, which suggests appropriate biocompatibility of the system.

After assembly, the gravity-driven setup was sterilized by circulating 70% ethanol through the entire fluidic circuit for 5 min. Ethanol was removed by air drying within a sterile hood and then flushing with two cycles of sterile DI water. After sterilization, the seeded IBIDI μ -Slide VI microfluidic channels were connected to the gravity-driven setup within a sterile hood. The hydrostatic chamber was then programmed to maintain a constant 40 mm fluid-height (392 Pa inlet pressure) to drive flow through the chip which had its outlet at atmospheric pressure through the dripping recirculation circuit. Laminar flow was assumed given the imposed pressure gradient and the rectangular design of the IBIDI device channel (length = 17 mm, width = 3.8 mm and height = 0.4 mm). The Poiseuille equation was used to calculate the expected flow rate given a hydrostatic pressure difference $Q = \Delta P/R$; where Q is the volumetric flow rate, ΔP is the hydrostatic pressure difference (392 Pa for a 40 mm media height) and R is the total resistance of the fluidic circuit ($R_{\text{channel}} + R_{\text{tubing}}$). For the IBIDI channel, which has a rectangular cross section, the fluidic resistance can be calculated as:^{44–46}

$$R_{\text{channel}} = \frac{12\mu L}{wh^3 \left(1 - 0.63 \frac{h}{w}\right)} = 6.21 \times 10^8 \text{ Pa s m}^{-3}$$

where μ is the dynamic viscosity of media which was assumed to be close to that of water (6.94×10^{-4} Pa s at 37 °C), L is the length of the channel, w is the width and h is the

height. For the tubing, which has a circular cross-sectional area with inner diameter 1/32 inch and total length of 20 cm, the fluidic resistance can be calculated as:⁴⁵

$$R_{\text{tubing}} = \frac{8\mu L}{\pi r^4} = 1.48 \times 10^{10} \text{ Pa s m}^{-3}$$

where r is the inner radius of the tubing, L is the length of the tubing, μ is the dynamic viscosity of media which was assumed to be close to that of water (6.94×10^{-4} Pa s at 37 °C). Using this information, we calculated the flow rate Q to be $2.64 \times 10^{-8} \text{ m}^3 \text{ s}^{-1}$ or 1.59 mL min^{-1} at this specific height. The wall shear stress, τ , imposed on the endothelial cells under this flow rate can be calculated as:⁴⁷

$$\tau = \frac{6\mu Q}{h^2 w} = 1.8 \text{ dyne per cm}^2$$

here τ is in dyne per cm^2 , μ is in poise, Q is in $\text{cm}^3 \text{ s}^{-1}$ and h and w are in cm and are the height and width of the IBIDI channel respectively. After 24 hours of culture, cells were fixed with 4% PFA, stained with DAPI and rhodamine and imaged under an EVOS inverted microscope (Life Technologies, Woburn MA, USA) with a 20 $\times$ objective.

II.c Statistical analysis

All validation experiments were conducted in triplicates. Error bars represent standard deviation in all figures. Calculations and plots were generated with Graphpad Prism 7 (GraphPad Software Inc.; La Jolla, USA).

III. Results & discussion

III.a Accurate capacitance fluid sensing and fluid tracking

The results of the initial characterization experiments used to assess the accuracy of capacitance readings and basic fluid level tracking capabilities of our sensing circuit are shown in Fig. 4. Measured values of known capacitors directly connected to the $C_{\text{sense}(+)}$ and C_{exc} terminals in our sensing circuit appear to match those of the E4981A capacitance meter within <0.1 pF error (Fig. 4A). Readings from the capacitive sensing circuit also appear to be linearly related to those obtained from the calibrated reference meter suggesting appropriate implementation of the sensor at the PCB level. Fig. 4B shows the aggregated results for three basic fluid-level tracking challenges using the fluid chamber with our proposed excitation-sensing-excitation inter-digitating capacitive sensor arrangement (ESE-ID) as depicted in Fig. 2C. The dashed red profile in Fig. 4B is the known fluid-height as imposed by the calibrated syringe pump. Black data points refer to the averaged capacitive sensor output for the three replicates at each time point. The average standard deviation across all samples in this experiment was <250 μm when compared to the fluid height imposed by the calibrated syringe pump. Fig. 4C shows both measured and estimated flow-rates in our gravity-driven pump as a function of set



fluid height. While experimental measurements appear to closely follow the theoretical values predicted by Poiseuille's equation, there also seems to be a slight overestimation of flow rate near the most upper level of the capacitive electrodes. While not fully explored in this work, such effect appears to be caused by an unmolded fringing capacitance at this boundary, which may be further corrected *via* software using a linear calibration curve. Fig. 4C shows an apparent minimum fluid height of 54 mm for this specific test; nonetheless, this included a 53 mm offset from the measuring outlet as described in section II.b.3. Considering this offset, it can be recognized that the actual minimum fluid height controlled for this test corresponded to approximately 1 mm. Despite the fact that our capacitive sensor was able to detect complete emptying of the fluid chamber, we selected this minimum fluid level in order to avoid partial emptying or significant meniscus effects such as edge pinning during this test. Error bars are shown in green for all measurements in Fig. 4. If further error reduction is required, averaging techniques and low-pass filtering may also be implemented in longer experimental time scales.

III.b Capacitance readings depend on fluid conductivity

Fig. 5A shows the results from the titration experiments conducted to characterize performance of our capacitive sensor based on fluid conductivity. Capacitance readings for upper ($H_1 = 30$ mm) and lower ($H_0 = 10$ mm) levels were smallest and closest together for deionized (DI) water and increased as electrolyte concentration increased to $1\times$ PBS (conductivity @25 °C = 1.6 S m^{-1}). Small steel rods of both lengths were also placed inside the monitored reservoir to assess signal in the presence of a known perfect conductor. Higher dilutions ($0.5\times$ to $0.0625\times$ PBS) led to a range reduction and lower absolute capacitance, reaching a minimum in DI-water lacking solutes (conductivity @25 °C = $0.055\text{ }\mu\text{S cm}^{-1}$).

This behavior can be explained by the model shown in Fig. 5B and C. In the case of water with dissolved solutes forming free ions (Fig. 5B), a large number of sensing fringing paths become terminated near the fluid-reservoir boundary leading to higher capacitance measurements. Conversely, in the case of pure water (Fig. 5C), fringing capacitance takes longer uninterrupted paths through the bulk of the fluid, generating smaller capacitance measurements. Fig. 5D shows the simulated displacement flux density around the $1\times$ PBS solution ($\epsilon_{\text{r,PBS}} = 80$, $\sigma_{\text{PBS}} = 1.45\text{ S m}^{-1}$). The charge relaxation time of the $1\times$ PBS solution is $\tau_{\text{PBS}} = \epsilon_{\text{r,PBS}} \epsilon_0 / \sigma_{\text{PBS}} = 488\text{ ps}$, which corresponds to a break frequency $f_{\text{PBS}} = 2.04\text{ GHz}$. This break frequency is orders of magnitude higher than the 32 kHz sensor excitation frequency used for the sensor.

Since $f_{\text{PBS}} \gg f_{\text{EXT}}$, the $1\times$ PBS solution behaves like a conductor, accumulating free charges on its surface and increasing the apparent capacitance seen from the sensor electrodes. The induced surface charges terminate the flux density vectors, and therefore the flux density vectors are invisible inside the $1\times$ PBS solution in Fig. 5D. For the case of

DI-water ($\epsilon_{\text{r,DI}} = 80$, $\sigma_{\text{DI}} = 5.5\text{ }\mu\text{S m}^{-1}$), Fig. 5C shows the field lines schematically, while Fig. 5E shows the simulated flux density field. The charge relaxation time of the DI-water is $\tau_{\text{DI}} = \epsilon_{\text{r,DI}} \epsilon_0 / \sigma_{\text{DI}} = 129\text{ }\mu\text{s}$, which corresponds to a break frequency $f_{\text{DI}} = 7.76\text{ kHz}$. Since $f_{\text{DI}} < f_{\text{EXT}}$, the DI-water behaves like an insulator, which explains why the flux density vectors induced inside the DI-water follows the pattern shown in Fig. 5C and E. These results show that calibration is required to adjust for potential differences in fluid conductivity. Further sections of this work make use of sensors calibrated for $1\times$ PBS and culture media.

III.c Accurate flow-rate tracking and calibration of external pneumatic micropumps

Fig. S3 of the ESI† shows the results of an extended series of experiments carried out to verify if accurate flow-rate calculations were achievable from analyzing fluid-height changes over time using our capacitive sensor. From these experiments, it was confirmed that a variety of flow-rate conditions can be inferred based on this sensor's output and that these values correspond to the programmed settings in the calibrated syringe pump used to impose flow. Fig. 6 shows the results of the characterization and calibration experiment of two open-loop pneumatic diaphragm micropumps (Fig. 6A) feeding and extracting fluid from the same monitored reservoir at a nominal rate of $1\text{ }\mu\text{L s}^{-1}$. Over the course of 40 min, a 0.2 mL decrease in fluid volume was observed in the monitored reservoir (Fig. 6B), showing that the output pump flow-rate was slightly greater than the input pump flow rate despite being actuated at the same frequency.

Independent flow-rate characterization of each of these micropumps using our capacitive sensor revealed an 8.3% mismatch between the output and input micropump flow rates. This difference explains the observed decreasing height drift and is well within the 10–15% expected error usually reported for these type of pumping systems.²⁴ After recalibration of the actuation frequency for both micropumps based on these readings, fluid-height drift appeared to be corrected for the same 40 min period as compared to the uncalibrated behavior. In general, long-term drift is expected to be present for a wide range of open-loop micropumps, due to back pressure, use-induced stress or solute deposition may sporadically change stroke volume. Thus, our sensor could be a valuable addition to open-channel microfluidic systems requiring accurate flow control, or as a new way to assess flow-rates and perform pump calibrations on demand.

III.d Reliable closed-loop control of gravity driven pump

From available approaches for fluid handling in micro- and mesofluidic devices, gravity-driven systems have historically been considered among the most robust, simple and convenient to use.¹³ Reservoirs acting as gravity-driven pumps are relatively low-cost, can achieve a wide range of flow-rates, rarely lead to bubble stagnation and usually do not require external power to impose flow.^{13,48} However, traditional



The constant, sine, triangular, saw tooth and step waveforms were all recognizable and accurately followed with less than 5% error. Overshooting decaying oscillations were observable at the high-frequency transitions for both step-like cycles, which is characteristic of many second order systems using closed-loop feedback control as it reflects the control loop dynamics. In our augmented gravity-driven setup, fluid height appears to be a useful target variable allowing us to accurately control both pressure and flow-rate in this system. While these variables are commonly measured and used to control large-scale gravity-dominated fluidic systems (volumes > 10 L),^{52,53} this is the first time, to our knowledge, that capacitive sensing has been adapted to control such parameters in an open-well microfluidic system. Thus, we believe that previous limitations associated with sensing accuracy, miniaturization, ease of use and cost of implementation, may be addressed by our proposed sensor design.

Finally, Fig. 8A shows the assembled and sterilized gravity driven setup used for our cell culture proof-of-concept. Optical analysis of both static (Fig. 8B) and gravity-driven

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