Multiplex pressure measurement in microsystems using volume displacement of particle suspensions

Kwanghun Chung,† Hyewon Lee† and Hang Lu*

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We demonstrate a simple image-based method to measure pressure in microsystems using volume displacement of fluorescent particle suspensions. These micro pressure-sensors are composed of two layers with a poly(dimethylsiloxane) (PDMS) membrane in between: the flow layer includes a flow channel and the sensor layer contains a detection channel filled with suspensions of fluorescent particles. The pressure increase in the flow channel deflects the membrane, and this membrane deformation can be quantified by measuring the cross-sectional areas at specific focal planes. These simple sensors have the advantage that a broad sensing-range can be achieved by tuning the mechanical property and the geometry of the membrane during design and fabrication, and even simpler by tuning the focal plane or the pressure of a reference chamber while in operation. We also demonstrate here a pressure transduction scheme coupled with the image-based sensing method as a multiplex pressure measurement tool for simultaneously detecting pressures in multiple locations in a microsystem. Overall, the image-based pressure sensing method has high precision when operated in both direct and remote detection modes. Compared to conventional mechanical methods of pressure detection, this technique is inexpensive because it does not require complex off-chip equipment to quantify the pressure-dependent membrane deformation. In addition, the image analysis using the software code developed here is fast, and it generates data that are simple to interpret.

Introduction

In microfluidics and lab-on-a-chip systems, control of fluid motion is essential in almost all applications, such as on-chip detection, analysis, mixing, separation, and reactions.¹⁻⁵ Fluid flow in microsystems can be monitored through pressure measurements; conversely, fluid flow can also be driven by pressure control. In many instances, accurate pressure control is required in loading and handling biological or chemical samples in microsystems with minimum fluctuation.⁶⁻¹² For example, pressure-driven flow was used in cell loading into target microchannels for single cell studies,⁶⁻⁸ transferring *Caenorhabditis elegans* for rapid phenotyping with high resolution (applied pressure range: 2-5 psi),^{9,10} and supplying perfusion media for long-term cell culture (0.4–10 psi).^{11,12} Besides controlling the flow, pressure measurement has been used to characterize hydrodynamic resistance of microchannels for studying mechanic properties of cells. For instance, the rheological properties of red blood cells and white blood cells in the flow of microchannels were studied by measuring pressure drop variations at the outlet of the test channel (5-10 psi).^{13,14} In these examples, the response of the flow system depends on cell type, the number of cells, and drug-induced changes in mechanical properties of the cell membrane; the pressure fluctuation in the system can be used to infer these changes. In other applications, pressure is critical in the generation and

manipulation of monodisperse bubbles in a continuous liquid stream, in various reactions such as polymerase chain reaction (10 psi),¹⁵ hydrophobic–hydrophilic patterning in microchannels (0.16–1.1 psi),¹⁶ and manufacturing contrast agents for ultrasonic imaging (3–10 psi).¹⁷ Gas–liquid segmented flow is often used in the microfluidic systems to enhance mixing and transverse channel transport by inducing a recirculation motion in the liquid. In multiphase flow in microchannels, the size of the gas bubble is highly dependent on the applied pressure in the gas stream.^{18,19} Thus, pressure sensing in microfluidics is important and necessary in many applications.

From a practical point of view, however, accurate pressure measurement inside microfluidic devices is not so straightforward without impeding the system operation, because of the small feature sizes in microchannels. To achieve on-chip pressure detection, a number of measurement methods have been developed.13,20-26 A microfluidic differential manometer was used to detect pressure drop by measuring displacement of the interface of two streams,¹³ one of which is a sample flow and the other a reference fluid. The movement of the interface as a function of pressure change in the sample flow was measured by image analysis. This differential manometer can measure the rapid fluctuations of pressure, and it is suitable for the identification of target objects in flow to enable studies of physical state of individual cells; however, it requires the sample fluid to be in contact with the reference fluid, and does not measure absolute pressure. In another approach, an in-situ pressure sensor used trapped air compression to detect static pressures for both liquid and gas samples precisely. While accurate, this design requires long indicator channels for a large sensing range

School of Chemical & Biomolecular Engineering, Georgia Institute of Technology, Atlanta, GA, USA. E-mail: hang.lu@chbe.gatech.edu; Fax: +404 894 4200; Tel: +404 894 8473 † Equal contribution

and large chambers for high resolution of small pressure changes. These size constraints in the device layer may not fit in highly integrated microchips. In addition, the application of this method is limited because trapping air in the indicator channels requires the use of non-gas-permeating materials, such as glass, which is more difficult to fabricate compared to standard soft lithography. Yet another common pressure measurement method uses membrane deflection to detect applied pressure. Piezoresistive,^{21,22} capacitive,²³ or optical²⁴ sensors are typically used to detect the change in membrane deflection. It has been known that these mechanical methods show high sensitivity and precision. However, there are a few drawbacks to this approach as well: first, these measurements may have substantial dead volumes; second, they require complex electrical/electronics control²¹⁻²³ or expensive optical equipment such as lasers²⁴ and position- or intensity-sensitive detectors to detect membrane displacement; third, these devices may not be readily integrated with any existing microfluidic devices because of the multistep fabrication processes, most commonly based on silicon or other semi-conductor fabrication processes.²¹⁻²⁴ Alternative PDMS pressure sensors fabricated by soft lithography were also developed.25,26 However, these sensors require either optical equipment with complicated analysis²⁵ or multiple valves and additional electronics for control.²⁶ Additionally, to multiplex these existing pressure sensors (e.g. to measure pressure at multiple locations) would also require additional hardware and take longer time.

To address the need for an integrated on-chip pressure sensor that is inexpensive and easy to use, we developed a simple image-based method using fluorescent particles to quantify the deformation of membranes as a function of applied pressures. The pressure sensor we designed consists of a flow channel layer, a sensor layer, and a PDMS membrane in between the layers. A pressure increase in the bottom microchannels results in a deflection of the membrane toward the upper channel ("detection channel") filled with nanoparticle suspensions. This pressure-dependent membrane deformation is quantified by measuring the diameter of area that represents in-focus particles. The image processing is performed by software that was developed in-house, which is simple to use and yields easy-tointerpret data. Although the poor chemical compatibility of PDMS limits applications of this particular implementation of the sensor to a few solvents, other polymeric materials can be used to perform the analysis with little modification of the general concept and method. This method offers several benefits over existing methodologies. First, it is very easy to integrate the pressure sensor without complicated fabrication processes or expensive off-chip equipment. Second, the membrane with a proper elastic modulus and aspect-ratio (and thus its deformability) contributes to the sensing range and sensitivity of the sensor. Third, the pressure sensing range of this measurement method can be easily optimized by tuning the focal planes of measurement and we demonstrated accurate pressure measurement in a range of 0–10 psi, which is relevant to many of the microfluidic applications described earlier. To extend the sensing range, we can simply pressurize or depressurize the reference chambers. Fourth, pressure sensing signals can be transferred through a transferring channel without signal loss, resulting in the capability to simultaneously monitor pressures

at multiple locations with a single read-out. Lastly, this detection scheme (including the multiplex scheme) is simple and fast, which is an attractive feature in enhancing the throughput of microfluidic devices especially for highly integrated multifunctional devices.

Experimental method and materials

Fabrication of devices

Multilayer soft lithography was used to fabricate all two-layer devices in poly(dimethylsiloxane) (Dow Corning Sylgard 184, Midland, MI).²⁷ To make all masters, features on transparency masks were transferred to a SU-8 2025-spin-coated wafer (or SU-8 and AZ 50 XT-spin-coated wafer) by standard UV photolithography. The sensor layer contains sensing chambers (located adjacent to the flow channel), detection chambers (where images are acquired), transferring channels (where volume displacement in the sensor chamber is transferred through to the detection chamber), and valves. The flow layer includes flow, reference, and valve control channels. To fabricate masters for the sensor layers, SU-8 2010 was used for a 20 µm-height transferring channel, SU-8 2025 for 50 µm-height chambers, and AZ 50XT (AZ Electronic Materials USA Corp, Somerville, NJ) to make the channel closable by control valves. The mold for the 40 µm-thick flow layers were fabricated using SU-8 2025. The wafer surface was treated with tridecafluoro-1,1,2,2-tetrahydrooctyl-1-trichlorosilane vapour (United Chemical Technologies, Inc, Bristol PA) to facilitate release of PDMS from the molds. To form a 15 µm-thick PDMS membrane on top of the flow layer, a mixture of PDMS (part A and B in a 20:1 ratio) and toluene in a 4:1 ratio was spin-coated on the mold of the flow layer. This layer was allowed to reflow for 1 h at room temperature to make the PDMS membrane flat. The layer is then partially cured at 65 °C for 15 min. For the sensor layer, a 4A : 1B weight ratio PDMS mixture was poured onto the sensor-layer master to give a 5 mm thickness which was cured at 70 °C for 20 min. After peeling off the 5 mm PDMS sensor layer, this sensor layer was aligned onto the flow layer and cured at 70 °C for 2 h. The devices were then cut into shape and access holes were punched in the PDMS before the devices were bonded to the cover glass. In order to measure the thickness of the membrane, the devices were cut vertically, and the membrane thickness was measured at five points along the membrane using an optical microscope with a $20 \times$ objective. The acquired images were processed using ImagePro (MediaCybernetics, MD). The thickness of all the membrane used in the experiments was $\sim 15 \,\mu m$. The standard deviation in this measurement is less than 1 μm.

System preparation and operation

For all the experiments, carboxylate-modified polystyrene particles (500 nm) (FluoSpheres®, Invitrogen, Carlsbad, CA) were suspended in DI water (0.5 wt%) and sonicated for ~30 min to break aggregates. These particle suspensions were then introduced into the detection channel and the fluid channel was filled with DI water (Fig. 1a–c). The particles in DI water have a net negative charge preventing them from aggregation and minimizing adsorption on the PDMS surface. Due to the



Fig. 1 Design and operating mechanism of an image-based pressure measurement method. (a) Optical micrograph of the microdevice: yellow, detection channel filled with a nanoparticle suspension; blue, flow channel. (b), (d), (f) and (h) show the sensor before pressure is applied in the fluid channel below, and (c), (e), (g) and (i) after. (b) and (c), Schematic of the cross-sectional views before, (b), and after, (c), applying pressure in the fluidic channel showing membrane deformation as a function of applied pressure. (d) and (e), Schematic of the top views at a particular focal plane before, (d), and after, (e), applying pressure in the fluid channel, the latter showing pressure-dependent membrane deformation, *i.e.* a decrease of an area enclosing in-focus particles. (f)–(i), Raw and processed images of 500 nm fluorescent polystyrene particles correlating with applied pressure. (f) and (g) represent raw images showing in- and out-of-focus particles before, (f), and after, (g), the pressure is applied. (h) and (i) represent processed images showing only in-focus before, (h), and after, (i), the pressure is applied. A minimum circular boundary that encloses all the in-focus particles is calculated and drawn. The diameter of the area devoid of particles is a strong function of the applied pressure, and can be automatically measured rapidly and accurately by the software.

neglegible density difference between water and the particle settling of the particle occurs very slowly and, therefore, does not affect measurement.

Before imaging, the vertical position of the membrane surface was first found by identifying stationary particles located on the membrane surface. To confirm that the membrane is initially flat, two images at 1 µm above and 1 µm below the membrane surface were obtained and processed. We know the membrane is flat because in-focus particles occupy the entire sensor area in the image taken 1 µm above, but no in-focus particle was found in the image taken 1 µm below. For the direct pressure measurement, the inlet of the flow channel was connected to a nitrogen tank and exposed to pressures in the range of 0-10 psi. In order to precisely control the applied pressure, digital pressure sensor (AP-C33 K, Keyence, Osaka, Japan) with a resolution of 0.01 kPa was used. The deformation of the membrane was then quantified as described in the following section. For the remote pressure measurements, the sensing chamber, the detection chamber, and the transferring channel in the sensor layer were filled with the particle suspension. An elastomeric pneumatic on-chip valve was then gradually closed by slowly increasing the valve pressure to keep the total volume of the nanoparticle suspension constant. The flow channel and reference channel in the sensor layer were filled with DI water. Pressure ranging from 0-11 psi was then applied in the flow channel and deformation of the membrane of the detection chamber was quantified. For the multiplex pressure measurement, the device was prepared as described previously. In order to calibrate the four sensors, the outlet of the flow channel was closed and pressure ranging from 0-11 psi was applied in the flow channel. Images were acquired under multiple pressures and processed. After calibration, the outlet of the flow channel was opened and the fluid was connected to a pressure source. Pressure drop along the flow channel was then analyzed by measuring the pressure at four points of the flow channel simultaneously.

Image analysis of in-focus particles

The fluorescent nanoparticles at a focal plane were monitored via optical microscope (Leica DM-IRB) with a $100 \times$ or $63 \times$ oilimmersion objective, and images were captured using a Hamamatsu C9100-13 EM CCD camera. A 0.5× coupler was used to mount the camera onto the microscope. The captured images were analyzed with software code developed in Matlab®. The analysis involves three steps: locating possible particle centers, identifying in-focus particles, and defining a minimum circular boundary that encompasses all the in-focus particles. During the first step, the original image is filtered via a spatial bandpass filter to remove pixel noise and smooth the image.²⁸ The brightness-weighted centroid algorithm then determines all local maxima regardless of whether they represent a real particle. After identifying all local maxima, cut-off criteria based on combinations of brightness and geometry were applied to identify in-focus particles: in this experiment, when 500 nm particles were imaged using a $63 \times$ oil lense, particles located within 1 μ m from the focal plane were identified as in-focus particles.²⁹ Lastly, distances between the center of the detection chamber and each in-focus particle are calculated and the average of the first ten longest distance was chosen as a diameter of a minimum circular boundary that encloses all the in-focus particles. Since a large number of in-focus particles are present near the surface of deformed PDMS membrane this method can accurately quantify the membrane deformation. The standard deviation of the first ten longest distance was less than 0.5% of the diameter of

detection chamber. For the multiplex pressure measurement, images were discretized into four equal-sized square domains. Each domain shows a quarter of one of the four sensors. For each domain, distances between the corner of the domain (the center of the detection chamber) and in-focus particles were calculated and a diameter was determined as described previously.

Numerical model for the deformation of the detection membrane

To aid the design of the pressure sensors, three-dimensional deformation model of a thin PDMS membrane was developed using a structural mechanics module of the finite element modeling software, COMSOL (Stockholm, Sweden). The membrane of the detection region was represented by a disk with 80 µm radius and 15-30 µm thickness, which has the same dimension as the actual devices we fabricated. In the simulation, the value of Poisson ratio was ~ 0.5 and the Young's modulus was chosen to be 0.1 or 1 MPa: both values matched well with those in the literature.^{30,31} The side surface of the membrane was assumed to have either no displacement in any direction or 5 µm in all directions, and the applied pressure was 0-10 psi, uniformly distributed along the bottom surface of the membrane. At each pressure, the deformation of the membrane (Fig. 2a) was quantified by reading the normalized cross-sectional diameter of the deformed membrane at a particular vertical position. The simulation results are plotted alone (Fig. 2b, c) and together with the experimental data for comparison (Fig. 3c, d).

Results & discussion

The mechanism of the image-based pressure detection method

In order to detect pressure by a simple image-based method, we developed a microfluidic pressure sensor consists of two layers and a PDMS membrane in between. This device has a sensor layer including a detection channel filled with a fluorescent particle suspension and a flow layer containing a flow channel (Fig. 1a-c). We use a simple fluorescence microscope to capture images located within the detection chamber at particular focal planes, a few microns above the membrane (Fig. 1b, c), to quantify the applied pressure. Before applying pressure in the flow channel (bottom), the membrane is flat (Fig. 1b), and therefore at the focal plane, the image shows that the area enclosing all in-focus nanoparticles is the same size as that of the detection chamber itself (Fig. 1d, f, h). When pressure is applied to the fluid in the flow channel, the membrane deflects upwards (Fig. 1c) and displaces the particle suspension fluid and thus the in-focus particles in the center of the image (Fig. 1e, g, i); the image then shows only a donut-shaped area containing fluorescent particles that are in focus. Note that the raw images (Fig. 1f, g) also show fluorescence from particles that are out of focus with dimmer and more diffuse signals. It is through image processing that we can quantify the membrane deformation as a function of applied pressure. The Matlab® algorithm we developed automatically processes the raw images, identifies in-focus particles (Fig. 1 h, i), and calculates the diameter of the area devoid of the in-focus particles as described in the Experimental Methods section. This entire process takes less than 0.25 s, allowing for almost instantaneous pressure detection. Moreover, the



Fig. 2 Numerical models of membrane deformation. (a) The deformation of the disk-shaped membrane is a strong function of pressure. The membrane is 160 μ m in diameter and 15 μ m thick. At a low pressure (5 psi, left/front), the membrane has a smaller deformation, as compared to a higher pressure (10 psi, right/back). Color scale represents the vertical displacement of the membrane in microns (μ m). (b) and (c), The normalized cross-sectional diameter of the dome-like deformed membrane also depends on membrane properties, including the Young's modulus (b) and aspect ratio as defined by ratio of diameter to thickness (c). By tuning these properties, the sensitivity of a pressure sensor can be maximized for a desired pressure range. The solid squares and circles represent data for sensors with the membrane edge anchored. The hollow squares and circles are data for sensors with 5 μ m edge displacement in the membrane.

simplicity of the sensing mechanism and the sensor design allow the sensor to be easily integrated in microdevices, particularly in multilayer PDMS devices, without complicated fabrication processes.

Numerical modeling of the membrane deformation

To aid the design and to optimize the performance of the pressure sensors, we developed numerical models using the finite



Fig. 3 Tuning the focal position for different sensing ranges using a single sensor. (a) and (b) Schematic of the extent of the membrane deflections as a function of applied pressures at a short (a) and a long (b) focal distance. The lower focal point allows the accurate detection of lower pressures because it is sensitive enough to show differences at low pressures, but is likely to have large errors at higher pressures. In contrast, the higher focal point is better for higher pressures while it will not be able to detect very low pressures. (c) The diameter of the area that is devoid of in-focus nanoparticles as a function of pressure 4 μ m above the membrane surface. The error bars are standard deviations in five measurements using identical sensors. The signal is a strong function of the applied pressure between 0 and 2 psi in the microchannel, which can be fitted to $P = 0.8563-0.8377e^{-1.8042d}$. The sensor in this range has an excellent sensitivity. (d) The diameter of the area devoid of focused-nanoparticles as a function of pressure at 24 μ m above the membrane surface. The signal is highly dependent on the pressure in the range 2–10 psi, which can be fitted to $P = 0.8327-1.5642e^{-0.4166d}$. The experimental results agree very well with the numerical calculations.

element tool, COMSOL. The geometries of the membranes were simplified to a disk; pressure was assumed to be applied uniformly at the bottom surface of the membrane. Fig. 2a shows dome-like deformations of the membranes when two different pressures are applied; as expected, lower pressure produces less deformation (front left membrane). Similar to how membrane deformation in the experiments was quantified, the deflection of the membrane in the models was measured by reading the normalized cross-sectional diameter of the deformed membrane (defined as cross-sectional diameter of the deformed membrane divided by the original diameter) at a position 24 µm above the membrane's resting position (Fig. 2b, c). We show, using the models, that the sensing range and sensitivity of the pressure detectors are strong functions of the membrane deformability, which can be designed a priori and controlled in the fabrication processes.

The membrane deformability is largely determined by two parameters: Young's modulus and the dimensions of the membrane.³⁰⁻³³ Numerical models were used to explore the sensing ranges and sensitivity of different designs of the pressure sensor. Experimentally, Young's modulus of PDMS can be varied easily by changing the mixing ratio of pre-polymer to curing agent, curing temperature, and curing time.^{30,31} The range we used in the models, from 0.1 MPa to 1.0 MPa, is well within values that can be easily obtained experimentally.

As shown in Fig. 2b, the membrane with a low Young's modulus (0.1 MPa) undergoes large deformations at low

pressures (between 0 and 3 psi), showing significant increase in normalized cross-sectional diameter in this range. In contrast, the membrane with a high Young's modulus (1 MPa) does not have detectable deformation at pressures below 3 psi when the measurement was taken at 24 μ m above the membrane's resting position. As the pressure increases above 3 psi however, the one with a low Young's modulus displays a saturation behavior where further increase in pressure does not contribute to appreciable amount of further deformation, while the membrane with a high Young's modulus shows greater deformation and noticeable displacement.

In addition to the mechanical properties of the membrane material, we examined the effect of membrane thickness on the sensor behavior. Thickness is also a parameter that can be easily varied, *e.g.* by changing the viscosity of the pre-polymer mixture or by changing the spin-speed during the spin-coating step to make the PDMS membrane. In the simulation, the diameter of the membrane was fixed as 160 μ m (as in our experiments), and two membrane thicknesses, 15 and 30 μ m, were considered.

As expected, the 15 μ m thick membrane is more flexible and thereby shows high sensitivity at low pressure whereas the thicker membrane experiences greater deformation at high pressure (Fig. 2c). Thus, without changing the masks or the design of the sensor, it is possible to maximize the sensitivity of a sensor in a pressure range of interest by varying membrane thickness and Young's modulus.

Using tunable focal plane to broaden sensing range of individual pressure sensors

The numerical models demonstrate that Young's modulus and the thickness of a membrane can be tuned for a desired pressure range. An additional attribute of our sensing method is that it is possible to perform pressure measurement at multiple focal planes using a single sensor for different pressure regimes: we can use large focal distances (or higher focal planes) for higher pressure ranges, and short focal distances (or lower focal planes) for lower pressure ranges; Fig. 3a and b illustrate such a scheme. To implement the scheme experimentally, we fabricated a device with a membrane 160 µm in diameter; images at two focal planes, 4 µm and 24 µm from the surface of the membrane, were acquired and processed for pressures ranging from 0 to 10 psi. At the lower focal plane (4 µm), pressure in the lower operating range (0–1.6 psi) can be measured with a high degree of accuracy (Fig. 3c). Similarly, pressure measurement at the higher focal plane (24 µm) shows high sensitivity and the sensing range extends from 1.6 psi to 10 psi (Fig. 3d). For both sensing regimes, the measured values fit well empirically to exponential functions $(P = 0.8563 - 0.8377e^{-1.8042d}$ with $R^2 = 0.9992$ for the lower focal plane, and $P = 0.8327 - 1.5642e^{-0.4166d}$ with $R^2 = 0.998$ for the higher focal plane, with P being pressure (psi) and d being the normalized diameter of the area devoid of the in-focus particle). For comparison, simulation results are also plotted together in Fig. 3c, d. Here, the normalized cross-sectional diameter in the models is equivalent to the normalized diameter of the area devoid of the in-focus particles in the experiments. In both cases, the trends of experimental data are well within those of the simulation results, demonstrating that the behavior of the pressure sensors is completely predictable and designable. For this set of measurements, atmospheric pressure was used as a reference to measure absolute pressures in the microsystems. To further extend the sensing range, one can simply pressurize or depressurize the nanoparticle suspension channel. The results shown here demonstrate that broad ranges of pressure can be measured using a single sensor by tuning focal positions or a reference pressure without re-design or fabrication.

Although calibration for each device is required due to (i) batchto-batch variation in raw materials and (ii) precision limitations of fabrication processes, the calibration of the device takes only 2–3 min, and once calibrated the device can be repeatedly used. In order to demonstrate that this sensor can be used for repetitive detection without recalibration, we performed repeated measurements at 3.1 psi. For this measurement the flow channel in Fig. 1 was repeatedly pressured/depressured by a computer controlled solenoid valve. As shown in Fig. 4, the sensor was able to accurately detect the pressure to at least 1000 times. We note that this image-based sensing method uses intensity contrast between particles and local background to identify and locate the center of in-focus particles and does not rely on quantitative analysis of fluorescence intensity. Therefore, photo bleaching caused by repeated imaging does not affect accuracy of the measurement.

Remote pressure measurement

For applications that require pressure measurement outside of or far from the flow channels, we developed a volume-



Fig. 4 Repeated detection (3.1 psi) in a sensor, showing that the detector can accurately measure the pressure at least 1000 times without recalibration. The error bars represent standard deviations in five measurements using the same device.

displacement transduction method using a system with two connected chambers (Fig. 5a-c). The two chambers - the sensing chamber on the flow channel and the detection chamber on the reference chamber – are connected to each other by a transferring channel, and all are filled with a nanoparticle suspension. An on-chip valve for the sensor layer is closed to keep the total volume of the nanoparticle suspension constant (after the injection of the nanoparticle suspension). Both chambers have a thin membrane (diameter : thickness ~ 32 : 3). These membranes are made of a PDMS mixture (A : B, 20 : 1) as before and are therefore much more deformable, whereas the other parts of the chambers and the transferring channel are made of much stiffer PDMS (A : B, 4 : 1). In our experiments, the focal plane where images were obtained is located in the reference chamber, 8 µm below the membrane of the detection chamber.

Before pressure in the flow channel is applied, both membranes are flat (Fig. 5b) and therefore particles are not visible in the field of view (Fig. 5d, f). As pressure increases in the flow channel the membrane of the detection chamber deflects upward and displaces a volume of particle suspension. This volume displacement is transferred to the detection chamber through the transferring channel and leads to the deflection of the detection membrane (Fig. 5c, e, g). The deformation of the detection membrane is then quantified as described previously (Fig. 5g). In an ideal sensor one would like to have no loss of the pressure in the transduction process, so the pressure measured in the detection region is the same as the one in the sample fluid channel. Deformation of the chamber walls and the transferring channel could be sources of concern; this capacitance may reduce the transferred volume and therefore reduce the deflection of the detection membrane, which leads to lowering of the sensitivity. By using much more rigid material for the rest of the device other than the membrane as we have done in our experiments, however, we observed that the loss of signal in our setup is negligible. Using this method we measured pressures remotely, in contrast to the direct sensing methods in Fig. 1-3, ranging from 3 to 11 psi with good accuracy (Fig. 5h). We note that the sensing range of the remote pressure sensor narrows somewhat at the low pressure end as compared to that of the direct detection method.



Fig. 5 Method for remote pressure measurement. (a) Optical micrograph of the microdevice: yellow, detection channel filled with nanoparticles in flow layer; blue, flow channel; red, valve for closing detection channel; green, reference channel. (b) and (c) Schematics of the cross-sectional views before (b) and after (c) applying pressure in the flow channel, showing the membrane deflection and transferring volumetric displacement of nanoparticle suspension. (d) and (e) Schematics of the top views showing the focal plane before (d) and after (e) the applied pressure. (f) and (g) Processed images of in-focus fluorescent particles before (f) and after (g) applying pressures. (h) The normalized diameter of the area that is in-focus nanoparticles as a function of pressure in a microchannel, showing that pressures in the range 3–10 psi can be measured accurately using this method. The error bars are standard deviations in five measurements using identical sensors.

This is because deflecting two serially connected membranes requires slightly higher pressure. However, as described previously, the sensing range can be extended by using multiple focal planes or depressurizing or pressurizing the reference chamber should there be a need.

Multiplex pressure measurement

Using the remote pressure measurement method, pressures in various parts of the flow channel can be transduced and the signals transferred to a single location, allowing us to detect pressures in multiple locations simultaneously. To demonstrate the potential application for measuring pressure in various parts of a chip, we performed a multiplex pressure measurement using a microdevice shown in Fig. 6a. The flow channel consists of large chambers connected by serpentine-shaped long and narrow channels (Fig. 6a). Each sensing chamber is located on a large chamber in the fluid path, and all detection chambers are assembled in one location on a reference channel (Fig. 6a). The field of view shows a quarter of each detection chamber (Fig. 6b); because of the symmetry of the circular membrane it provides all the necessary information for calculating four pressure values.

We first calibrated the four sensors as a function of the applied pressure in the flow channel with its outlet closed so that the pressures we measured were static pressures. After the calibration, the outlet was opened and a flow was driven through the channel by a constant back-pressure (P = 10.48psi). A raw image showing a quarter of each of the four sensors was then obtained and processed to calculate the pressure values in the detection region. As expected, the processed image shows that the area enclosing in-focus particles decreases as pressure of the flow decreases along the channel (Fig. 6c). To validate the measurements, we compared the experimental data with theoretically predicted values (Fig. 6d). The theoretical pressure values are numerically calculated by a resistive circuit model.34-36 Calculated resistance of the serpentine-shape channel is over 2000 times bigger than that of the 50 cm long tubing connecting the pressure source and the microfluidic chip, and over 800 times bigger than that of the large chambers in the flow channel. Hence the hydrodynamic resistances of the tubing and the large chambers are neglected, and we can simplify the circuit as shown in the inset of Fig. 6d. The theoretical calculations show excellent agreement with the measured values. In this multiplex measurement, image acquisition and processing were automated and took less than a second to read out the four pressure values. Thus, we believe this simple and fast detection scheme will improve the throughput of microfluidic devices especially for multi-functional or highly integrated devices.

Conclusions

We developed an on-chip pressure measurement method, which uses volume displacement of fluorescent particle suspensions to detect the membrane deflection. Unlike other mechanical methods, our system does not require expensive off-chip equipment or a complicated fabrication process, and is simple to use. The image analysis to measure the diameter of the area



Fig. 6 Method of multiplex pressure measurements. (a) Optical micrograph of the microdevice fabricated using multilayer soft lithography: yellow, detection channel filled with nanoparticles in flow layer; blue, flow channel; red, valve for closing detection channel; green, reference channel where four detection chambers are collected in a single location. The zoomed-in view shows the detection chambers and field of view where the image is captured. The flow is from left to right in the fluid channel when multiple pressures are measured. (b) Zoomed-in view of the detection chambers. (c) Processed image showing four areas of in-focus particles correlated to the pressure at various locations in the flow channel. Chamber 1 is most upstream and chamber 4 is most downstream. (d) Pressure measured by the diameter of the area containing in-focus particles, as a function of positions in the flow channel. The inset figure shows a resistive circuit model corresponding to the microchannel network. Experimentally measured pressure is in good agreement with the numerical calculations based on the resistive circuit model. The error bars are standard deviations in five measurements using identical sensors.

containing in-focus particles was sufficient as a read-out of pressure. This image processing is easy to use and produces readily readable data that can be simply interpreted. Via simulation and experimental analysis, we observed that there are three simple ways to modulate the sensing range and sensitivity of the pressure sensor: changing the membrane deformability by varying its Young's modulus and aspect ratio (diameter to thickness), tuning focal planes, and using different reference pressures. We show that it is possible to obtain highly accurate pressure measurements that are also predictable from simple models. These advantages of our pressure sensor allow it to be integrated with various microfluidic components for different applications. Moreover, the most unique advantage of this method over conventional methods lies in the ability to measure pressures at multiple locations simultaneously in a microsystem with a single read-out, which enhances the throughput of microsystem operation. Although mechanical and chemical properties of PDMS constrain application of this method, the idea of using volume displacement can be extended to other materials, while using the exact same image processing/ analytical method and the general design. Therefore, our method adds to the toolbox of non-invasive measurements, particularly for simultaneously monitoring dynamics of biological or chemical processes with pressure changes in multiple channels, as it can effectively decrease the overall detection time for analyzing a large number of samples.

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