Dynamic Size-Tracking of Single Cells Using Microfluidics-Integrated Microwave Sensors

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ABSTRACT

Cellular size is one of the most important biophysical indicators of the state of a cell. Cellular size can be used as a sensory signal for determining cellular growth and decay trends and can facilitate the testing of novel drugs on cancerous cells. While size measurements have traditionally been performed using optical microscopy and quantitative phase imaging, there is still ample space for sensors with higher resolution and throughput, as well as lower cost. In this respect, microwave resonators have been suggested for detecting single cells in a label-free manner; however, little has been done to systematically compare the microwave signal output induced by single cells to the cellular size obtained by optical microscopy. Here and for the first time, we demonstrate that the cellular size measurements conducted by microscopy and a microfluidics-integrated microwave sensor indeed correlate, at the single cell level. We further study in real-time the changes in size of single cells that are dynamically trapped around the sensing region and chemically induced to shrink. The strong correlation between the electronic and optical size results indicates that microwave sensors can be used for detecting and sizing single cells in real time.
KEYWORDS: Cell Size, Microwave Resonators, MIMS, Quantitative Phase Imaging, Suspended Microchannel Resonators

INTRODUCTION

Precise measurements of cellular size at high speed have opened up avenues for personalized medicine and the early diagnosis of diseases.\textsuperscript{1,2} To measure the cellular size, quantitative phase microscopy\textsuperscript{1,3,4} can be used to trap a cell and continuously observe the dynamics of size change. However, for point-of-care applications, a system with a smaller form factor, lower cost and ease of use is needed. This need has driven a significant amount of research efforts for developing sensors capable of sizing single cells.\textsuperscript{5-16} Among the advances with such microscale sensors include the observation of fast mass fluctuations at the single-cell scale\textsuperscript{14} and the measurement of the differential drug response of cancer cells derived from different patients.\textsuperscript{15}

The focus of the aforementioned sensors has been on mass measurements of single cells, as mass is the most suitable metric for the total accumulated material inside a cell. To detect mass changes, micromechanical structures are used that are directly affected by the total mass of the structure, including any attached analyte. However, the performance of micromechanical sensors in a fluidic environment is hindered by the high viscous dissipation of mechanical motion and diffusion-limited capture rate of analytes.\textsuperscript{17} As an effective solution to both problems, resonant sensors based on suspended microfluidic channels have been developed. These sensors, suspended microchannel resonators (SMRs), enclose the fluid inside and are operated under vacuum conditions. Originally developed\textsuperscript{5,18,19} and optimized\textsuperscript{7,8,20-22} mainly by the Manalis group, SMRs are now pursued by numerous research groups.\textsuperscript{23-28} However, the need for obtaining a suspended hollow microchannel structure imposes stringent conditions for microfabrication and drives the cost of each sensor.
Remarkably, mass is not the only parameter suitable for determining the total material content inside a cell. As a cell synthesizes proteins and biological polymers, such as cytoskeletal proteins and phospholipids, and maintains a concentration imbalance of different electrolytes in its cytosol with respect to the external medium, the total electrical polarizability of the cell is expected to become markedly different than the electrical polarizability of its external environment.\textsuperscript{29-31} This contrast in electrical polarizability depends on the total material accumulated inside the cell and exhibits itself as a change in the effective dielectric constant and capacitance with respect to the fluid it displaces. Therefore, an electrical sensor capable of resolving minutiae capacitance changes induced by a single cell can be used for sizing single cells \textit{in principle}. However, at low electrical frequencies (e.g. smaller than 1 GHz), extraneous effects such as Debye screening and cell membrane polarization dominate the response, and techniques working in this range, such as conventional impedance spectroscopy, do not directly yield cellular size. On the other hand, electrolyte ions cannot follow the electromagnetic field and cease to interfere with the response, when the electromagnetic oscillation frequency is faster than the relaxation rates. Relevant relaxation times\textsuperscript{32} in this case are i) the bulk diffusion, ii) diffuse layer charge relaxation and most stringently iii) Debye time scales: in practice, frequencies larger than 1 GHz are sufficient to \textit{short out} the response of the electrolytes and probe into the cellular structure. At these frequencies, hydrogen bonds in the water can still follow the electrical oscillations up to approximately 20 GHz.\textsuperscript{33} As a result, a large contrast difference emerges between the extracellular medium (mainly composed of water) and intracellular biostructures (such as cytoskeleton, nucleus and organelles) at the lower end of the microwave frequencies (1-20 GHz). This contrast difference enables microwave sensors with much larger dimensions to be used to measure cells compared to mechanical sensors.\textsuperscript{34-39} However, the question remains whether the microwave signal induced by a passing cell indeed correlates with the
**geometrical size of the cell.** By using simultaneous microscopy measurements, we verify that this is indeed the case. We then proceed to use these sensors for sizing single cells in real-time. Our results demonstrate the emerging potential of microfluidics-integrated microwave sensors (MIMS) for single-cell biophysical measurements.

**RESONANT SENSOR**

To utilize the high sensitivity of microwave frequencies, a coplanar waveguide (CPW) resonator on a fused silica chip was designed to match the 50 Ω impedance of the electronic measurement system (Figure 1a). The CPW narrows down into a bowtie-shaped sensing region that coincides with a microfluidic channel. The on-chip CPW has a signal path of a 3.4 cm length and a 1.6 cm width. The gap between the ground and signal line is 400 µm, which narrows down to 50 µm at the sensing region (Figures S1). In the sensing region where the inter-electrode distance shrinks, the local electric field intensifies to enhance the sensitivity of the detection (Figures S2). As cells pass through the sensing region one by one, they induce distinct frequency shifts in the microwave sensor while being imaged using optical microscopy (Figure 1b).

![Figure 1](image.png)

**Figure 1.** (a) The designed CPW resonator has a ground – signal – ground (GND-SGN-GND) circuit...
configuration patterned on a 250 µm thick fused silica wafer. A PDMS-based microfluidic system was bonded on the fused silica wafer, to create a microchannel passing through the active region of the sensor. While microwave sensor data were being recorded, the sensing region was simultaneously monitored via an optical microscope. (b) Typical response of the resonator to the transition of single cells through the sensing region. Slow drift in the background was subtracted using a sixth-order IIR digital high pass filter. Each precipitous spike is induced by a single-cell event. Here, three cells with different sizes pass through similar positions: the change in the resonance is different for each case: 0.8, 1.17, and 1.28 kHz, respectively, which correlates with their sizes observed under the microscope. (c) Close up view of the middle event in part (b).

EXPERIMENTAL SETUP

In our experimental setup, there were three subsystems: an optical microscope, a microfluidics-integrated microwave sensor, and a fluidic control system that transfers cells to the sensing region (Figure S3). Initial measurements to find the resonance frequency and the quality factor of the sensor were determined using a vector network analyzer (Figure S4). To increase the sensitivity of the microwave sensor, we employed a narrow-band detection scheme that is centered around the first resonance frequency of the CPW structure.\textsuperscript{40} Phase-sensitive detection was performed with a lock-in amplifier and due to the frequency limitation of the lock-in amplifier, we constructed an external heterodyne circuitry\textsuperscript{34,35} to continuously track the resonance frequency (Figure S5). With the PLL, the phase of the resonator was locked to 0 degrees with a proportional-integral (PI) controller. Any deviation from 0 degrees emerges as an error signal updating the frequency of the signal generator. With this method, we can continuously track the resonance shifts.
The fabricated MIMS chip, together with microwave and microfluidic connections, was placed under the optical microscope stage. While the sensing region was observed by the microscopy, adherent MDA-MB-231-Luc2-GFP cells were detached from the culture plate using Trypsin. The suspended cells were added to a reservoir, and trypsin was neutralized by adding cell medium. The cells were then transported to the sensing region with a controllable pressure pump (Flui gent; MFCS-EZ) and passed through a flow sensor with typical flow rates of 0.3 - 1 µL/min which strike a balance between the response time of the measurement setup and accurate optical imaging of the cell.

**EXPERIMENTAL RESULTS**

For the first device having a channel height of 110 µm, the resonance frequency (at 2.19 GHz) was monitored with an Allan deviation of $1.8 \times 10^{-8}$ and frequency modulations induced by single cells were recorded. The amount of the shift in the first resonance mode, which corresponds to the electrical volume of a cell, can be related to the geometrical size of a cell (Supplementary Note 1). While electronic data were being measured, a video for the cells transiting the sensing region was recorded simultaneously for correlating the optical and microwave signals (Supplementary Video 1).

Using the bright field images of the cells, the number of pixels for each single cell was estimated digitally using built-in functions on MATLAB. The size of a pixel was estimated by calibrating the number of pixels with device dimensions. The mean and standard deviation of the size of each cell was obtained by measuring the same cell on many successive frames. The pixelated area was then converted first into the actual area, and then assuming a spherical shape, into the cell volume.

By matching the calculated volume of the cell from microscopy images and the induced microwave frequency shifts, we observed an almost linear relationship (Figure 2a). Indeed,
for cells that passed through the middle of the channel and hence did not overlap with the sensing electrodes (red data points in Figure 2a), the fitting to the linear trend was much stronger than the trend seen in cells whose trajectory overlaps the sensing electrodes (blue data points in Figure 2a). The RMSE (root-mean-square error) values are 0.10 kHz and 0.44 kHz for the non-overlapping and overlapping cells, respectively, and the overall RMSE value is 0.26 kHz. We note that all cells larger than a specific size in Figure 2a have trajectories that overlap with the electrodes inevitably. Although the trend for larger cells shows larger dispersion around the fitting line, there does not seem to be any nonlinear effect emerging at the single-cell level. The RMSE value, excluding these overlapping large cells, is 0.25 kHz. A comparison plot taken with another device is provided in the Supplementary Information (Figure S6), which exhibits similar behavior.

The measurement bandwidth of our system was set to 2 Hz to strike a balance between the Signal-to-Noise ratio and throughput. As a result, a small number of cells which passed through the sensing region faster than 500 ms did not produce full response. These cells were excluded from the analysis since the data from both the microwave sensor and the optical microscopy image were not reliable. On average, the responsivity of the sensor in Figure 2a turns out to be $0.33 \frac{Hz}{\mu m^3}$. With an Allan deviation of $1.8 \times 10^{-8}$ and resonance frequency of 2.19 GHz, the minimum resolvable frequency is $\sim 60$ Hz, which results in a minimum resolvable change in volume of $182 \mu m^3$. This level translates into a minimum detectable change in the cell radius of $\sim 145$ nm, for a mean cell having radius of $10 \mu m$.

We have also investigated the effect of channel height on frequency shifts. To observe this effect, we fabricated another device with a channel height of 55 $\mu m$ (which was 110 $\mu m$ in previous device). We repeated the same experimental and data analysis procedure to obtain the relation between volume and frequency shifts (Figure 2c). Similar to Figure 2a, we have seen a linear relation, however, the responsivity of the new device was larger than that of the
previous one (Figure 2d). The RMSE value for non-overlapping cells is 0.43 kHz and for overlapping cells 1.23 kHz. The mean RMSE is 0.92 kHz and the sensitivity of the new device on average was 1.2 $Hz/\mu m^3$. This sensitivity and noise level in the experiment (230 Hz) corresponds to $\sim$152 nm change in the cell radius for a cell having 10 $\mu m$ radius.

Figure 2. (a) Comparison of the optical volume of the cell and induced frequency shift. The relationship between the volume and frequency is almost linear. Red data points: cells passing through the middle of the channel without overlapping the electrodes. Blue data points: cell trajectory overlaps one of the electrodes. (b) Data points within the rectangle in Part a. The error in the frequency was calculated by the minimum resolvable frequency, and the error in the optical size was estimated from repetitive pixel measurements. (c) Data taken with 55 $\mu m$ channel height. Cells were classified in accordance with transition position with red data points again indicating cells non-overlapping with the electrodes. The relation seems again
linear especially for cells that pass between two electrodes. (d) Comparison of two different channel height. When the cells having similar size are compared in Part (a) and Part (c), induced frequency shifts are larger where channel height was 55 \( \mu m \) (green data points).

To elucidate the positional dependence of the frequency shifts, we used 20 \( \mu m \) polystyrene particles (Sigma-Aldrich, Supelco - 74491) which constitutes a more homogeneous sample compared to a cell population. We diluted the original solution with deionized water at a ratio of 40:1. Using another device with a resonance frequency of 2.52 GHz, we repeated the same experimental procedure as with the cells and recorded the frequency modulations at different locations for particles of the same size (Figure 3a).

We trapped each particle near the sensing region and repeatedly moved it vertically back and forth many times (at least 40 times) along the transition position by reversing the direction of the applied pressure with respect to the reservoir. While the pressure was reversed, we did not observe a significant change in the horizontal position of the trapped particle. To span the sensing region, we collected the statistics of eleven different particles, each of which transited the sensing region at a different position. The positional sensitivity is shown in Figure 3a where the sensing region is partitioned into eleven different subsections – along the most densely visited trajectories of the particle flow for each experiment. In Figure 3a, the blue data points indicate a vertical transition path along the sensing electrodes, the red data points are for particles passing in between two electrodes without any overlap, and the green data points indicated the cases where a fraction of the particles overlaps with the top of the signal or ground electrode.

The shift in the resonance frequency turns out to have a local maxima near the edge of each electrode, in line with earlier observations.41 The frequency responsivity varies by more than half over the signal electrode. The trend in Figure 3a was further investigated by a similar
device as detailed in Figure S7. Remarkably, the responsivity at the center of the sensor, where there is no overlap with the electrodes, is constant within the noise level (Figure 3a). This result indicates that microwave measurements can be used for size measurements, without the need for additional microscopy for position determination, as long as the particle flow is confined within the central region (which can be accomplished by e.g., hydrodynamic focusing, modifying the device design, or introducing position measurement capabilities\textsuperscript{40,42}).

Polystyrene particles were further used to investigate the effect of channel height on the responsivity of the sensor. For instance, when the channel height was reduced to 35 µm, the frequency shifts induced by each particle climbed up to approximately 40 kHz, with a frequency noise ~ 325 Hz at the measurement bandwidth (Figure 3b). These numbers yield an SNR of 123 for frequency shift measurement, defined as the ratio of the frequency shift induced by the particle over the frequency noise level. However, repeating the experiment over an ensemble of identical particles and calculating the standard deviation within the ensemble yields a larger dispersion for two reasons. Firstly, particles with different trajectories induce frequency shifts of different magnitudes as mentioned before. Secondly, a standard deviation of 0.35 µm in diameter for the commercial microparticle sample limits the ensemble SNR to a maximum of 22.3 (Methods). The performance metrics for three different values of channel height are listed in Table 1 on page 24.

After the single microparticle experiments, we turned our attention to studying the dynamics of single cells. A single cell can also be trapped and made to pass back and forth between the sensing electrodes by fine-tuning the applied pressure dynamically (Supplementary Video 2). The fluorescent optical images of the same cell were recorded during the experiments and then processed by a custom image processing algorithm. The algorithm counts the number of white pixels for 20 consecutive frames and estimates the mean and standard deviation in the
geometrical size of the cell. This way, the transition position, dwelling time and velocity of the cell can also be obtained (Figure 4).

Figure 3. (a) Comparison of the frequency shift and the position of a standard-sized particle in the sensing region. The horizontal position of each data point coincides with the center of the trajectory of each particle (which moves back and forth, vertically). The vertical lines near the sides indicate the boundary of the microfluidic channel. The positions of the electrodes and particles were matched from the optical images. The region above the ground electrode turns out to be more sensitive to the resonance modulations. (b) Frequency shifts induced by three polystyrene particles, 20 µm diameter, successively passing through the sensing region of a channel of height 35 µm. SNR in frequency shift is more than 120 in this case. The inset shows the region marked with the black rectangle in more detail.

By recording data for 25 minutes and continuously reversing the applied pressure, we obtained the mean value for the frequency shift to be 4.87 kHz (for a resonance frequency of 2.51 GHz) with a normalized standard deviation of 0.09, where normalized standard deviation, \( \hat{\sigma}_1 \), is defined as the standard deviation over the mean (Figure 4c). The data do not indicate any directional asymmetry of the sensor for the cell moving forward or backward. In Figure 4a, the position of the cell was tracked by fitting a circle to the grayscale version of the
fluorescent image, and each data point represents the center of this fitted circle. The applied pressure to the cell reservoir was set to two specific values, so that the cell passed with a similar velocity (Figure 4a color bar).

**Figure 4.** (a) The position of the cell inside the microchannel in each transition. The amount of frequency shift is concentrated around a certain value (4.87 kHz) as the cell kept its position along the electrodes. The velocity of the cell in each transition through the electrodes was also considered in the analysis (color map). Since the applied pressure was the same in each time, transition velocities were similar in general. (b) Frequency shifts from the same
cell observed over time. Frequency shift statistics remained stable within 25 minutes. (c)

Comparison of the size distributions obtained from frequency shift measurements and through fluorescence microscopy. The histograms for volume and frequency shifts are also projected to their respective axes and in both cases a distinct peak appears as expected.

The size of the trapped cell was estimated by an image processing algorithm and compared with the frequency shift statistics. We expect the algorithm to yield similar volume values as the same cell was processed in different frames. In fact, Figure 4c illustrates the volume concentrated to approximately $1.18 \times 10^4 \mu m^3$ with a standard deviation normalized to the mean value ($\tilde{\sigma}_2$) of 0.17, which can be seen at the bottom histogram as well in Figure 4c. Remarkably, the microwave sensor results indicate a relatively narrower distribution for the size of the cell.

**MEASURING CHANGES TO CELL SIZE IN REAL TIME**

The ability to dynamically monitor the effects of a drug delivered to diseased cells is of utmost importance. One such dynamical effect of certain drugs is the shrinkage of a cell, which can be detected by MIMS. To this end, after we have shown that single cells can be trapped and their frequency shift statistics measured, we designed another experiment to detect the biophysical change in a single cell. We tracked the resonance frequency of a MIMS device (at approximately 2.72 GHz) with frequency stability of $1 \times 10^{-8}$.

To demonstrate that microwave sensors can track the real-time dynamics of individual cells, we chose to use dimethyl sulfoxide (DMSO), a chemical that accelerates dehydration, to induce a dynamical change in the size of the cell. We observed that the effect of DMSO is optimal when treating the cells for approximately half an hour in their culture plate. For the experiment, cells were pretreated for 15 minutes to capture the initial dynamics of cell shrinkage. After the pretreatment, the cells were transferred to the microchannel, and a target
cell among the population was selected and trapped around the sensing region. Then, the applied pressure was directed in either direction continuously and the electronic data were recorded (Figure 5).

![Figure 5. Trend in the frequency shifts when a target cell was treated with DMSO or growth medium and trapped around sensing region. (a) Cells were pretreated with DMSO for 15 minutes. The moving average of the shift was 6.43 kHz at the beginning, later, declined to 4.64 kHz after half an hour (b) Control run with the same device. This time cells were treated with the culture medium instead of DMSO for 15 minutes and the change in the signal average remained close to initial levels (5.46 kHz to 5.11 kHz). As DMSO causes a contraction of the interfacial region between water-lipid boundary, we expected to see a shrinkage in the lipid bilayer of the cells causing smaller sizes. In Figure 5a, the ten-point moving average of the shifts decreased from 6.43 kHz to 4.64 kHz (Figure 5a) within thirty minutes. For the control run, another cell population was processed in the same way, replacing DMSO with cell culture. Using the same device, another cell from the control cell population was tracked for more than twenty minutes. Throughout the experiment, the average frequency shift remained close to the original signal level (5.46 kHz to 5.11 kHz).]
Analytically, we estimated the amount of shift that is caused by the cell in between the electrodes (Supplementary Note 1). In the calculations, we have utilized the permittivity contrast between the cells and fluid that modulates the capacitance of the overall system. Using the dimensions and resonance frequency of the fabricated devices, the change in the resonance turns out to be approximately a few kHz and in accordance with the experimental results as in Figure 2a. Usually, the relative changes in the cellular size as a function of control parameters (e.g., introduced drugs) are more relevant than the absolute size determination. To accurately determine the cellular size from a given sensor, a calibration procedure using analytes of known size and permittivity values can be conducted first (similar to the microparticle measurements of Figure 3). By utilizing MIMS technology, the drug sensitivity of diseased cells (e.g., cancerous cells) to various remedies can be predicted with lower cost and reliable sensitivity.

**CONCLUSION**

In this work, we have demonstrated how the frequency shift signal of MIMS can be used to obtain the size of single cells by correlating the signal with optical microscopy measurements. Changes in size of single cells that were dynamically trapped around the sensing region were also tracked and the effect of cell dehydration and size shrinkage monitored. As we propose the first account of the microwave-optic correlated sensors in real-time and at the single-cell level, we have studied the positional dependency of the sensor's response in detail. The elimination of the positional dependency, by designing sensing regions with more homogeneous electric fields, is one of the first priorities in optimizing MIMS technology. Considering the ease of fabrication for MIMS sensors and their brief development period, quick progress is expected for reducing the sensor volume and eliminating noise processes. With the seamless integration with microfluidics technology and ease of fabrication, MIMS technology offers important benefits for single-cell sizing applications.
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METHODS

**Microwave Measurement.** The resonance frequency of the chip was determined considering the S21 response of the sensing region with circulator (PE8401) on the vector network analyzer (Rohde & Schwarz ZNB40). The driving signal (around 2 - 3 GHz) from the signal generator (Rohde & Schwarz SMB 100A) was first up-converted with the output signal (~ 2 - 3 MHz) from the lock in amplifier (Zurich Inst. MFLI). Then, the signal was sent to the circulator to effectively drive and read the response of the resonator. Similarly, the response was down-converted and filtered with a low pass filter (MiniCircuits 4.5 MHz)

**Cell Culturing.** MDA-MB-231 was purchased from ATCC (Manassas, VA, USA) and was labeled with green fluorescent protein (GFP) to be able to visualize under fluorescent light. Cells were cultured in Dulbecco’s modified Eagle’s medium (Biowest, Nuaille, France), supplemented with 10% fetal bovine serum (FBS, Biowest), 1% non-essential amino acid (Biowest) and 50 U/ml penicillin/streptomycin (P/S, Biowest). For the standard experiments, cells were washed with phosphate-buffered saline (PBS, Biowest) and trypsinized (Biowest) to detach from the plates. To assess the changes in the size of the cells, cells were pre-treated with 100% DMSO (ChemCruz, TX, USA) or growth medium (DMEM) as control for 15 minutes and then collected for the analysis.

**SNR Calculations.** In experiments with 20 µm polystyrene particles (Sigma-Aldrich, Supelco - 74491), for each channel height, the maximum frequency shifts induced by each particle ($f_i$) is measured. The mean frequency shift is calculated as $\bar{f} = \frac{1}{N} \sum_{i=1}^{N} f_i$ where $N$ denotes the number of microparticles measured for a given channel height (for all cases $N>50$). The Ensemble Standard Deviation ($\sigma$) is calculated as $\sigma = \sqrt{\frac{1}{N} \sum_{i=1}^{N} (f_i - \bar{f})^2}$. Noise Level is obtained by calculating $\sqrt{2} \times \sigma_A(\tau_{PLL})$ where $\sigma_A(\tau_{PLL})$ stands for the Allan Deviation at the
PLL time scale $\tau_{PLL}$, just prior to introducing microparticles into the sensor. SNR in frequency shift is calculated as the mean frequency shift over the noise level. SNR of the Ensemble is calculated by dividing the Mean Frequency Shift to Ensemble Standard Deviation. The dispersion of the commercial microparticle sample is reported as $\sigma_R = 0.3 \mu m$ in diameter, with a mean diameter of $\bar{R} = 20.1 \mu m$. The microwave frequency shift signals are proportional to the mass (and not the radius) of the microparticles. The mass of the microparticles scales as the cube of the diameter ($m \sim R^3$), hence the standard deviation of the mass distribution ($\sigma_m$) is $\sigma_m = 3\bar{m} \times \frac{\sigma_R}{\bar{R}}$. Here, $\bar{m}$ denotes the mean value for the mass, and the relationship is obtained by using standard formulas for relating the variances of random variables with narrow distributions. Plugging in the numbers, $\frac{\sigma_m}{\bar{m}} = 3 \times \frac{0.30 \mu m}{20.1 \mu m} = 0.045$. As a result, the SNR that can be obtained from this sample cannot be larger than $\frac{\bar{m}}{\sigma_m} = 22.3$.

**Image Analysis.** Transportation of cells on the sensing region is observed via an optical microscope (Zeiss Axio-Imager 2) with both bright field and fluorescent light. To quantify cell size and location, MATLAB built-in image processing functions were utilized. A computer equipped with 8 GB RAM was used to analyze videos recorded with 30 frames per second. In order to effectively utilize image processing algorithm, videos were recorded under fluorescent light due to easier threshold determination. Image processing code consists of the steps illustrated in Supplementary Note 3.

**DATA AVAILABILITY**

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

**CODE AVAILABILITY**

The computer codes generated during the current study are available from the corresponding author on reasonable request.
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COMPETING INTEREST
MSH and RTE are founders of Sensonance. The other authors declare no competing interests.

ADDITIONAL INFORMATION
A Supplementary Information document containing further details and data about measurements is available. Two supplementary videos combining the fluorescent microscopy
| Channel Height [µm] | Mean Frequency Shift [kHz] | Noise Level [kHz] | SNR in Frequency Shift | Ensemble Standard Deviation [kHz] | SNR of the Ensemble |
|---------------------|-----------------------------|-------------------|------------------------|-----------------------------------|---------------------|
| 35                  | 40.4                        | 0.325             | 124                    | 2.26                              | 17.9                |
| 55                  | 28.9                        | 0.291             | 99.2                   | 2.13                              | 13.6                |
| 110                 | 12.8                        | 0.096             | 134                    | 1.18                              | 10.8                |

Table 1: The effect of channel height on frequency shifts. Particle was trapped between the electrodes in each case and their responses were recorded. As the channel height was increased, the intensity of the electric field was reduced and the responsivity of the device decreased. In all cases, at least 50 nominally identical particles were flown through the active region. By using the maximum frequency shifts induced for each particle for a given channel height, Mean Frequency Shift and Ensemble Standard Deviation are calculated. Noise Level is obtained by calculating $\sqrt{2} \times \sigma_A(\tau_{PLL})$ where $\sigma_A(\tau_{PLL})$ stands for the Allan Deviation of the device for the PLL timescale, $\tau_{PLL}$, just prior to introducing microparticles into the sensor. SNR in frequency shift is calculated as the mean frequency shift over the noise level. SNR of the Ensemble is calculated by dividing the Mean Frequency Shift to Ensemble Standard Deviation.