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Title: Microwave sensing and heating of individual droplets in microfluidic devices

Our research primarily focuses on fundamental studies of microfluidic transport phenomena and Lab-on-a-Chip technology for a wide range of applications. Here we present a novel microwave system for simultaneous sensing and heating of individual nanoliter-sized droplets in microfluidic devices.
Microwave sensing and heating of individual droplets in microfluidic devices†

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Droplet-based microfluidics is an emerging high-throughput screening technology finding applications in a variety of areas such as life science research, drug discovery and material synthesis. In this paper we present a cost-effective, scalable microwave system that can be integrated with microfluidic devices enabling remote, simultaneous sensing and heating of individual nanoliter-sized droplets generated in microchannels. The key component of this microwave system is an electrically small resonator that is able to distinguish between materials with different electrical properties (i.e. permittivity, conductivity). The change in these properties causes a shift in the operating frequency of the resonator, which can be used for sensing purposes. Alternatively, if microwave power is delivered to the sensing region at the frequency associated with a particular material (i.e. droplet), then only this material receives the power while passing the resonator leaving the surrounding materials (i.e. carrier fluid and chip material) unaffected. Therefore this method allows sensing and heating of individual droplets to be inherently synchronized, eliminating the need for external triggers. We confirmed the performance of the sensor by applying it to differentiate between various dairy fluids, identify salt solutions and detect water droplets with different glycerol concentrations. We experimentally verified that this system can increase the droplet temperature from room temperature by 42 °C within 5.62 ms with an input power of 27 dBm. Finally we employed this system to thermally initiate the formation of hydrogel particles out of the droplets that are being heated by this system.

When operating as a sensor, the resonator is able to remotely detect the content of each droplet based on its electrical properties (permittivity, conductivity); and when operating as a heater, the resonator provides rapid, localized, selective, and self-triggered heating of individual droplets. The resulting resonator architecture should find uses as a sensor in monitoring reaction output and as a thermal actuator in various material synthesis strategies.

Temperature control is one of the key functions required for many droplet-based microfluidic applications such as polymerase chain reaction, thermally based protein analysis and thermally initiated material synthesis. Various external heater designs (resistive, joule, Peltier modules) have been incorporated into microfluidic devices; however, these methods rely on thermal conduction to deliver energy and do not provide sufficient localization to heat up a single droplet. Laser based techniques are able to achieve sufficient spatial localization but require complicated configurations to perform simultaneous heating and sensing (infrared laser, excitation laser for fluorescence, etc.). Microwave heating, in contrast, can selectively deliver energy to or probe a single droplet without affecting its carrier fluid by taking advantage of the disparity in dielectric properties between droplets. Microwave technology has been applied to material spectroscopy, sensing and dielectric heating, especially for drug discov-
Microwave heating of droplets in microfluidic chips has also been demonstrated; however, the reported techniques cannot achieve selective heating of individual droplets due to their working mechanisms\(^\text{16,17}\) as explained below.

The reported microwave techniques\(^\text{16,19}\) employ microwave cavities or transmission line resonators that are based on the constructive interference of propagating waves to deliver energy for heating purposes. In order to heat up individual nanoliter-sized droplets, such working mechanisms require extremely high resonance frequencies which require the resonators to be fabricated to an extremely high accuracy adding prohibitive challenges to practical operations. This is because the size of such resonators is on the order of \(\lambda/2\), where \(\lambda\) is the operating wavelength and normally ranges from 1 mm to 1 m. Their corresponding operating frequencies range from 300 GHz to 0.3 GHz. The smaller the operating wavelength, the higher the resonance frequency required. If this type of resonator is chosen for heating up a single nanoliter-sized droplet (\(i.e.\) a 100 \(\mu\)m sphere) with a high efficiency, its operating wavelength and corresponding resonance frequency would be on the order of 200 \(\mu\)m and 1500 GHz, respectively. Such a high resonance frequency is practically prohibitive because of the extremely high accuracy needed in fabrication of electrodes. In addition, the electric field distribution within the resonator is a sinusoidal function which prevents localized heating with a high efficiency. Therefore, a different working mechanism is needed for selective heating of individual droplets, which prompts this study.

**Experimental**

**Device fabrication**

The device consists of two parts, a base with the microwave components and a polydimethylsiloxane (PDMS) mold with the designed microchannels for making droplets, which are fabricated separately and then bonded together. The electrical traces for the microwave components were fabricated using a combination of photolithography and electroplating (see Fig. S6 and S7, ESI\(^f\)). Briefly, the positive photoresist, S1813 (Rohm-Haas), is spin coated at 1500 rpm for 60 s onto the 50 nm thick copper film (EMF Corporation) that is pre-deposited on a glass slide and then baked at 120 °C for 75 s. The resonator design is patterned into the photoresist via UV lithography and subsequently developed with the developer, MF-319 (Rohm-Haas). The patterned slide is then immersed in an acidic copper electroplating solution (0.2 M CuSO\(_4\), 0.1 M H\(_3\)BO\(_3\), and 0.1 M H\(_2\)SO\(_4\)) and electroplated at 2 mA and 4.3 V for 2 min and then 7 mA and 15 V for 24 min. After electroplating, the photoresist is removed with acetone leaving an electroplated copper film approximately 5 \(\mu\)m thick. Next, the original base layer of copper is removed by etching with dilute ferric chloride (MG Chemicals). A passivation layer of a PDMS variant (EG-6301, Dow Corning) is then spin coated at 4000 rpm onto the glass to protect the electrical traces. A subminiature version A (SMA) connector (Tab Contact, Johnson Components) is then soldered to the electrodes of the microwave components to provide an external connection to the microwave function generator (HMC-T2100, Hittite) for heating or to the vector network analyzer (VNA) (MS2028C, Anritsu) for characterizing the microwave sensor (Fig. S4, ESI\(^f\)). Microchannels are fabricated from PDMS using standard soft-lithography techniques. The PDMS is mixed in a 10 : 1 ratio of base to curing agent and moulded against SU-8/ silicon masters and then cured at 90 °C for 2 h. The moulds are then cut out from the masters and fluidic access holes are made using a 500 \(\mu\)m biopsy punch. Both the finished microwave parts and the PDMS chip are then treated with oxygen plasma at 29.7 W, 500 mTorr for 45 s. The plasma treatment process renders the PDMS hydrophilic; however, for water in oil droplets, the PDMS channels should be hydrophobic to form the oil phase. To do so, the chip is heated at 200 °C for 24 h.

**Materials**

All aqueous NaCl and glycerol solutions were prepared with double-distilled water. Similarly, 2 mM aqueous fluorescein (Invitrogen) solutions were buffered at 7.1 pH with Tris-HCl and at 9.0 pH with sodium bicarbonate, for heating and mixing experiments, respectively. For droplets we used Fluorinert FC-40 with 2% perfluoro-1-octanol surfactant (Sigma-Aldrich) as the continuous phase.

**Microwave characterization and control**

Resonance of the microwave device was characterized by measuring the reflection coefficient (dB) for a range of frequencies using the VNA. For heating, the resonator was connected to the high power microwave signal generator. Each chip was mounted onto an inverted epifluorescence microscope system (Eclipse Ti, Nikon) with a high speed CMOS camera (Phantom v210, Vision Research) and a low speed, high sensitivity CCD Camera (Retiga 2000R, QImaging). Fluids were connected to the device by ETFE (ethyltrifluoroethylene) tubing (Tefzel, Upchurch Scientific) and driven by a high precision microfluidic pressure system (MFSC-8C, Fluidgine) or a dual syringe pump (Pump33, Harvard Apparatus).

**Temperature measurements**

The temperatures of single-phase fluids and droplets in the channels were experimentally measured using a microscopy fluorescence thermometry technique.\(^\text{20}\) Fluorescein dye was dissolved in 10 mM Tris-HCl buffered at pH 7.1. Although fluorescein is normally a poor candidate for thermometry measurements because its quantum efficiency shows weak temperature dependence, it does possess strong pH dependence in the pH range of 6–7. Concurrently, Tris-HCl has a strong pH-temperature dependence, which allows the pH–fluorescence behaviour of fluorescein to be converted into a temperature dependent fluorescence response. A calibration curve was obtained using the methods outlined previously.\(^\text{20}\) Images of the microchannel with the microwave heater on are normalized against images with the heater off to remove artifacts associated with variation in geometry, illumination and detector sensitivity. For droplet experiments, long
exposure times (4 s) and frame averaging (160 frames) were employed to obtain steady profiles due to the periodic nature of the flow.

Results and discussion

Sensor and heater design

To meet the urgent need for a new working mechanism that enables heating of individual nanoliter-sized droplets, we propose the use of electrically small microwave resonators because they allow the maximized power to be transferred to a small volume.21 Electrically small resonator structures are based on metamaterial unit cell designs, 22,23 which were originally developed to engineer artificial materials with specific electromagnetic responses and to control plasmonic behaviours at frequencies lower than the optical spectrum.24,25 Resonator structures have been previously used for sensing purposes,26 although we use such a structure for both heating and sensing of droplets.

The proposed microwave resonator consists of two electrical traces as shown in Fig. 1: (i) the resonator, a circular inner loop with a small gap through which the microchannel is to be aligned; (ii) a concentric excitation loop that supplies microwave power to the resonator. The resonator was designed to operate below 3 GHz as microwave components in this frequency range are inexpensive and widely available. Microwave energy is inductively coupled to the resonator by the excitation loop and there is no physical contact between the two loops. Here we fabricate the excitation and resonator loops in a coplanar arrangement for easy alignment; however, the excitation loop can be made independent and the resonator can be activated off-chip allowing for remote control.

In such a microwave structure, the electric field energy is stored within the capacitor of the resonator (the gap region) and the magnetic field energy is stored in the inductor of the resonator. If a microfluidic channel is aligned with the capacitive gap, the passing fluid interacts with the electric field generated by the capacitor causing a frequency shift as illustrated in Fig. 2. When there is a perturbation in the permittivity of the medium, the resonance frequency shifts by27

$$\Delta f = \frac{-\int_{V_0} \Delta \varepsilon E \times E_0 \, dv}{\int_{V_0} \left(\varepsilon E \times E_0 + \mu H \times H_0\right) \, dv}$$  (1)

where $E_0$ and $E$ are the electric fields before and after the perturbation, $H_0$ and $H$ are the magnetic fields before and after the perturbation, $f$ is the resonance frequency before the perturbation, $\varepsilon$ is the permittivity of the medium and $\mu$ is the permeability of the medium. This shift can be used to sense the presence of a single droplet and its content. In addition, if power is sent at the shifted frequency, only the droplet is internally heated without affecting its surrounding medium (such as oil) or the chip material. A powerful feature of such a design is that power transfer can be set to a specific frequency related to the content so that the appearance of a droplet of the right type triggers the heater.

Sensing performance

As shown in eqn (1), the frequency shift, which also indicates the sensitivity of the sensor, is a function of the permittivity contrast between the droplet and its surrounding medium and...
the volume of the droplet. It can be seen that the sensitivity increases with the permittivity contrast. The effect of the droplet size on the sensitivity depends on the relative size of the droplet and the sensor. As a result of the confinement of the electric field in the sensing region, when the size of the droplet is larger than the sensing region, droplet size does not affect the resonance frequency and thus the detection sensitivity. The focus of this work is to demonstrate the feasibility and potential of the microwave technique for simultaneous sensing and heating of individual droplets, so the droplet size is chosen to be larger than the sensing region.

To determine the resonator’s sensing capability, we measured the reflection coefficient of the resonator for various fluids in microchannels as a function of frequency as summarised in Fig. 2. It can be seen that the sensor is able to differentiate between fluids of different permittivity (Fig. 2a) and conductivity (Fig. 2b). The resonance frequency shift with respect to air was found to be 18.5 MHz for silicon oil, 12.5 MHz for FC-40, 174.5 MHz for water and 149 MHz for the 25% and 50% glycerol–water mixtures (wt.%), respectively.

To demonstrate the sensor’s effectiveness in handling non-ideal solutions, we tested the sensor against various dairy fluids (Fig. 2c). Each fluid shows distinct profiles that may be used to identify fat and dissolved salt content, both of which are important parameters in dairy herd health.28,29 In addition, the microwave resonator was also applied to detect the presence of water droplets (Fig. 2d), suggesting that it could potentially be used as a coulter counter in many applications.
Heating performance

The resonator dissipates energy by three mechanisms. The first is by radiation, which is negligible for a small resonator due to its low radiation resistance. The second mechanism is the conductive loss in the resonator, which is spread through the loop, therefore heating due to the conductive loss is not localized. The third mechanism is the dielectric loss within the capacitive gap region. According to our numerical analysis of the microwave resonator structure, 45% of the energy is dissipated within a 2 nL volume in the capacitive gap region. According to our numerical analysis of the microwave resonator structure, 45% of the energy is dissipated within a 2 nL volume in the capacitive gap region (see Fig. S1, ESI† for the details on the design and characterization of the resonator), which shows that the electrically small resonator can deposit the microwave energy with a high efficiency.

To quantitatively evaluate the resonator’s heating performance, several single phase fluids and then water-in-oil droplets were heated using the resonator. Pumping only pure water through the microchannel, the temperature at the exit of the resonator was measured for a range of the applied powers and frequencies as shown in Fig. 3a. The resonance frequency was 2.69 GHz. The blue points present the measured data and the black line is the average of four different measurements. (c) The temperature distribution over the channel where the droplets are being heated at different frequencies shows that the temperature reduces by 20 °C with a shift of 60 MHz in the operating frequency. (d) The temperature distribution along the channel at 2.69 GHz is plotted for 27 dBm and 25 dBm input powers. The temperature increases with the operating frequency within the heating zone. In this experiment, the droplets are 367 μm long generated based on flow rates of 11.63 μL min⁻¹ and 7.71 μL min⁻¹ for the continuous and dispersed phases, respectively, in a 200 μm wide, 50 μm high channel.
temperature rise as a function of the applied power (at the resonance frequency) was recorded as shown in Fig. 3b. Micrographs of the fluid temperature distribution near the resonator for two input powers are presented in Fig. 3c. The images show that droplets are being heated slightly before the heater due to the fringing fields generated by the capacitor and heat conduction through the surrounding chip material. Nevertheless, as droplets pass through the capacitance region they are heated very rapidly. In this example, the droplets (367 μm long) have a residence time of 5.62 ms in the heating region and their temperature increases by 42 °C with an input power of 27 dBm. The average energy in each droplet is estimated to be 0.647 mJ by assuming the specific heat of water is 4.186 kJ kg⁻¹ K⁻¹ and the water droplets are rectangular cuboids of 367 μm × 200 μm × 50 μm. By changing the residence time (through decreasing the speed) or the applied power, the temperature of the droplets can be finely tuned (Fig. 3b).

A sudden and well controlled heating pulse has the potential to initiate thermal based polymerization within droplets. The developed microwave heater has the capability to provide controlled heating pulses. To demonstrate this, we applied the microwave heater to polymerize poly(N-isopropylacrylamide) (PNIPAM) hydrogel particles within microdroplets. Precursor chemicals, 0.5 g mL⁻¹ N-isopropylacrylamide monomer, 0.02 g mL⁻¹ N,N'-methylenebisacrylamide as a cross-linking agent, and 4,4-azobis (4-cyanovaleric acid) for the thermal initiator, were combined in water and used as the dispersed phase. Hexadecane was used as the continuous phase. By targeting the resonance frequency of water, the initiator would disassociate into radicals and the hydrogel is generated by free radical polymerization. The results can be seen in Fig. 4, where a precursor droplet approaches the microwave generator. Subsequently, a separate, round hydrogel particle is formed behind the droplet. The hydrogel, due to being heated, exhibits temperature dependent hydrophobicity and repels itself out of the aqueous droplet. Moreover, the cohesion of the hydrogel, in addition to it repelling both water and the surrounding hexadecane oil, causes it to self-assemble into a circular shape at the back end of the droplet.

Another interesting application of confined heating is the manipulation of droplets using Marangoni stresses created by temperature gradients. Previously, laser based localized heating has been applied to stop, direct, merge and mix droplets in microchannels using this technique. It was hypothesized that the same manipulations could be achieved with the microwave resonator structure. However, we found that the temperature gradients were insufficient to provoke such manipulations [see ESI S3].

Conclusions

The microwave structure presented here can be used effectively as a sensor and heater and should find broad applicability in various microfluidic devices. For example, it could be used to detect the outcome in various conductivity based bioassays in microdroplets where conventional contact electrodes would lead to cross-contamination. It can also be applied to detect conductive metal particles or traces in oil streams as a feedback control tool for engine systems to avoid unnecessary oil replacements and testing.

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Notes and References