5-2023

Microwave Characterization of Polymeric Microparticle Morphology

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An Undergraduate Thesis Presented to the Clemson University Honors College
in Fulfillment of Departmental Honors in Electrical and Computer Engineering

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May 12th, 2023
Clemson, SC, USA

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***Abstract*—Biological cell function and overall health are highly defined by the cell's morphology. Sorting cells based on their shape is recently a major interest to the biomedical field for the future of medical treatments and health diagnostics. This work presents a new shape-based particle sorting technique using a novel microwave sensing system as a step toward biological cell sorting. The new sensing system consists of a grounded coplanar waveguide (GCPW) transmission line that simultaneously serves as a single-particle microfluidic channel and a microwave sensor, which is paired with a microwave interferometer to boost signal-to-noise ratio (SNR). Polystyrene microspheres, a common organic polymer, are differentiated into different aspect ratios (ARs) using a custom stretching technique to simulate non-spherical biological cells, ideal for isolating volume-effects on the microwave signal due to the low coefficient of variation (CV < 2%) of particle size. Although some microwave measurement data suggests the signal is dependent on microparticle shape, more data points and a machine learning algorithm is needed. The GCPW sensor demonstrates a previously-unattainable level of sensitivity. An early investigation into the effects of orientation of elongated particles on the microwave signal is also explored, as well as the design of an improved GCPW sensor.**

***Keywords*—biological sensor, microwave design, coplanar waveguides, microparticles, microfluidics.**

I. INTRODUCTION

Cell shape or morphology is a fundamental cell property that is an indicator of cell health [1], regulates stem cell lineage commitment [2], enables cells to adapt to the changing local chemical environment [3] and induces microorganism pathogenesis, such as in C. albicans [4]. Therefore, measuring cell shape is important in biology [5] and health care service [6], among others. Microscopy and optical imaging have been standard methods for cell shape measurement, though fluorescent labels are often needed that present phototoxicity problems. Therefore, developing a label-free and non-invasive method of characterizing cell morphology is of great interest. Lower frequencies than optical, such as microwave and radio-frequencies (RF), may have untapped potential in non-invasive sensing due to their ability to penetrate mediums like the human body. If these frequencies can detect shape-dependent features of particles, signal data could be paired with a machine learning algorithm to allow for label-free sorting. In this work, it is shown that microwave techniques are promising to be such a method through the measurement of single polystyrene particles of different aspect ratios (ARs). The first use in available literature of a coplanar waveguide's gaps as a microfluidic channel is demonstrated in this experiment. Additionally, the concept of measuring microparticle shape using microfluidics is new, which the novel sensor design is used to explore.

This work is the culmination of my last two years of work in Dr. Pingshan Wang's Microwave Lab-on-chip laboratory. This project also draws on knowledge I have built on since starting work in Dr. Wang's lab in the summer of 2019 on my first work and publication, the bulk-measurement of yeast cells using a microstripline RF sensor [7]. I continued work on studying biological cells using a new microstripline sensor to observe the effects of RF fields on neurons. COVID-19 then disrupted access to the lab, and upon return to the lab in January 2021, I shifted work to non-biological particles to support the undergraduate biologists we recruited. The microparticle experiment was formed with the intention of developing an electrical sensing platform for biological cell sensing. Microparticle shape was chosen for its interesting properties and potential to uncover new science. Over the last few years, my work on this project has involved finite-element analysis, multiphysics simulations in HFSS and ADS, as well as SOLIDWORKS designs for new transmission lines, microwave interferometer design, 3D-printing, programming in MATLAB and Python language, PCB design, materials science, biology, microfabrication and far more. This paper for the Clemson Honors College is a brief summary of the last project I have done for Dr. Wang's group, but I hope the work I have done over the last four years will leave a lasting impact in our group, especially to my mentees.

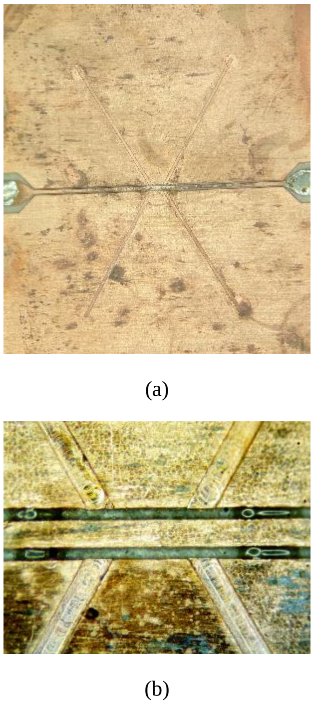


Fig. 1. A top-down view of the coplanar waveguide sensor. 140 μm-wide microfluidic channels are machined into the copper-substrate stackup. The two inlet-outlet channel pairs are machined into the copper ground planes and take the shape of a "cross" (a). Each pair diagonally approaches a 0.6 mm-long sensing zone parallel to the signal line (b).

II. SENSOR AND MICROWAVE INTERFEROMETER

A. *Coplanar Waveguide Sensor*

In this experiment, the channels in between the ground planes and the signal line of a grounded coplanar waveguide (GCPW) transmission line are used as microfluidic channels for single particle measurements. The sensor used in this experiment is pictured in Fig. 1, though multiple iterations and an experimental new iteration of this sensor design were created. The sensing zone consists of a 0.6 mm section parallel to the signal line that is confined by ultraviolet glue "walls" on either side to prevent fluid leakage. The long channels that approach the sensing zone in a diagonal direction serve as inlets and outlets. The sensor was fabricated using micromachining tools with Clemson Machining and Technical Services on Rogers 3003 PCB material. To take measurements, one side of the sensing zone is filled with water, and the other is filled with polystyrene particles suspended in water. The sensor achieves its sensitivity through the breaking of symmetrical wave transients along the signal line. When a particle enters one channel, the propagation velocity of microwave transients on one side of the signal line changes due to the particle's presence, causing a disruption in S-parameter signal [8]. This disruption serves as the basis for our sensing technique, which we demonstrate shows a significant improvement in sensitivity than other microwave sensor designs.

The channels are covered with a flat slab of polydimethylsiloxane (PDMS), a polymer often used for microfluidic applications, held in place with an acrylic lid applying uniform pressure so that the PDMS can relax into and block crevices causing leakage. Fig. 2 presents the broadband S_{21}-parameter measurement of the sensor with the PDMS cover, acrylic lid, and water in both channels. The S_{11} measurement is not shown but is consistently below -10 dB around the frequencies of interest (1 MHz – 10 GHz). Broadband testing suggests high-sensitivity the GCPW design due to relatively low S_{21} losses (forward transmission) and low S_{11} (reflections).

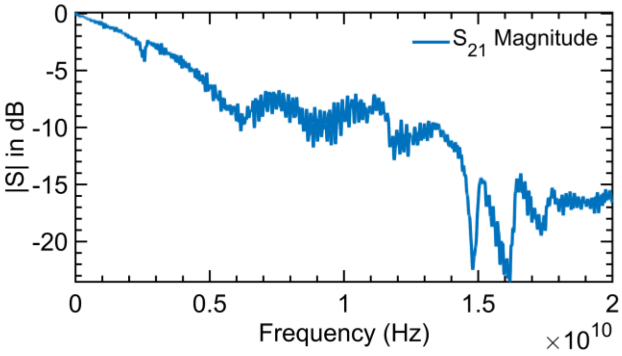


Fig. 2: S_{11} and S_{21} parameter measurements of sensor with PDMS covering and water in both channels.

B. *A Tunable Microwave Interferometer*

To identify shape-based characteristics for polymeric microparticles and eventually biological cells, it is desirable to measure many frequencies to capture frequency-dependent information. Additionally, signals are expected to be small for 20 µm particles passing thorough a 140 µm channel. A microwave interferometer is used to boost signal-to-noise ratio (SNR) for highly sensitive measurements paired with a digitally tunable attenuator for frequency tuning and balancing the loss between the two branches [9]. The measurement setup is shown in Fig. 3.

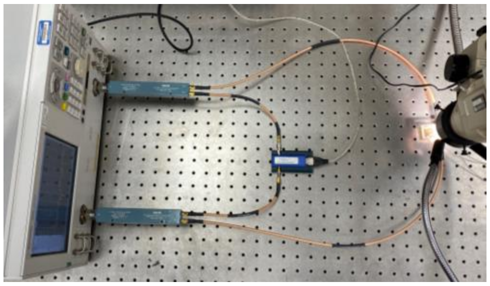


Fig. 3: Microwave interferometer with digitally tunable attenuator in the shorter branch and the sensor in the longer branch with microscope above.

III. PARTICLE PREPARATION

Particle volume must remain agnostic from the characterization process. In other words, the experimental design must be able to accurately assume that particle shape is the dominating factor of measurement differences. For this reason, 20 µm polystyrene (PS) microspheres with low diameter variation (CV < 2%) from MilliporeSigma were chosen as the candidate for the experiment. The spherical particles could then be differentiated into ellipsoidal particles by stretching, using a similar technique to Champion et al [10]. The stretching process is as follows: particles are immersed in a high-grade polyvinyl alcohol (PVA) aqueous solution mixed with glycerol (plasticizer), which then form elastic films upon evaporation of water content. Spherical particles are then stretched in one-dimension by heating the films past the glass transition temperature of PS ($T_g = 100°C$) in a vacuum oven and applying mechanical force in opposite directions with clamps driven by a stepper motor. The stretched films are cooled at room temperature, and particles are extracted by dissolving the PVA films in water at 80°C. Examples of stretched particles are pictured in Fig. 4.

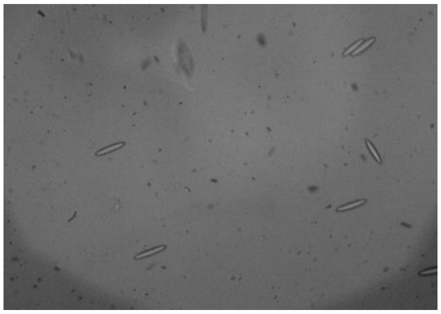


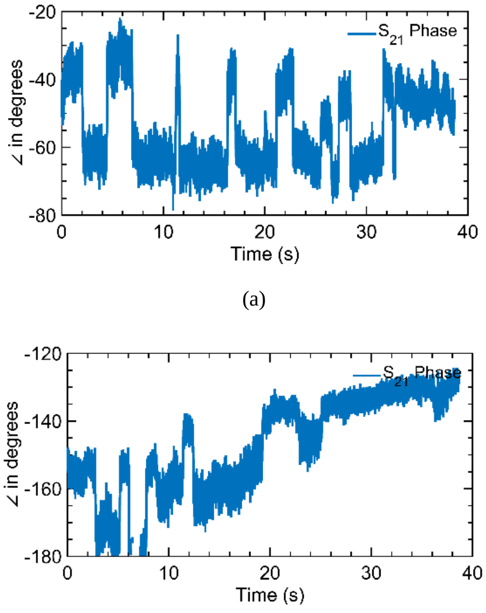
Fig. 4: Stretched 20 µm polystyrene particles (AR = 4)

IV. MICROWAVE MEASUREMENT RESULTS

A. *Stretched Particle Morphology*

A stretched particle "soup" was prepared by mixing stretched particles of many ARs. This soup was flowed through the sensor to correspond measurements with video footage with the plan to reveal shape-dependent microwave signal changes. By timing the pumping action of the inlet tubing, the same particle could be measured multiple times. "Pumping" was accomplished manually by using a syringe or by elevating the silicone tubing external to the sensor. Flexural stresses of fluid flow through the silicone tubing caused the tubing to expand when the syringe is pushed and to contract when the syringe pressure is relieved. A passive flow of fluid backwards is the result of this action, which can be utilized to have a single particle flow through the sensing zone multiple times, picking up multiple signals for the same particle. The particle soup was thus useful to measure many different ARs multiple times. Particles could also be measured multiple times without syringe action by elevating and lowering the inlet and outlet silicone tubing. Gravity does the work of changing the direction of the passive capillary-action flow of the fluid in the silicone tubing.

While multiple frequencies between 2 GHz and 3.1 GHz were tested on both spherical and stretched particles, 2.1 GHz resulted in the highest SNR. While differences were minimal in terms of S_{21} magnitude, example data in Figure 5 shows the clear differences in S_{21} phase observed between spherical and stretched AR = 3 particles. The difference is notably higher than the extremes of particle size difference caused by a CV < 2%, leading me to believe that these signal change observations are not volume-dependent, but shape-dependent. More measurements need to be taken at a larger range of frequencies to conclude the relationship between signal phase, signal magnitude, and particle shape. It is interesting that S_{21} magnitude changes are minimal, even though the interferometer boosts SNR significantly and reduces phase SNR. I suspect that measurement data could improve by taking measurements without the interferometer setup.



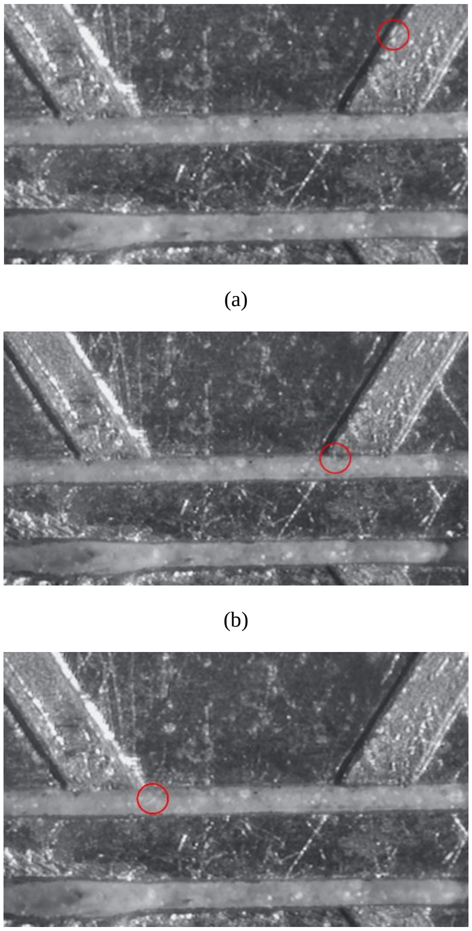
(a)

(b)

Fig. 5: S_{21} phase difference between spherical (a) and stretched AR = 3 (b).

B. *Stretched Particle Orientation*

Particles tend to follow the same hydrodynamic path due to laminar flow within the microfluidic channel. Thus, as long as the path of flow is the same, the orientation of a spherical particle is indiscriminate of the direction of flow or the point of observation. In testing stretched particles on the other hand, it was found that despite closely following the same hydrodynamic paths, there is randomness to the orientation of the stretched particles. Preliminary observations show that ellipsoidal particles more often follow the hydrodynamically-favorable orientation with a higher velocity. The long length of the channel (0.6 mm) allows close observation of particles when moving slow, and video footage reveals rotation of the stretched particles while inside of the sensing zone. Fig. 6 shows an image taken of an AR = 2 particle that exhibits an unusual orientation, chosen to explore if particle orientation is correlated to changes in the microwave signal due to yet-unexplained electromagnetic effects, and whether the act of rotation of stretched particle orientation affects the signal.



(a)

(b)

(c)

Fig. 6: AR = 3 Particle flowing from top right inlet (a) to top left outlet. Particle orientation rotates as the particle enters the sensing zone (b) from nearly vertical to nearly horizontal (c).

C. *Clues of Orientation-Dependent Signal Change*

Initial results on orientation effects are interesting but require further exploration. Measurement at 2.1 GHz is shown in Fig. 7. The signal "wiggles" during the particle's flow inside the sensing zone is supplemented by microscope video footage of the stretched particle rotation (AR = 3). It is still unclear if and why these signal changes are due to the particle orientation changing or something unaccounted for, such as particle shape or environmental noise. Further, different stretched particles can enter the channel with different orientations, for which control can be achieved using the same particles multiple times and hydrodynamic focusing. Improvements to the measurement system's SNR and noise level are also a next step.

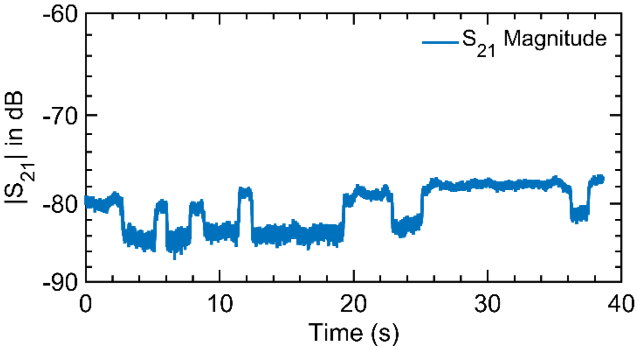


Fig. 7: S_{11} and S_{21} parameter measurements of sensor AR = 3-3.5 stretched particles. Signal length variation is due to manual speeding-up and slowing-down of the rate of flow.

V. DISCUSSION

This work has developed a promising platform for exploring the shape characteristics of single particles and biological cells, an exciting new contribution to the highly active research field of microwave sensing for biological applications.

Initial results on the ability of using microwave signals to detect microparticle shape are inconclusive but promising for future measurements, showing signal changes greater than the maximum variations of particle volume. Controlling volume-based signal effects is a major advantage of stretching PS microparticles rather than buying off-the-shelf microparticles of different shapes, which have different volumes than spherical particles of the same size. It is noteworthy that while the microwave interferometer paired with the GCPW sensor yields impressive S_{21}-parameter SNR, the signal differences between different ARs was seen in S-parameter phase. This leads me to believe the interferometer may be obscuring phase information that is essential to shape characterization. One of the original inspirations for our sensor that derives its sensitivity from field asymmetry [8] showed substantial time delay of RF transients around bends in a coplanar waveguide structure. In our experiment, these "bends" take the form of a particle's presence creating asymmetry between the two sensing channels. Transient phase may thus contain information worth studying.

The novel coplanar waveguide microfluidic sensor has already demonstrated higher SNR than in available literature, a marginal improvement from about 5:1 to 7:1 [11]. There is a significant practicality in using this new sensor on biological cells fit for biomedical studies, such as yeast and CHO cells, due to its long sensing zone, permitting microwave measurements longer than 10 seconds. The exploration of DC-biasing of AC fields to observe changes in single-cell membrane potential is also potentially interesting.

Even though improvements to the measurement system are necessary for future experiments, it is not likely that uncertainties from the measurement setup obfuscated the reported data. A gradual decay in SNR over time due to sensor clogging is unavoidable due to flaws in the current technique for sensor machining. This effect, however, becomes major after many hours, but the reported results occurred within nearly one hour of each other. It is likely that there is much more information to be gathered on particle morphology, and perhaps orientation, at higher frequencies, for which the system and sensor must be adapted. Further, the digitally tunable attenuator should be replaced with a non-active mechanical attenuator to reduce noise and further boost SNR.

To make these improvements to the experiment, a second iteration to the GCPW sensor design is proposed. Figure 8 shows an early draft of this sensor, with GCPW channels micromachined into the same Rogers 3003 PCB laminate as before.

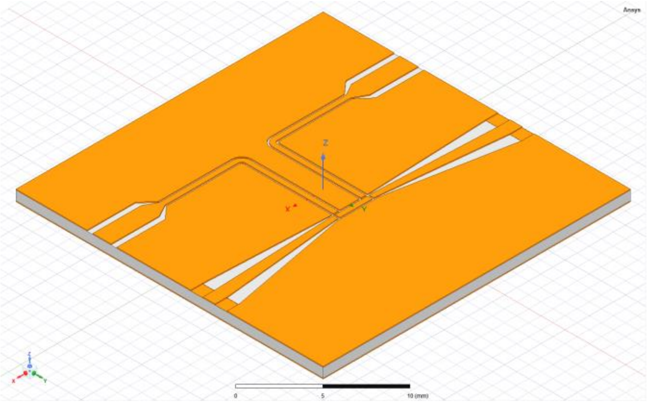


Fig. 8: Ansys HFSS simulation and model of new GCPW sensor with simplified microfluidics. The bend allows the "critical section" of the sensor to be approach microfluidic channels from a horizontal direction, as opposed to at slanted directions in the previous model. By minimizing the points for particles to clog the sensor, we hope to achieve greater levels of sensitivity.

To avoid the problems of gradual clogging and assembly misalignment, the main purpose of this second version is to greatly simplify microfluidics. The channel containing particles suspended in fluid should be straight (rightmost channel pictured in Figure 8) and with particles flowing in and out via horizontal inlets and outlets. The significant challenge in fabricating this design comes from the wear of drill bits with diameters on the order of thousandths of an inch. The first GCPW sensor design took many attempts at fabrication at the Clemson machine shop and came out with CPW channels twice as large as desired (140 μm instead of 70 μm). Other methods of fabrication are being explored, such as thin film deposition and laser engraving. The simulation results show promise in utilizing the symmetry-dependent sensing scheme described in the original motivation for the GCPW sensor. Figure 9 shows a cross-section of the field distribution at the center of the sensing zone, showing nearly-symmetrical fields in both the water and particle channel. Once fabricated, I hope to test the sensor with stretched particles, with and without the microwave interferometer system.

While the main motivation of this experiment was to develop a shape-based identification technique using a novel microwave sensor, interesting results were found arising from the rotating orientations of elongated microparticles. Although the change in signal is not yet explained, the work will be further explored, for what could be an impactful discovery in physics and electromagnetics.

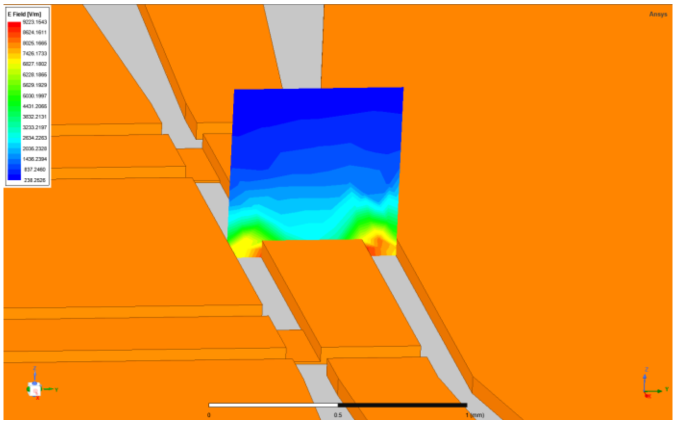


Fig. 9: Ansys HFSS electric field simulation of a cross-section of the new GCPW sensor in the sensing zone. The two microfluidic channels (running vertical on the image) have a nearly-symmetrical field distribution, a requirement of the sensing technique used that makes the GCPW architecture favorable.

ACKNOWLEDGMENT

I sincerely thank my faculty advisor Professor Pingshan Wang and my graduate student mentors Duye Ye, Saiful Islam, and Amir Nazeri. I also thank Professor Thompson Mefford, Professor Sarah Harcum, and ECE department technician David Moline for their guidance and contributions. This project would not have been possible without my experience in the Clemson Honors EUREKA! Program in 2019, the Clemson SURE Research Experience for Undergraduates (REU) in 2021, and constant support in funding and resources from the Clemson Honors College and Creative Inquiry program.

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