A microwave system for the extraction and measurement of *Candida* cells in blood

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***Abstract*— Rapid detection and identification of *Candida* cells in infected patient blood is needed for proper candidemia treatment. In this work, *Candida* spiked bovine blood was processed with two microfluidic devices– a mixer where blood cells are lysed and a separator where *Candida* cells are separated from the lysate. The extracted *Candida* cells were then injected into a broadband microwave sensing device to detect and differentiate two *Candida* species, *Candida tropicalis* and *Candida krusei*. The obtained scattering plot of microwave measurement signals of the *Candida tropicalis* and *Candida krusei* showed a clear separation between the two species. Thus, the system is successfully used to extract and measure *Candida* from bovine blood within 2 hours. The results show that microwave sensors are promising to extract, detect and differentiate *Candida* cells from infected blood.**

***Keywords*— Microwave sensing, *Candida*, microfluidic mixer, microfluidic separator, cytometry.**

I. Introduction

Candida species are the fourth most common nosocomial bloodstream pathogens [1]. Candidemia has an attributable mortality of 15–35% for adults and 10–15% for neonates, and the hospitalization cost for each episode is approximately US $46,000 [2]. A 12-hour delay of proper antifungal therapy can increase the mortality rate by up to 20% [3]. A timely diagnosis is of utmost importance, but the standard method of cell culture and microscopic observation for *Candida* detection and identification can take days to obtain results [4]. Non-culture methods like polymerase chain reaction (PCR) and next generation sequencing (NGS) can rapidly and accurately detect *Candida* in patient blood sample but these expensive methods still need pre-culture before measurement and lack phenotypic drug susceptibility test option [5].

Four species of *Candida* cells and their antifungal drug effects are characterized under flow conditions using a microwave flow cytometer at multiple frequencies [6]. The results show that microwave sensing is promising to address the limitations of current *Candida* detection techniques for rapid detection and identification of *Candida* species at single cell resolution as well as rapid testing of *Candida* antifungal drug susceptibility. Nevertheless, the number of *Candida* cells in patient sample, such as blood, is usually very low, e.g., on the order of 1 cell per milliliter (mL) while red and white blood cell concentrations are a few billion and million cells per milliliter, respectively. At the same time, single cell microwave sensing is usually low throughput, e.g., at less than 1-10 microliter (μL) per minute. Thus, intact *Candida* cells need to be extracted from blood sample, separated from blood lysate, and concentrated. Each of these steps is a difficult challenge, in addition to low yield in system integration. In this work, we use *C. tropicalis* and *C. krusei* as model cells to show the feasibility of a microwave system which uses microfluidic channel-based technique to rapidly extract, detect and identify *Candida* from blood in a continuous flow.

II. System Design Consideration

Fig. 1 is a block diagram of the microwave system, which consists of a microfluidic mixer where blood cells are lysed while *Candida* cells are intact, an inertial separator where *Candida* cells are separated from the blood lysate, and a microwave sensor where *Candida* species are detected and differentiated. A microfluidic channel-based cell concentrator, such as [7], can be developed to reduce *Candida* sample volume after separator, but is omitted in this presentation partly due to the use of high concentration of *Candida* spiked in bovine blood. The mismatch of the flow rate between the microfluidic separator and the microstrip sensor requires an intermediate reservoir, Fig. 1. As a result, a negative pressure pump can be used to pull sample from the reservoir for microwave measurement. Furthermore, a filter is used to remove large aggregates of cell debris.

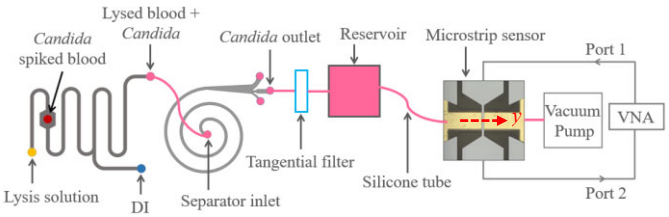

Fig. 1 A block diagram of the microwave system consisting of a microfluidic mixer, an inertial separator, and a microwave sensor.

A. Microfluidic mixer

Microfluidic mixers, Fig. 2, are considered to lyse blood cells with surfactants, i.e., sodium dodecyl sulfate (SDS) in this work, while keeping *Candida* cells intact [8, 9]. Long channels are needed for diffusion-based mixing [8] of chemicals (inlet 1) and samples (inlet 2). Towards the end of the mixer (inlet 3), de-ionized water is used to induce osmotic shock on the surviving blood cells. Meandered channel layout helps to achieve compact mixers where the curved bending ends generate vortices, which facilitate effective mixing.

The microfluidic mixer mold is built using a 3D printer. PDMS is used to build the device. Herringbone structures can further improve mixing efficiency by causing a turbulent flow in the channel [8, 10, 11, 12].

The meandered mixer is a hairpin like structure consisting of 47 rows of channels, each lined with a staggered herring bone

structure on the channel ceiling [8, 9]. The channels are 500 µm wide, 160 µm tall and the herringbone structures are 25 µm wide, 32 µm tall.

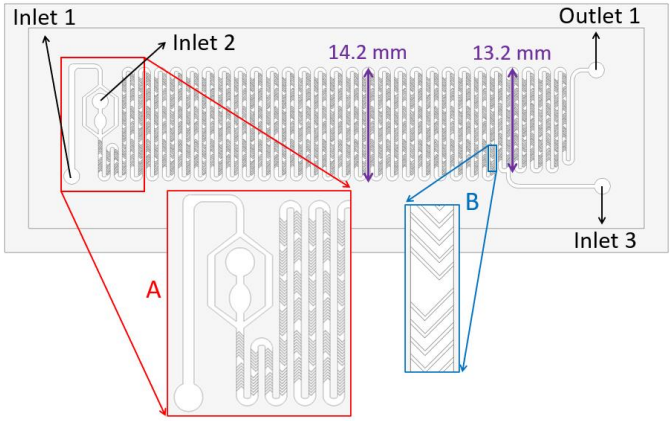

Fig. 2 A microfluidic mixer consisting of three inlets and one outlet. The ceiling of the mixer channel is lined with herringbone structure to facilitate mixing. Zoom-in region labelled as A shows a closer view of inlets 1 and 2 where *Candida* spiked bovine blood and 0.5% SDS solution first mix. Zoom-in region labelled as B shows the top view of alternating herringbone structure lining the ceiling of the microfluidic mixer.

B. Microfluidic separator

Fig. 3 shows a microfluidic separator consisting of an inlet, which is connected to the outlet of the mixer in Fig. 2. There are three separator outlets designed to collect lysed blood and larger debris at outlet 1, *Candida* yeast at outlet 2, and surviving blood cells in lysate at outlet 3. The spiral separator uses inertial method to separate cells/debris of varying sizes [10, 13]. The spiral separator is 400 µm wide, and the outlets 1, 2 and 3 are 1200 µm, 350 µm, and 300 µm wide, respectively. The inertial separator mold is fabricated in a cleanroom using SU-8 on silicon wafer, and the separator is built with PDMS.

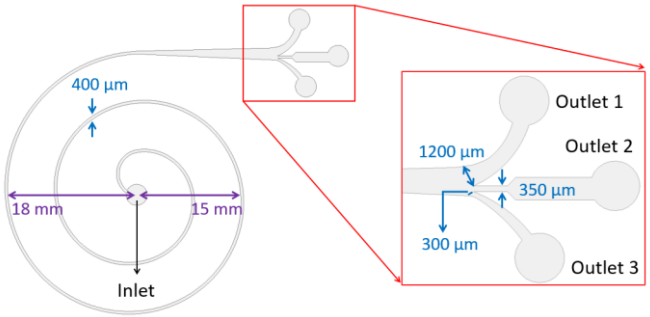

Fig. 3 Microfluidic mixer consisting of one inlet and three outlets.

C. Microwave Sensor

A vector network analyzer (VNA) and a microstrip line-based sensing electrode are used to measure *Candida* cells obtained from the separator, illustrated in Fig. 1. A microfluidic channel is incorporated into the microstrip line for *Candida* cell transport. Fig. 4(a) is a sketch of the cross-section. Ansys HFSS simulation shows that microwave E-field distribution in the sensing zone is reasonably uniform in both vertical, i.e., *z*, and horizontal, i.e., *x* (channel width), directions. When a 4.5 µm diameter polystyrene particle touches the channel top surface or bottom surface in the sensing zone, simulation shows a 6% microwave signal difference. In experiment, a less than 2.7% signal variation is observed when the same particle passes through the sensing zone multiple times [6]. Such small variations indicate reproducible measurement results. Repeatable and reproducible measurements are critical for microwave measurement-based cell analysis.

Fig. 4(b) shows E-field energy distribution along *y* direction. As expected, the distribution agrees well with measured single cell signal.

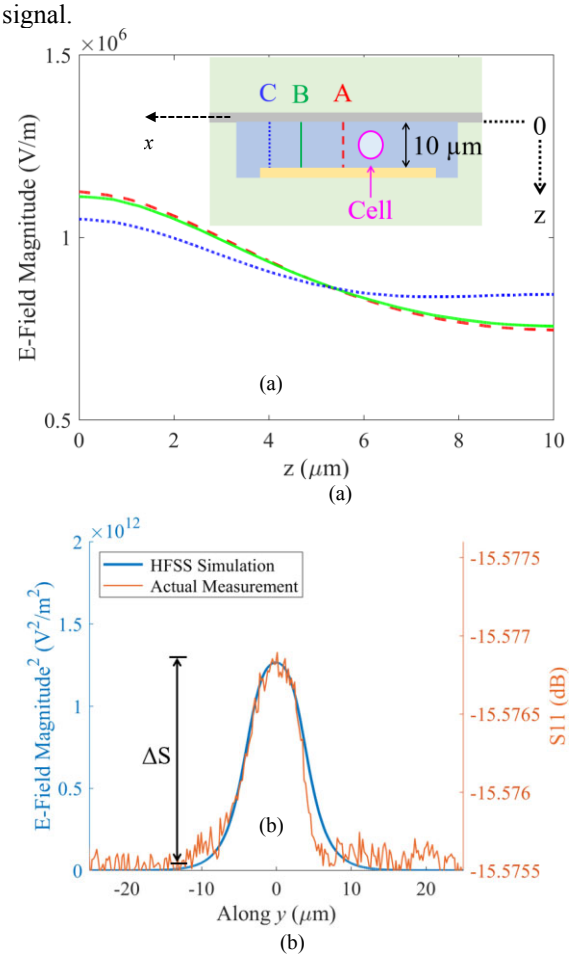

Fig. 4 (a) Simulated field distribution at different *x* locations, i.e., C, B, andA, which are indicated in the cross-section sketch of the sensing zone of the microstrip sensor in Fig. 1. (b) The distributions of simulated E-field energy and measured signal (S_{11}) magnitude. The origin of *y=0* indicates the center of the microstrip sensing zone in Fig. 1.

Coplanar waveguide structures, which are easier to build and more convenient to integrate with microfluidic channels, are not used in this work due to non-uniform sensing E-fields at CPW gaps and the difficulty to control cell location relative to CPW metal surface.

To further simplify the operation, direct two-port microwave s-parameter measurement is performed. Sensitivity enhancement methods, such as interferometers [6], are not used. Fig. 6(a) below shows a typical S-parameter (S11 dB) measurement showing signal changes corresponding to *Candida* cell passing in the sensing zone.

III. Candida Cell Extraction and Microwave Measurement

A. Candida sample preparation

Candida yeast species were purchased from American Type Culture Collection (ATCC). *C. tropicalis* (ATCC No. 13803) and *C. krusei* (ATCC No. 14243) are cultured in YPD (Yeast Extract–Peptone–Dextrose) by following the recommended processes [14]. *C. tropicalis* and *C. krusei* were chosen since they are two of the five most important *Candida* yeasts that cause candidemia cases [1, 2]. Together, they account for 11% of candidemia incidences. Cell viability was determined using trypan blue exclusion test before microwave characterization. In our measurement, more than 99% cells are viable.

B. Microfluidic mixer and separator

Prior to the experiment, fresh bovine blood is collected in 10 mL EDTA (Ethylenediaminetetraacetic acid, an anticoagulant) tube. Overnight *Candida* culture is mixed in blood in a 1:10 ratio (100 µL culture and 1 mL blood) such that approximately 5 million *Candida* cells are present in 1 mL spiked blood. Blood lysis solution (0.5% SDS) and *Candida* spiked bovine blood are injected into inlets 1 and 2, Fig. 5(a), respectively at a flow rate of 200 µL/min each. Deionized water is injected into inlet 3 at a flow rate of 400 µL/min, and the lysed blood and *Candida* exiting the outlet is guided into the separator inlet by a silicone tube.

Fig. 5(a) shows *Candida* spiked bovine blood lysing as it travels in the microfluidic mixer. As blood and lysis solution continue to flow, the red color gets fainter and wider indicating proper mixing and lysing. The even fainter red color towards the end of the mixer is due to further dilution by de-ionized water.

Fig. 5(b) shows the experimental setup where the mixer and the inertial separator are connected with a silicone tube. Centrifuge tubes are used as reservoirs to collect the separated *Candida* cells as well as lysate wastes. This interruption is needed to (*i*) pass the sample through a dead-end flow filter (not the tangential flow filter illustrated in Fig. 1) which in turn prevents sensor clogging and (*ii*) match much lower flow rate (approximately 10 µL/min) required for the downstream microwave sensor.

Outlet 2 of the separator does not yield pure *Candida* cell solution. Some blood cell debris in lysate and surviving white blood cells also remain. Outlets 1, 2 and 3 have 38.9%, 43.2%, and 17.9% *Candida* distribution. More importantly, outlet 1 has large blood debris, outlet 2 has mostly *Candida* cells and a very few white blood cells, and outlet 3 has many white blood cells as intended in the separator design consideration.

Fig. 5(c) shows, from left to right, microscopic images of *C. tropicalis* and *C. krusei* cells before spiking, and the lysed blood containing surviving cells at the separator outlet 2. The reddish coloration of the image on the right is due to lysed red blood cells, none of which survive the lysis process.

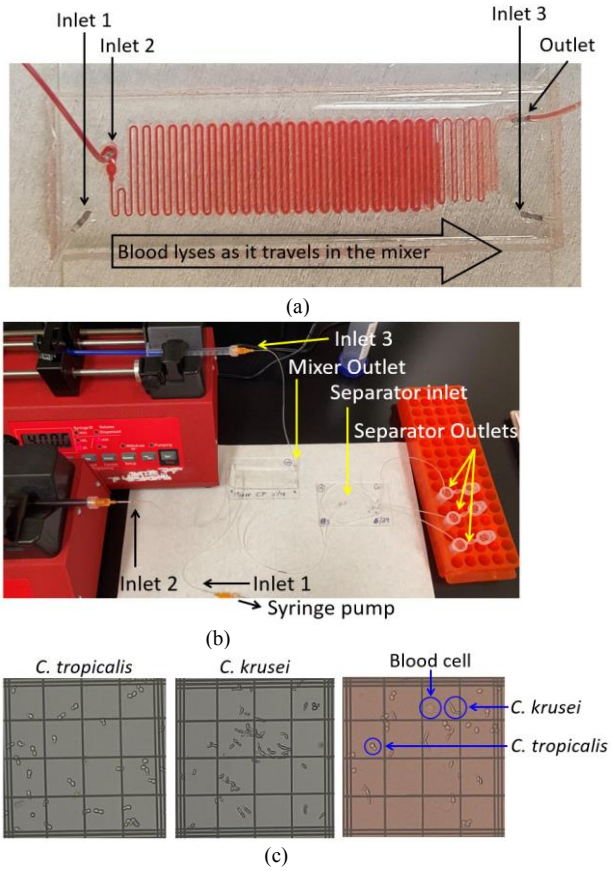

Fig. 5(a) Lysis of *Candida* spiked bovine blood in the microfluidic mixer. (b) Microfluidic mixer and separator system. The outlet of the mixer is connected to the inlet of the separator. (c) From left to right, microscopic images of *C. tropicalis* and *C. krusei* cells before spiking bovine blood, lysed blood containing surviving cells at the separator outlet.

The overall cell lysing and separation process took only a few minutes, as indicated by the operation flow rate. After separation, *Candida* yeast cells remain viable.

B. Microwave measurement

The lysed blood sample collected at the outlet 2 of the separator was filtered and injected into the microwave sensing device in Fig. 1, and scattering parameters were measured using a VNA. The peak values of real and imaginary S-parameters were calculated. Fig. 6(a) shows a typical S-parameter measurement where each time a *Candida* cell passes the sensing zone, a signal change is seen. Fig. 6(b) shows that the measurement is reproducible where a single *C. tropicalis* cell transported back and forth in the sensing zone for a period of 4 minutes shows a 2.3% measurement variation. In figs. 6 (c) and (d), normalized Δ (S-param imaginary) versus Δ (S-param real) were plotted for S11 and S21. Bovine blood spiked with *C. tropicalis* has a clear separation from bovine blood spiked with *C. krusei*. Some overlap in data points can be due to a wide range of cell size distribution for the two species.

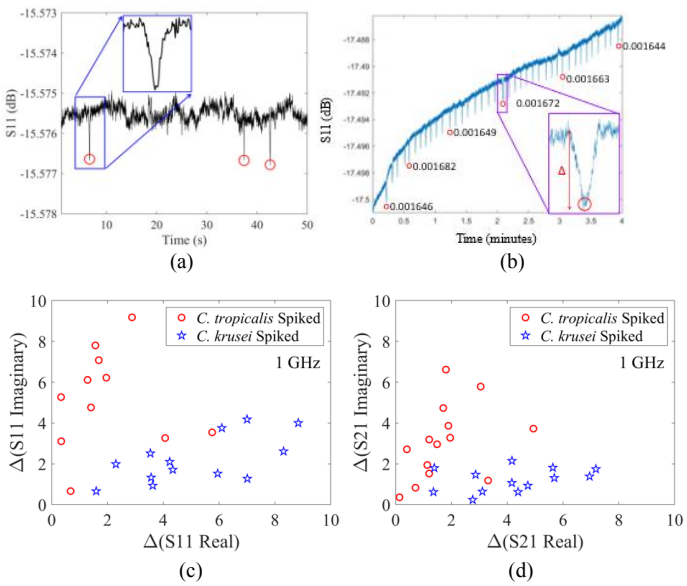

Fig. 6(a) A typical S-parameter (S11 dB) measurement showing signal changes corresponding to *Candida* cell passing in the sensing zone. (b) Measured S_{11} over a 240 seconds (4 minutes) span, which shows reproducible measurement results. (c) Δ (S11 imaginary) versus Δ (S11 real). (d) Δ (S21 imaginary) versus Δ (S21 real). In each plot *C. tropicalis* and *C. krusei* cells are extracted from bovine blood.

IV. Discussion and Conclusion

The above efforts are mostly focused on the key components of the microwave sensing system in Fig. 1. To achieve automatic and continuous system operation, the manual filtration step needs to be removed. Filters like tangential flow filtration (TFF) can be used. TFF can also help condensate *Candida* cell sample. Additionally, *Candida* extraction at high purity and recovery rate is needed. High accuracy microwave differentiation of cell species needs to be further examined.

Nevertheless, in this work, we showed a possible microwave system for rapid direct sample-to-results detection and identification of *Candida* cells in blood. The developed microfluidic module successfully lysed red and most white blood cells in bovine blood with 0.5% SDS. The obtained *C. tropicalis* and *C. krusei* cells are effectively detected and identified with a microstrip based microwave sensor. With further development, the achievable sample-to-results time is promising to be significantly shorter than 2 hours, which is the limit of current *Candida detection* methods.

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References

[1] H. Wisplinghoff, T. Bischoff, S. M. Tallent, H. Seifert, R. P. Wenzel, and M. B. Edmond, "Nosocomial Bloodstream Infections in US Hospitals: Analysis of 24,179 Cases from a Prospective Nationwide Surveillance Study," *Clin. Iinfec. Dis.*, vol. 39, pp. 309–317, 2004.

[2] J. Guinea, "Global trends in the distribution of Candida species causing candidemia," *Clin. Microbiol. Infec.*, vol. 20, suppl. 6, pp. 5–10, 2014.

[3] M.Morrell,V. J. Fraser, and M. H.Kollef, "Delaying the empiric treatment of Candida bloodstream infection until positive blood culture results are obtained:Apotential risk factor for hospital mortality," *Antimicrob. Agents Chemother.*, vol. 49, no. 9, pp. 3640–3645, 2005.

[4] M. M. Usaj, C. H. L. Yeung, H. Friesen, C. Boone, and B. J. Andrews, "Single-cell image analysis to explore cell-to-cell heterogeneity in isogenic populations," *Cell Syst.*, vol. 12, pp. 608 621, 2021.

[5] C. Zurl et al., "T2Candida magnetic resonance in patients with invasive candidiasis: Strengths and limitations," *Med. Mycol.*, vol. 58, pp. 632–638, 2020.

[6] N. Dahal *et al.*, "Spectroscopic Analysis of Candida Species, Viability, and Antifungal Drug Effects With a Microwave Flow Cytometer," *IEEE J. Electromagn. RF Microw. Med. Biol.*, vol. 6, iss. 4, pp. 566-573, 2022.

[7] J. M. Martel, "Continuous Flow Microfluidic Bioparticle Concentrator," *Sci. Rep.* 5, art. 11300, 2015.

[8] A. D. Stroock, S. K. W. Dertinger, A. Ajdari, I. Mezic, H. A. Stone, and G. M. Whitesides, "Chaotic Mixer for Microchannels," *Science*, vol. 295, 2002.

[9] S. Zelenin, J. Hansson, S. Ardabili, H. Ramachandraiah, H. Brismar, and A. Russom, "Microfluidic-based isolation of bacteria from whole blood for sepsis diagnostics," *Biotechnol. Lett.*, vol. 37, pp. 825–830, 2015.

[10] J. M. Martel and M. Toner, "Inertial Focusing in Microfluidics," *Annu Rev Biomed Eng.*, vol. 16, pp. 371-396, 2014.

[11] P. S. Fodor and M. Kaufman, "Time Evolution of Mixing in the Staggered Herringbone Microchannel," *Mod. Phys. Lett. B*, 2011.

[12] J. Aubin, D. F. Fletcher, J. Bertrand, C. Xuereb, " Characterization of Mixing Quality in Micromixers," *Chem. Eng. Technol.*, vol. 26, pp. 1262–1270.

[13] B. B. Fuchs, S. Eatemadpour, J. M. Martel-Foley, S. Stott, M. Toner, and E. Mylonakis, "Rapid isolation and concentration of pathogenic fungi using inertial focusing on a chip-based platform," *Front. Cell Infect. Mi.*, vol. 9, art. 27, 2019.

[14] N. Heintz and S. Gong, "Small-Scale preparations of yeast DNA," *Cold Spring Harb Protoc*, vol. 10, pp. 430–432, 2020.