Distinguishing the viability of a single yeast cell with an ultra-sensitive radio frequency sensor

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We propose and demonstrate a simple, ultra sensitive radio frequency (RF) sensor to detect a single yeast cell and distinguish its viability in a microfluidic channel. On-chip interference is used to cancel background probing signals to improve sensor sensitivity. Individual viable and nonviable yeast cells (~ 5.83 ± 0.85 μm in diameter) are measured with clear sensing and identification of these cells.

Sensing and identifying a single cell are essential for the operation of flow cytometers, which are basic tools for cell biology studies and clinical disease diagnostics.[1] Single cell analysis is also important for screening cell-based drugs,[2] differentiating cell states, and distinguishing cell responses to external stimuli and intracellular reactions.[3] Therefore, many efforts have been devoted to developing single-cell sensing and analysis tools through different approaches, including optical analysis,[4] chemical analysis,[5] and nanomagnetic particle (labeling) approach.[6] However, these techniques need labeling procedures, and they are incompatible with CMOS technologies, which provide a platform for low-cost, high-performance micro-total-analysis-system (μTAS)[7] development. As a result, electronic, label-free cell sensing and analysis have attracted a lot of recent interests even though such a system has yet to be developed.[8]

Based on Coulter principle (*i.e.* cell size differentiation), microfluidic devices have been developed to detect single polystyrene beads (to mimic single cells) of 15 μm in diameter.[9] Yet, cell analysis potential with this approach is limited due to limited information contents (*i.e.* cell size only). Over the years, electrical impedance spectroscopy (EIS)[10] has emerged as a main electronic approach for single cell sensing and analysis since cell interior information (*i.e.* cell cytoplasm) can be obtained with high frequency probing signals. Such efforts include differentiation of single polystyrene beads,[11] analysis of single latex beads, erythrocyte cells and ghost erythrocytes cells,[12] and analysis of leukocyte cell sub-populations.[8] The resonant structure with electronic barcode for cell identification[13] is another EIS approach. Probing signal frequencies in these research efforts are relatively low (around 100 MHz or below), partly limited by EIS system design challenges. For instance, at high frequencies, stray capacitance, instead of cells, could dominate the impedance changes.[11] On the other hand, cell dielectric properties at higher frequencies, *e.g.* GHz radio frequencies (RF), have rendered themselves as a promising mechanism for individual cell sensing and analysis since it is generally recognized that heterogeneous cells have different RF dielectric characteristics and homogeneous cells have similar properties.[14] These cell-specific RF properties include information of cell morphology, physiological state, and viability, dictated by different cell components, such as cell membrane, cytoplasma and organelles. High frequency probing signals are usually needed for their measurement since cell membranes and fluid medium would block lower frequency probing signals. Unfortunately, developing such RF sensors is very challenging since cell RF dielectric properties are usually close to that of medium fluids.[15] The small cell sizes further diminish the attainable signal levels. Consequently, sensing and identification of a single cell with an RF sensor have not been demonstrated so far.

In Ref. 16 we proposed an RF sensor, which uses an on-chip interference process to cancel background parasitic (probing) signals in order to improve sensor sensitivity. Based on similar mechanisms, we propose and demonstrate a new and simpler RF sensor with ~20 dB cancellation level (*i.e.* sensitivity) improvement in this work. We also demonstrate single yeast cell sensing with clear identification of viable and nonviable cells.

Fig. 1 shows the schematic of our proposed RF sensor. Two Wilkinson power dividers, instead of one power divider and one 180° rat-race hybrid as in Ref. 16, are used to compose the sensor. The use

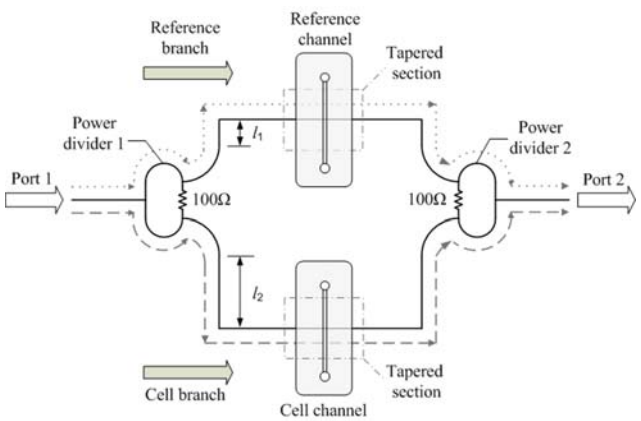

Fig. 1 Schematic of the proposed ultra sensitive RF biological cell sensor. The dotted and dashed lines indicate two main signal paths.

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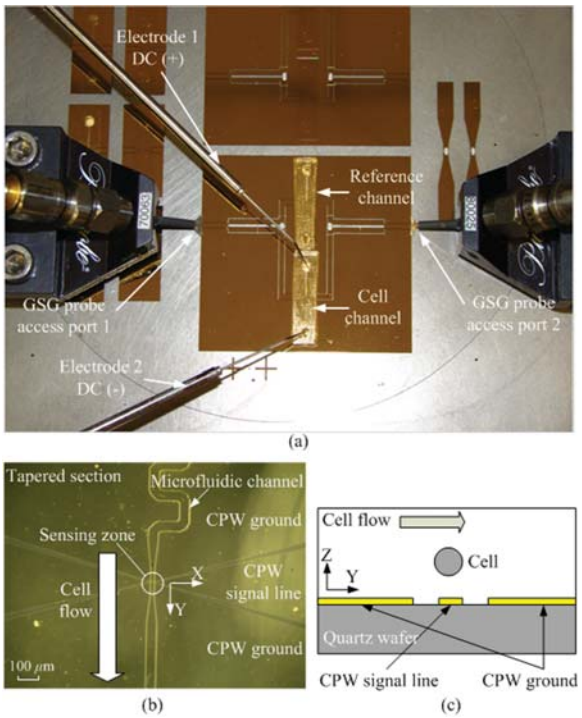

Fig. 2 (a) A photo of the fabricated RF sensor under test. (b) A zoom-in microscopic photo of the tapered section. (c) Cross section of the sensing zone to indicate possible cell positions.

of a second power divider simplifies the design and fabrication processes, in addition to improving the symmetry of the device. The required 180° phase difference is achieved by adjusting l_2 in Fig. 1.

Standard microfabrication procedures are used to build the RF sensor, shown in Fig. 2(a), with its tapered section shown in Fig. 2(b). The designed center frequency is 5 GHz. A copper coplanar waveguide (CPW), formed with chromium (8 nm)/copper (500 nm)/gold (20 nm) on a 500 μm thick quartz substrate, is used to build the RF components of the sensor. Gold wire bonding is performed for CPW ground connections.[17] Microwave thin film chip resistors (100 Ω) are attached to power dividers. Compared with our previous microstrip design,[16] the use of CPW improves device sensitivity. The tapered section further improves sensitivity by concentrating electromagnetic fields in a small sensing zone.

SU-8 is used to form the microfluidic channels of the RF sensor through a standard photolithographic process. Serpentine channel structures are designed to focus the cells.[18] The uniform sections of the channels are 25 μm high and 50 μm wide. The tapered sections of the channels, Fig. 2(b), are designed for higher sensitivity with better cell control. Two pieces of 2 mm thick polydimethylsiloxane (PDMS) rectangular blocks are used as the channel covers, which are put on the SU-8 channels manually. At each end of the PDMS blocks, a hole (*i.e.* well) is formed as inlet and outlet for test samples.

An HP8510C vector network analyzer and a Cascade probe station with ground-signal-ground (GSG) probes are employed to measure the scattering parameters. A full two-port calibration procedure is conducted before measurements. The yeast strain that is used in the measurements is *Saccharomyces cerevisiae* ATCC4098. The cells are cultured in Sabouraud Dextrose broth (Becton and Dickinson Company, USA) at 37 °C, harvested after 24 h and washed in distilled deionized (DI) water three times. Nonviable yeast cells are obtained by boiling viable cells for ten minutes and prepared for measurements by mixing them with DI water, as for viable cells. The boiling process kills 100% of the cells and does not change cell morphology when inspected under microscope. The average cell diameter is 5.83 ± 0.87 μm.

The reference channel and cell channel, Fig. 2(a), are filled with DI water and cell sample (viable or non-viable, separately), respectively. Two tungsten needles are inserted into the wells to drive the cells in the channel by applying a DC voltage between them. The DC voltage level also controls cell flow velocity.[18] A combination of low cell concentration level, serpentine channel and microscope visual inspection is used to guarantee that only one cell is in the sensing zone during measurement. Cell positions are determined by direct visual observations with a microscope. Then, the transmission scattering parameters S_{21} are recorded.

The obtained results are shown in Fig. 3. The three curves in Fig. 3(a) correspond to measurements with no cells, viable yeast cells and nonviable yeast cells in the sensing zone. Their minimum S_{21} points are labeled as P_A, P_B, and P_C. Fig. 3(b) shows P_A, P_B and P_C distributions for multiple measurements with different yeast cells. A few observations can be made. First of all, when there is no cell, neither viable nor nonviable cell, the minimum S_{21} is ~−80 dB, which indicates a ~20 dB parasitic (probing) signal cancellation improvement in comparison with our previous results.[16] As a result, the sensitivity of the RF sensor is dramatically improved. The improved sensitivity is key to the sensing and identification of single cells since devices with

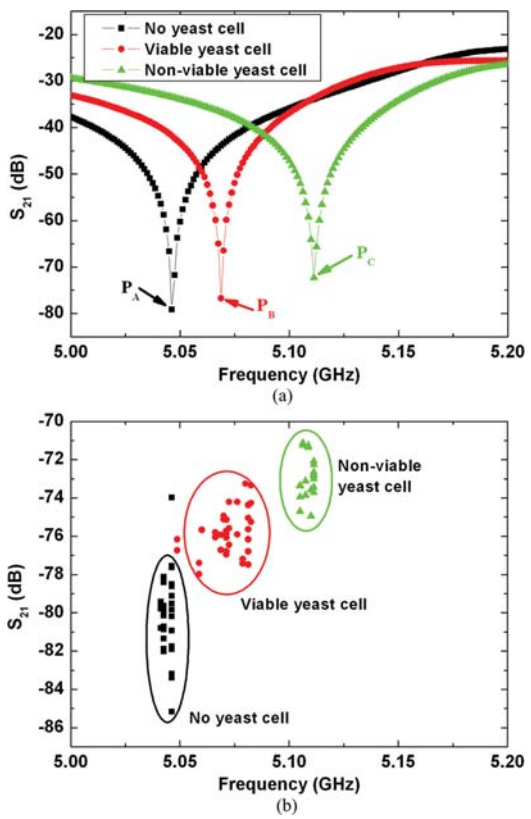

Fig. 3 (a) Measured transmission scattering parameters for different test arrangements. (b) P_A, P_B and P_C distribution for measurements with different yeast cells.

−40∼−50 dB cancellation level yield no distinguishable output signals that would indicate the presence of cells in the sensing zone. Second, a single viable cell at the sensing zone changes both the amplitude and frequency of the minimum S_{21} point by ∼3 dB and ∼15 MHz, respectively, when comparing points P_B and P_A. Both changes are significant. The peak point shifts to higher frequency indicates that the viable yeast cell has lower permittivity than that of DI water at ∼5 GHz, which is consistent with previous observations.[19] Furthermore, point P_B moves back towards point P_A when the cell is unloaded from the sensing zone. Therefore, both the frequency shift and amplitude change can be used as sensing indicators for a viable yeast cell in the sensing zone. Third, the occurrence of a nonviable yeast cell shifts point P_C from P_A by ∼7 dB and ∼65 MHz. Additionally, the separations between point P_C and point P_B are ∼4.0 dB and ∼40 MHz. This frequency shift also indicates that the permittivity of our nonviable yeast cells is lower than that of viable yeast cells. Protein denaturation and less polarized plasma membranes are probably the reasons for the reduced cell permittivity.[15] When the nonviable cell is moved away from the sensing zone, point P_C moves towards point P_A. The differences among the three different measurements indicate that nonviable yeast cells can be sensed and identified from viable yeast cells. Fourth, Fig. 3(b) shows a wide data distribution for different cell measurements even though the wide distribution does not change the above observations and conclusions qualitatively. Possible reasons for the broad distribution include measurement environmental disturbance, cell size variations, and cell position variations in the sensing zone as illustrated in Fig. 2(c). The obtained ∼−80 dB cancellation level makes the sensor ultra sensitive, yet prone to environmental influences (*e.g.* vibration), which should be isolated in the future. Additionally, we can hardly control cell sizes since cell size variations exist naturally. So, further work is needed to quantify cell size effects. Moreover, cell relative position in the sensing zone is not well controlled in our setup. The serpentine channels are only designed to focus the cells in X direction, shown in Fig. 2. As a result, cell position (both Z and Y direction) variations cause measurement fluctuations. Three-dimensional hydrofocusing channel could be employed to solve this problem in the future. With these and some other improvements, a correlation between cell size, position and RF spectra can be established and quantified. A time course of transmission signal intensity can also be obtained while a cell enter and leave the measurement site. Such time domain signals are more straightforward illustrations of the RF sensor operation. Lastly, the non-viable yeast cells show narrower data distribution (*i.e.* narrower frequency distribution), which may indicates more uniform cell dielectric properties (and sensing positions) than that of viable yeast cells. The data distribution differences between viable and nonviable cells suggests that cell property (and cell position) differences contribute significantly to the observed wide data distribution of viable yeast cells. Thus, the sensor has the potential for detailed single cell analysis.

In conclusion, we proposed and demonstrated an ultra sensitive RF sensor that can detect and identify single viable and nonviable yeast cells. The label free electronic detection and identification are based on the high-frequency dielectric property differences between viable and nonviable yeast cells in addition to their differences with water. Further work is needed to improve data measurement accuracy, to conduct broadband or multiple frequency measurements of single cells in order to establish a cell dielectric property library so that a label free, all electronic cell sensing and identification paradigm could be established and applied for portable flow cytometers.

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Notes and references

1 H. M. Shapiro, *Practical Flow Cytometry*, 4th ed., Wiley-Liss, New Jersey, 2003.
2 W. Z. Song, X. M. Zhang, A. Q. Liu and C. S. Lim, *Appl. Phys. Lett.*, 2006, **89**, 203901.
3 T.-C. Chao and A. Ros, *J. R. Soc. Interface*, 2008, **5**, S139–S150.
4 H.-H. Lai, P. A. Quinto-Su, C. E. Sims, M. Bachman, G. P. Li, V. Venugopalan and N. L. Allbritton, *J. R. Soc. Interface*, 2008, **5**, S113–S121.
5 N. J. Docichi and S. Hu, *Curr. Opin. Chem. Biol.*, 2003, **7**, 603–608.
6 B. H. McNaughton, R. R. Agayan, R. Clarke, R. G. Smith and R. Kopelman, *Appl. Phys. Lett.*, 2007, **91**, 224105.
7 D. R. Reyes, D. Iossifidis, P.-A. Auroux and A. Manz, *Anal. Chem.*, 2002, **74**, 2623–2636.
8 D. Holmes, D. Pettigrew, C. H. Reccius, J. D. Gwyer, C. Berkel, J. Holloway, D. E. Davies and H. Morgan, *Lab Chip*, 2009, **9**, 2881–2889.
9 D. K. Wood, S.-H. Oh, S.-H. Lee, H. T. Soh and A. N. Cleland, *Appl. Phys. Lett.*, 2005, **87**, 184106.
10 C. M. Lo, C. R. Keese and I. Giaever, *Biophys. J.*, 1995, **69**, 2800–2807.
11 S. Gawad, K. Cheung, U. Seger, A. Bertsch and P. Renaud, *Lab Chip*, 2004, **4**, 241–251.
12 S. Gawad, L. Schild and Ph. Renaud, *Lab Chip*, 2001, **1**, 76–82.
13 D. K. Wood, G. B. Braun, J.-L. Fraikin, L. J. Swenson, N. O. Reich and A. N. Cleland, *Lab Chip*, 2007, **7**, 469–474.
14 K. R. Foster and H. P. Schwan, *Crit. Rev. Biomed. Engin.*, 1989, **17**(1), 25–104.
15 K. Asami, T. Yonezawa, H. Wakamatsu and N. Koyanagi, *Bioelectrochem. Bioenerg.*, 1996, **40**, 141–145.
16 C. Song and P. Wang, *Appl. Phys. Lett.*, 2009, **94**, 023901.
17 L. Fan and K. Chang, *IEEE Trans. Microwave Theory Tech.*, 1996, **44**, 2411–2420.
18 J. Zhu, T.-R. J. Tzeng, G. Hu and X. Xuan, *Microfluid. Nanofluid.*, 2009, **7**, 751–756.
19 K. Asami and T. Yonezawa, *Biophys. J.*, 1996, **71**, 2192–2200.