TH1B-4

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

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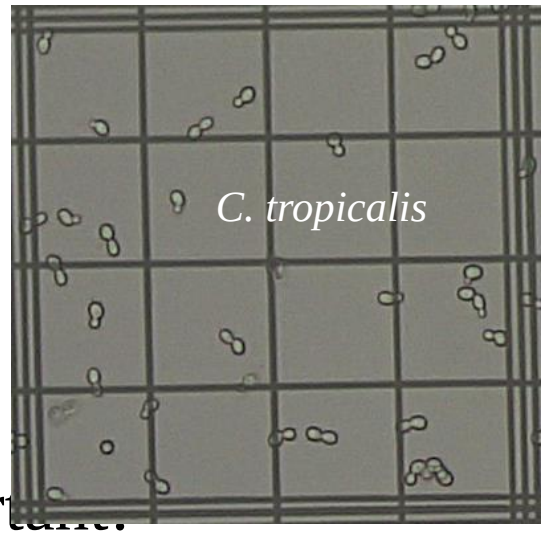
Outline

- Motivation
- System design consideration
- Candida cell extraction
- Microwave measurement of single *Candida* cells
- Summary

Motivation: *Candida* and Candidemia

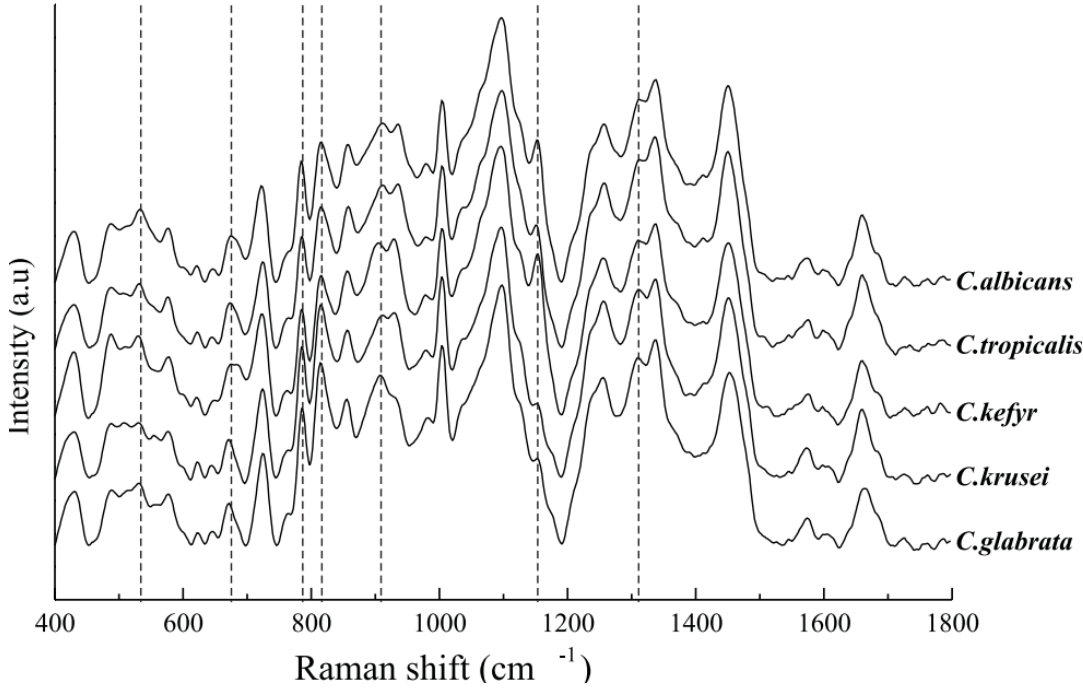
- *Candida* & Candidemia: the 4th most common nosocomial bloodstream pathogens[1],
  - Attributable mortality of 15–35% for adults and 10–15% for neonates
  - Hospitalization cost: approximately US $46,000 per episode[2].
  - A 12-hour delay of proper antifungal therapy can increase the mortality rate by up to 20%[3].
- *Candida* species and antifungal drug resistance
  - *C. albicans*, *C. tropicalis*, *C. parapsilosis*, *C. glabrata*, and *C. krusei* cells
  - The number of *Candida* cells in blood, <1 cell/mL for 50% patient blood
  - Antifungal drug resistance, such as *C. glabrata and C. auris*
  - Antifungal drugs
    - Limited choices: e.g., echinocandin (caspofungin, micafungin, or anidulafungin), fluconazole, amphotericin B
    - Serious side-effects: antifungal susceptibility testing (AFST)



Rapid, single cell detection and phenotypic AST is important.

Current Methods

- Standard methods: cell culture & microscopic observation[4]
  - Time-consuming: up to days
  - Not very sensitive or specific:
- Other methods
  - Non-culture methods: polymerase chain reaction (PCR), next generation sequencing (NGS), mass spectroscopy (MS), Raman spectroscopy
  - Flow cytometry: direct hybridization assay, 5 hours
  - T2Candida: PCR and magnetic resonance, 1 CFU/mL
    - Takes ~ 4 hours
    - Detect 5 *Candida* species in two groups, but with similar AFST pattern
    - No phenotypic AFST option

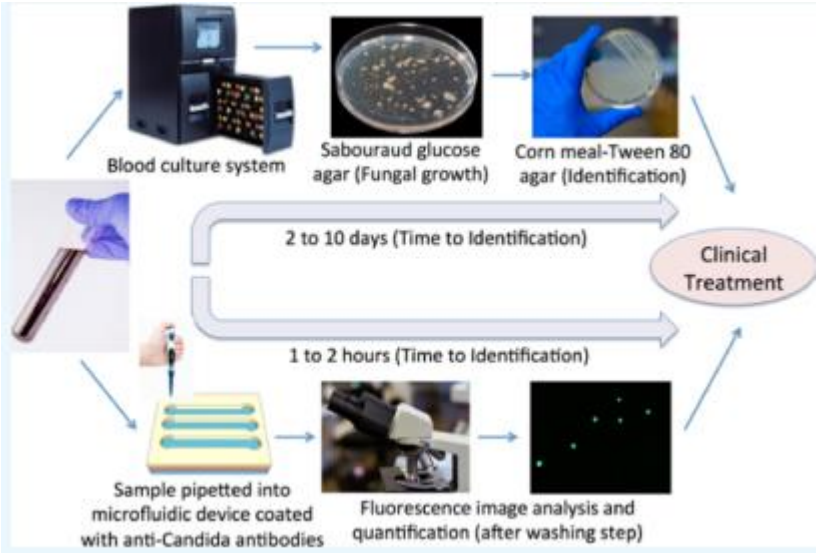


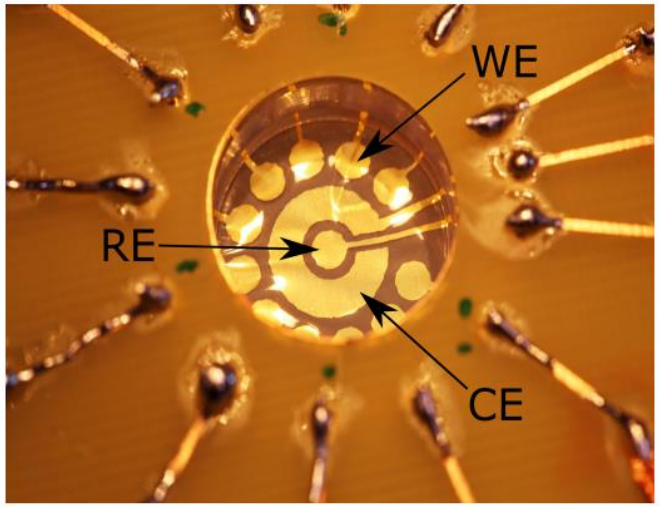
B. Tage and C. Kurtzman, "Rapid Identification of Candida Species and Other Clinically Important Yeast Species by Flow Cytometry," J. Clinical Microbiology, 2005

K. Maquelin et al., "Rapid Identification of *Candida* Species by Confocal Raman Microspectroscopy," 2002

Some Other Methods

- Cell wall components
  - polysaccharides, such as mannan and
  - β-D-glucan
- Microfluidics-based
  - 10 CFU/mL, 1-2 hours
- Impedance measurement
  - anti-C. albicans antibodies, 10 CFU/mL, 1 hour



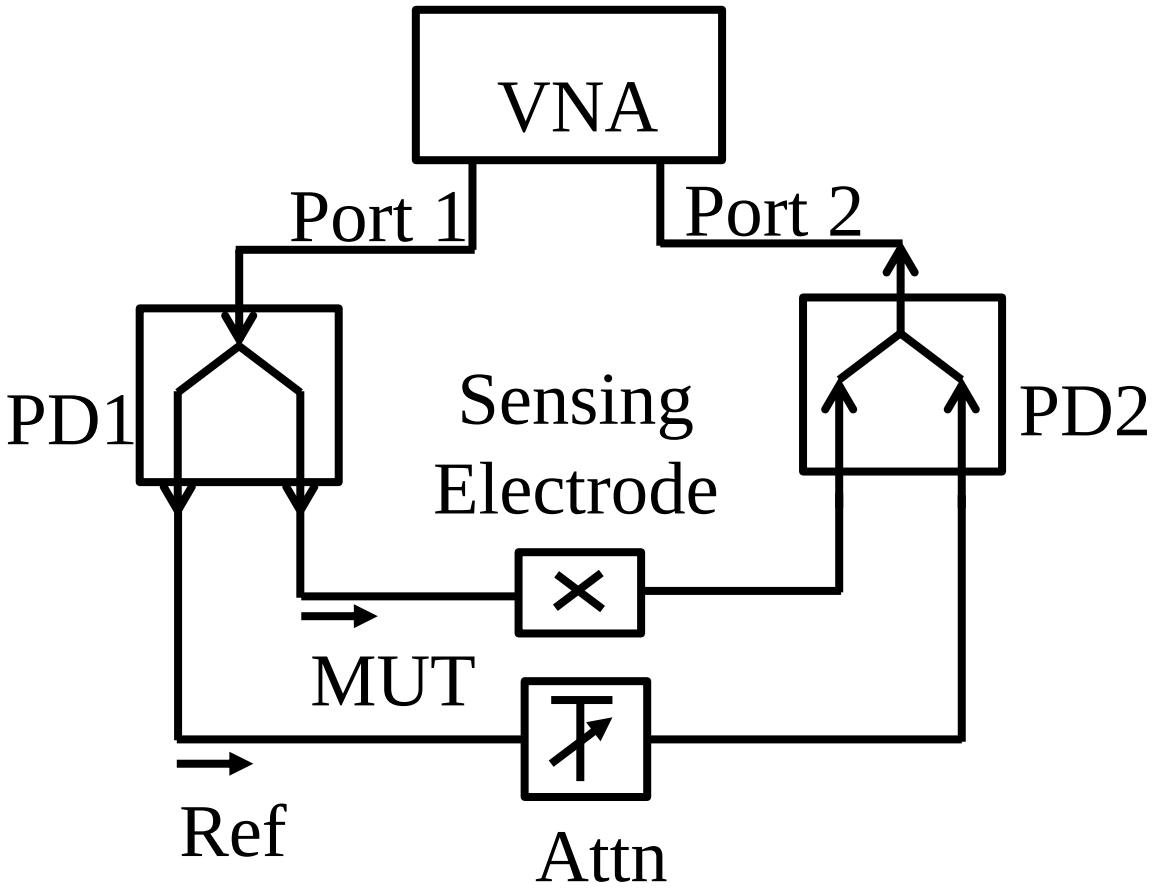


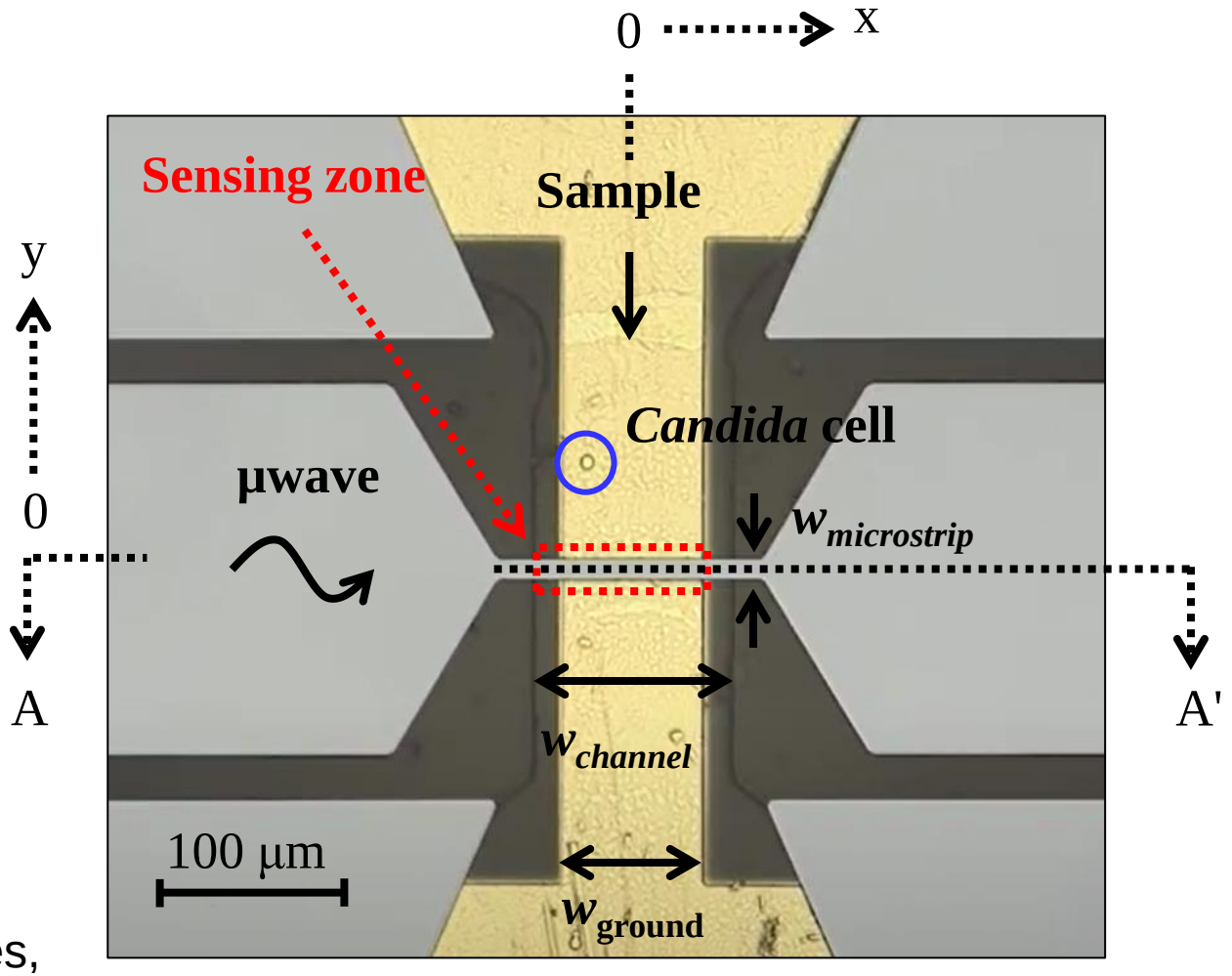
F. Richter et al., "Fungi-on-a-Chip: microfluidic platforms for single-cell studies on fungi," FEMS Microbiology Reviews, 2022

W. Asghar et al., "Microfluidic Chip for Detection of Fungal Infections," ACS OMEGA, 2019

D. Kwasny et al., "Direct Detection of *Candida albicans* with a Membrane Based Electrochemical Impedance Spectroscopy Sensor," Sensors, 2018

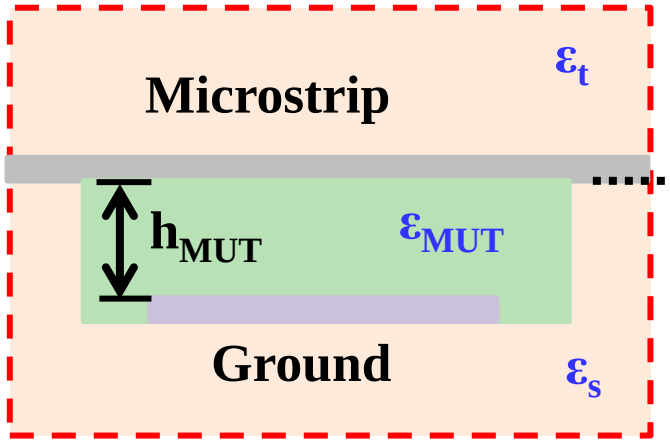
Microwave Flow Cytometry

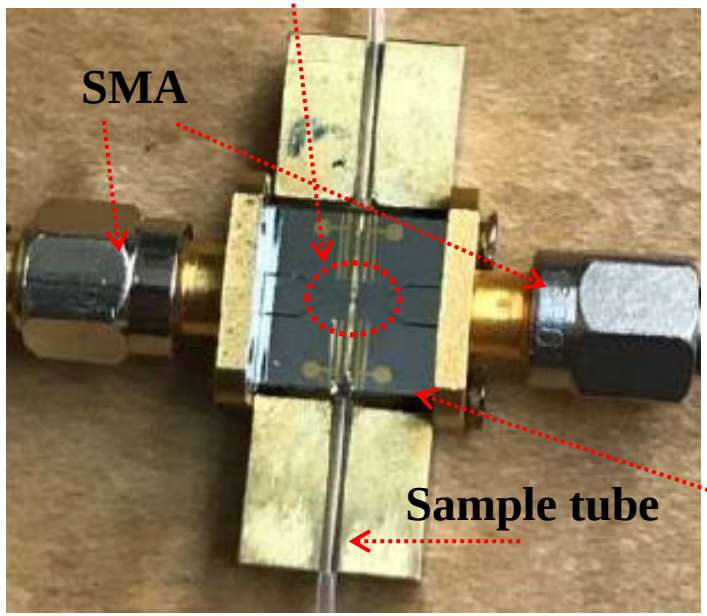




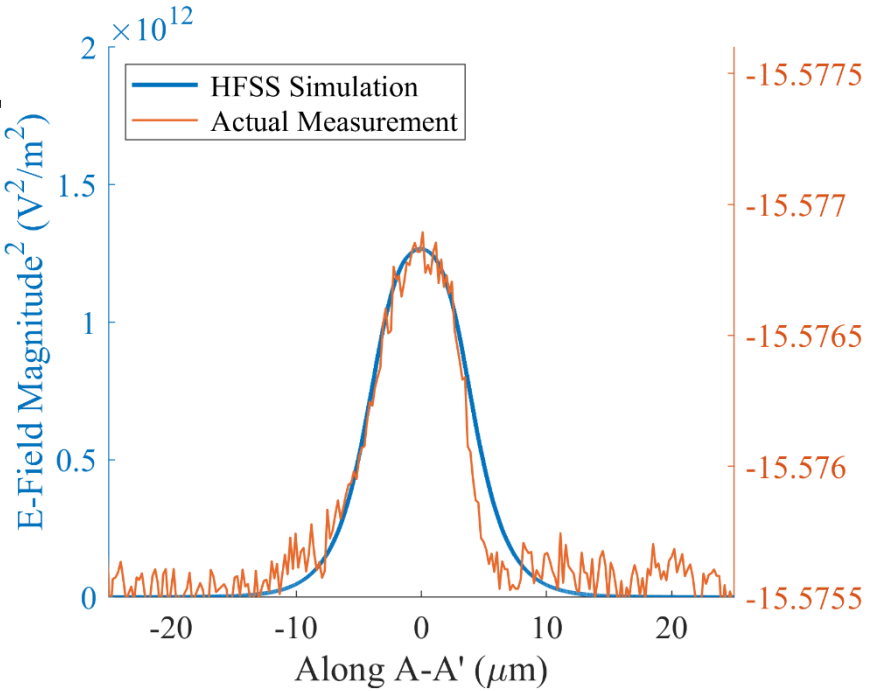
N. Dahal et al., "Spectroscopic Analysis of Candida Species, Viability, and Antifungal Drug Effects With a Microwave Flow Cytometer," 2022

Reproducible Particle Measurement

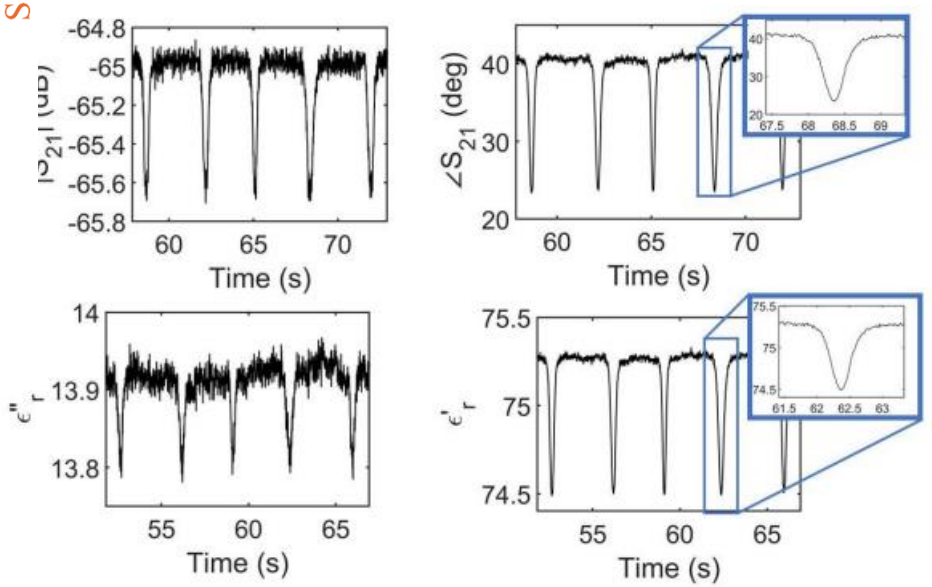




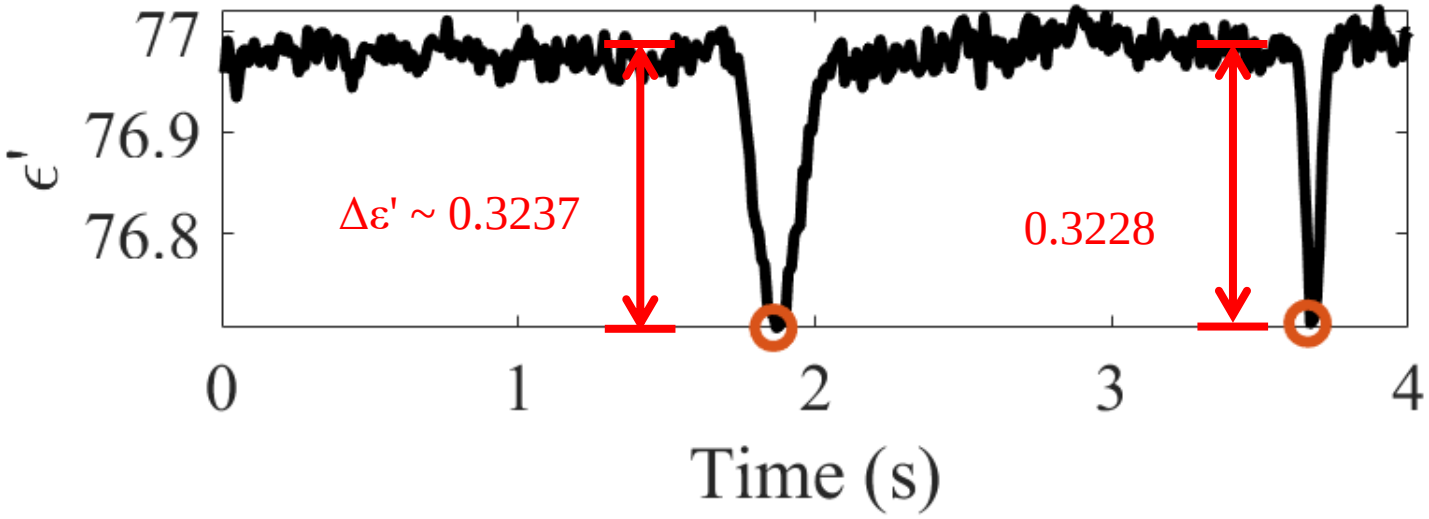
Assembled microwave sensor

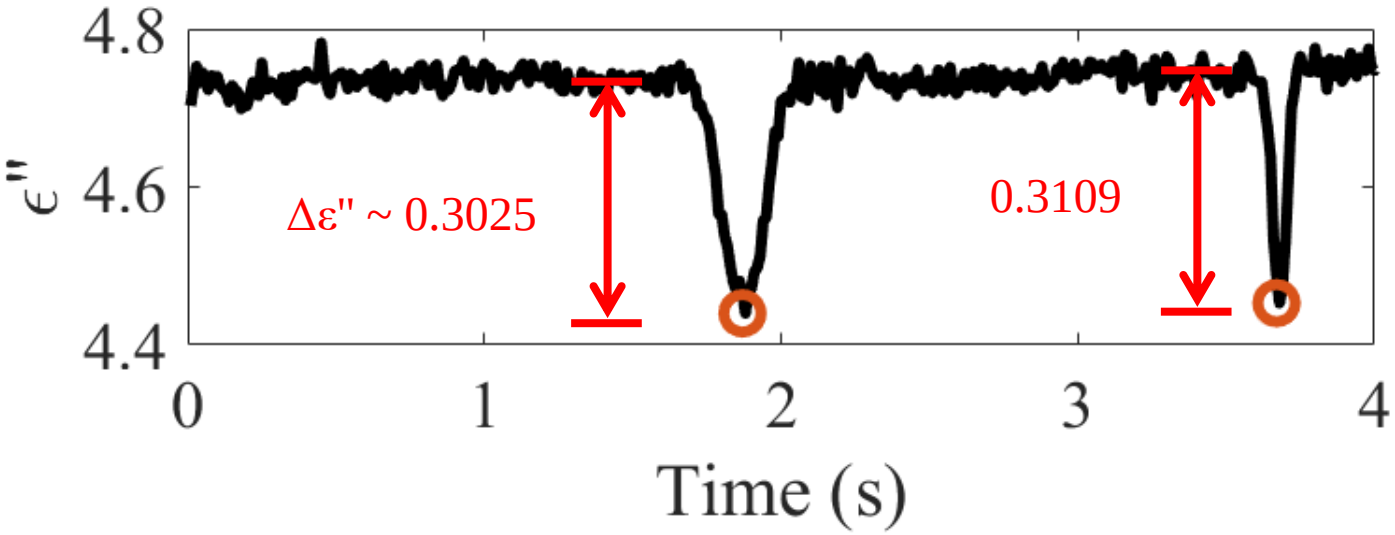


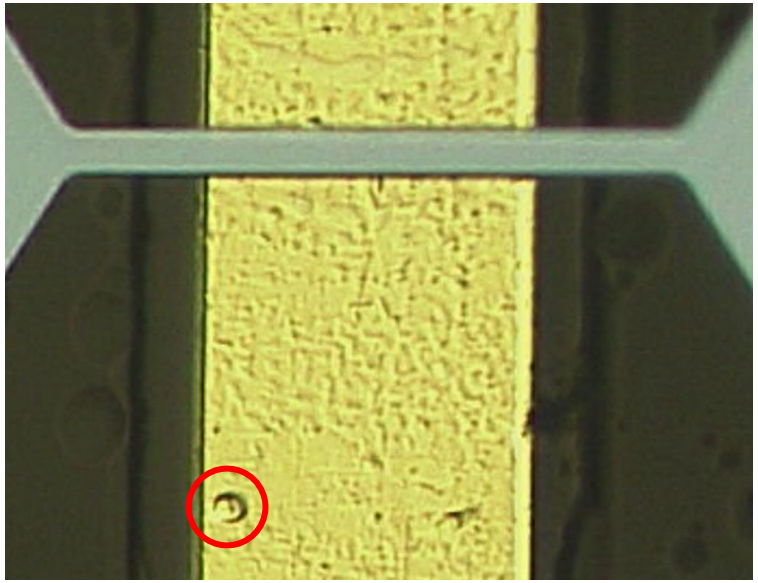
Reliable and repeatable measurement: the same 5.5-μm PS particle at 3.96 GHz.



Reproducible Cell Measurement

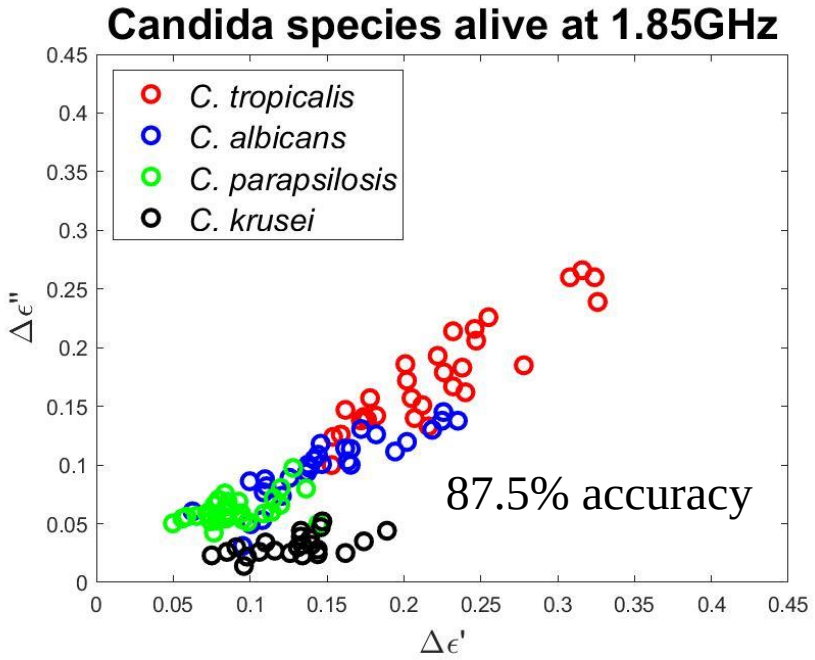






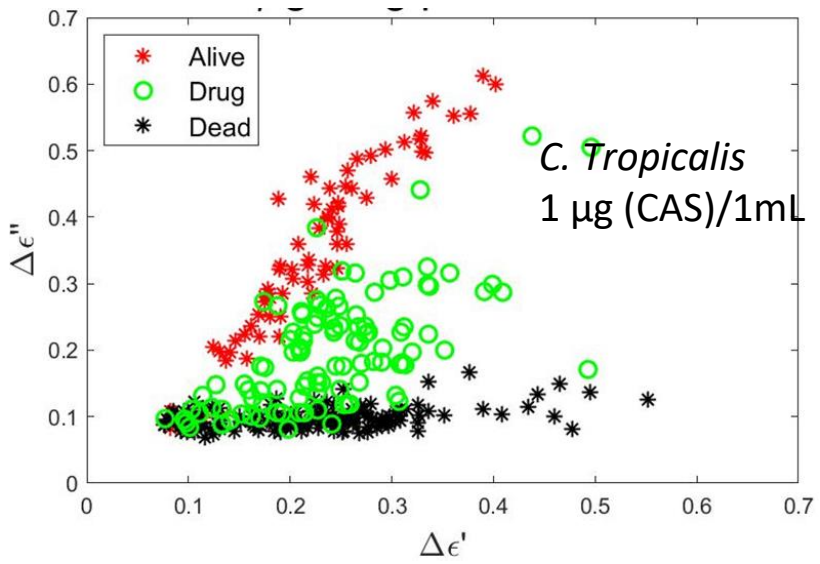
f= 1.85 GHz

Candida Species, Viability, and Drug Effects

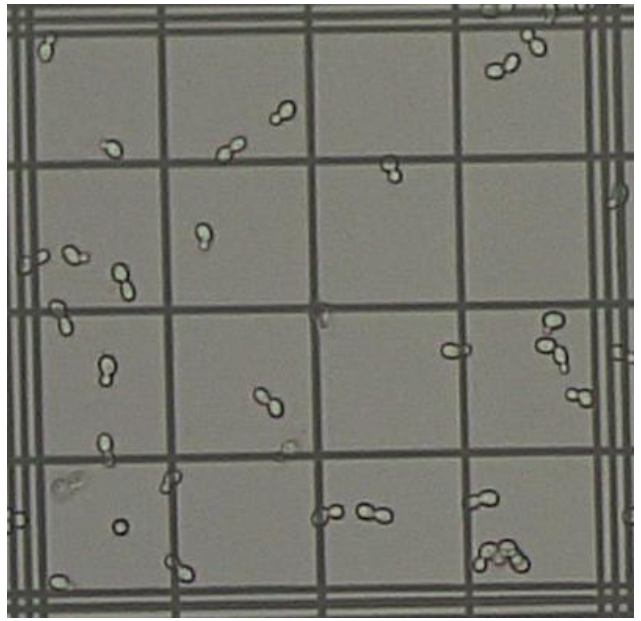


Different *Candida* species

@ 0.796 GH



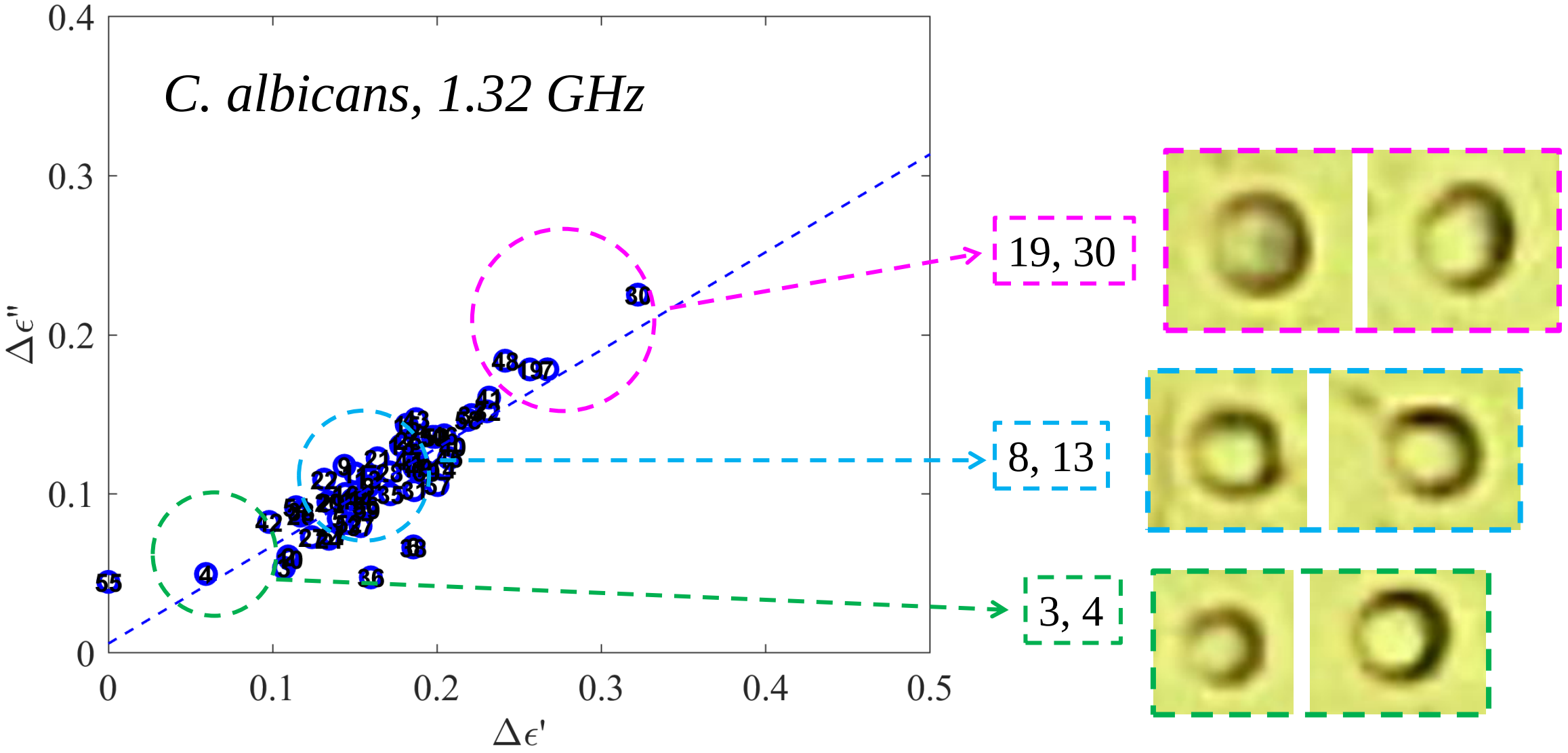
Viability & drug effects



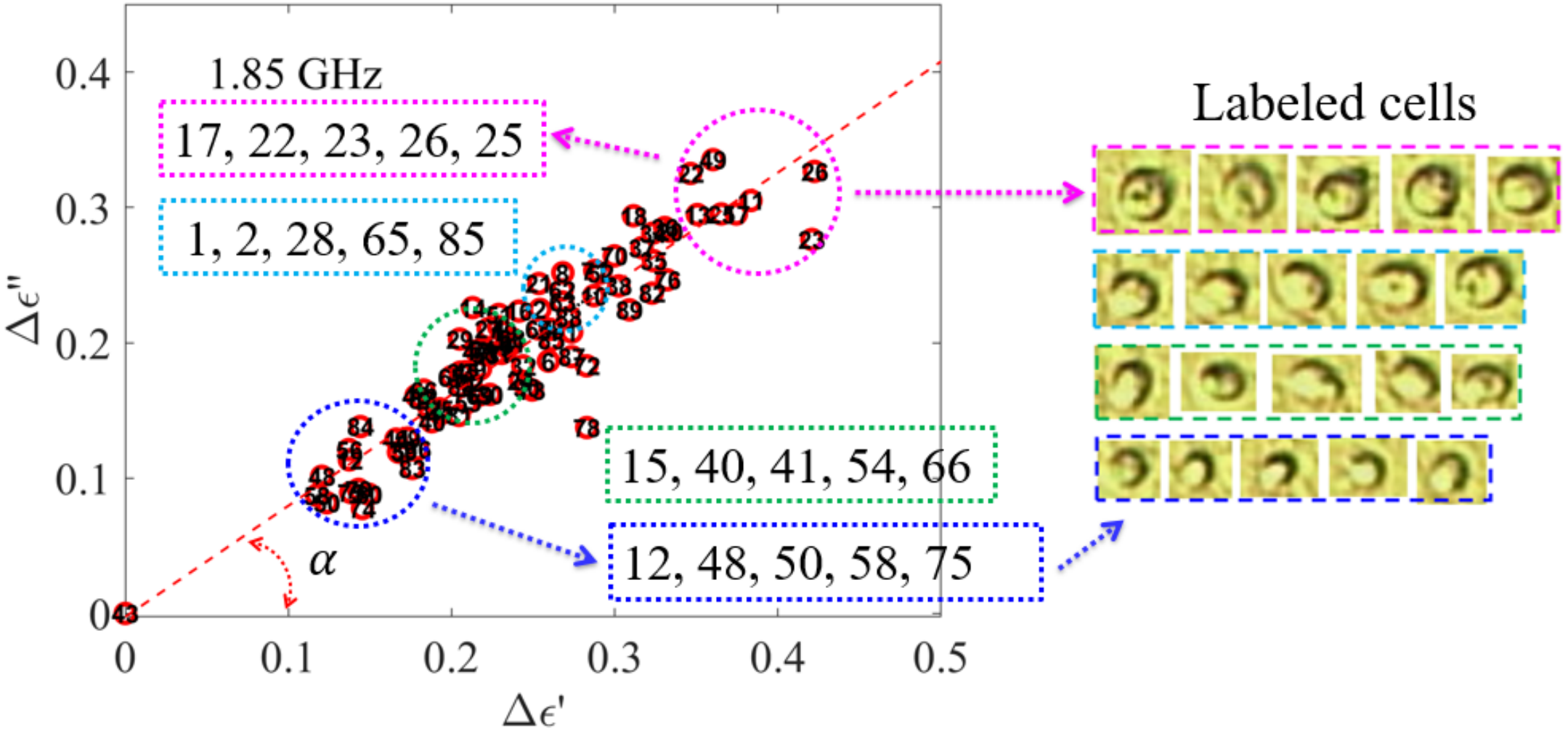
N. Dahal et al., "Spectroscopic Analysis of Candida Species, Viability, and Antifungal Drug Effects with a Microwave Flow Cytometer," *IEEE* 2022

N. Dahal et al., "Measuring yeast cell heterogeneity with a microwave flow cytometer," IEEE *IMS 2022*, Denver, CO

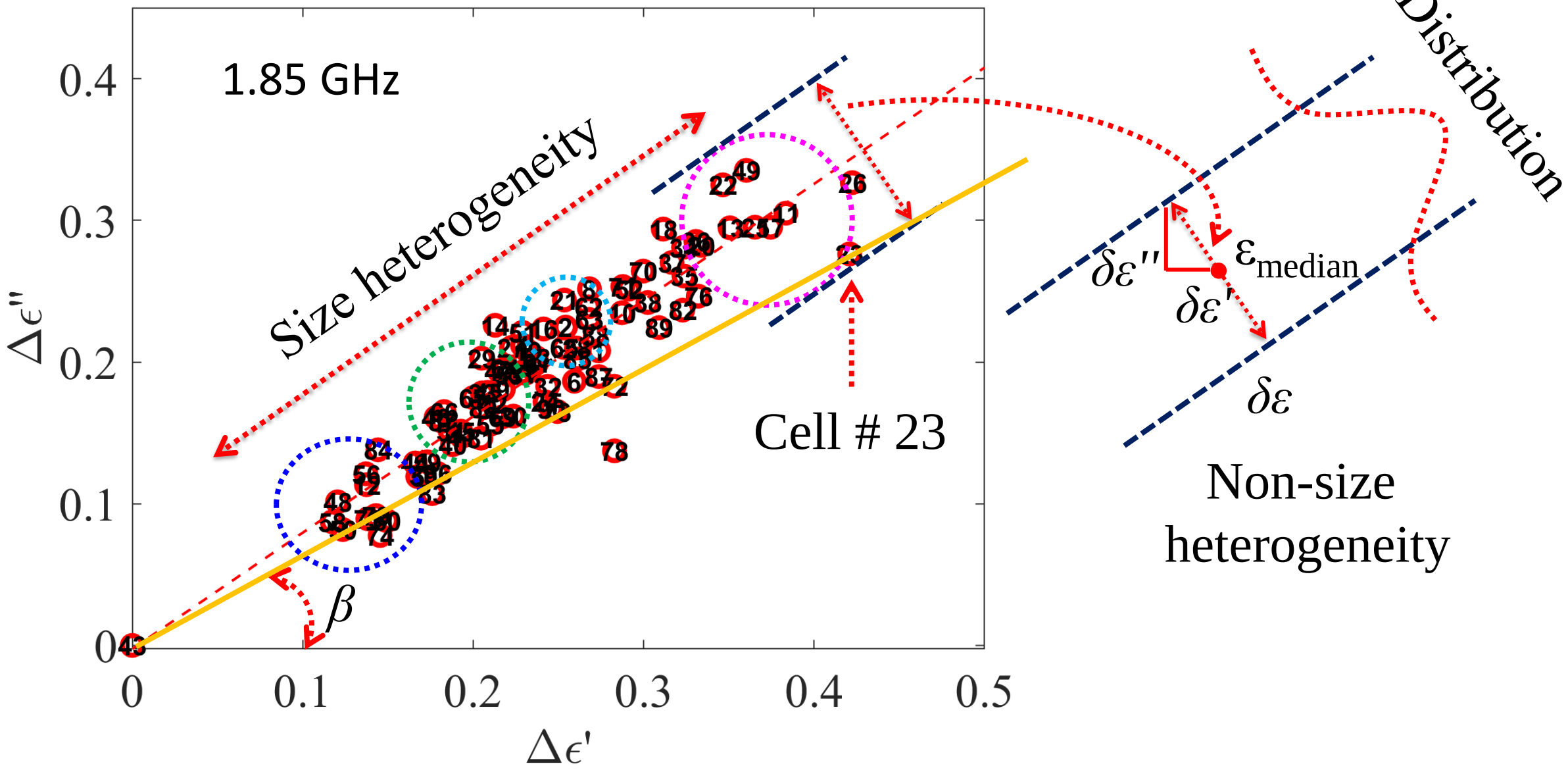
Cell Size: *C. albicans*



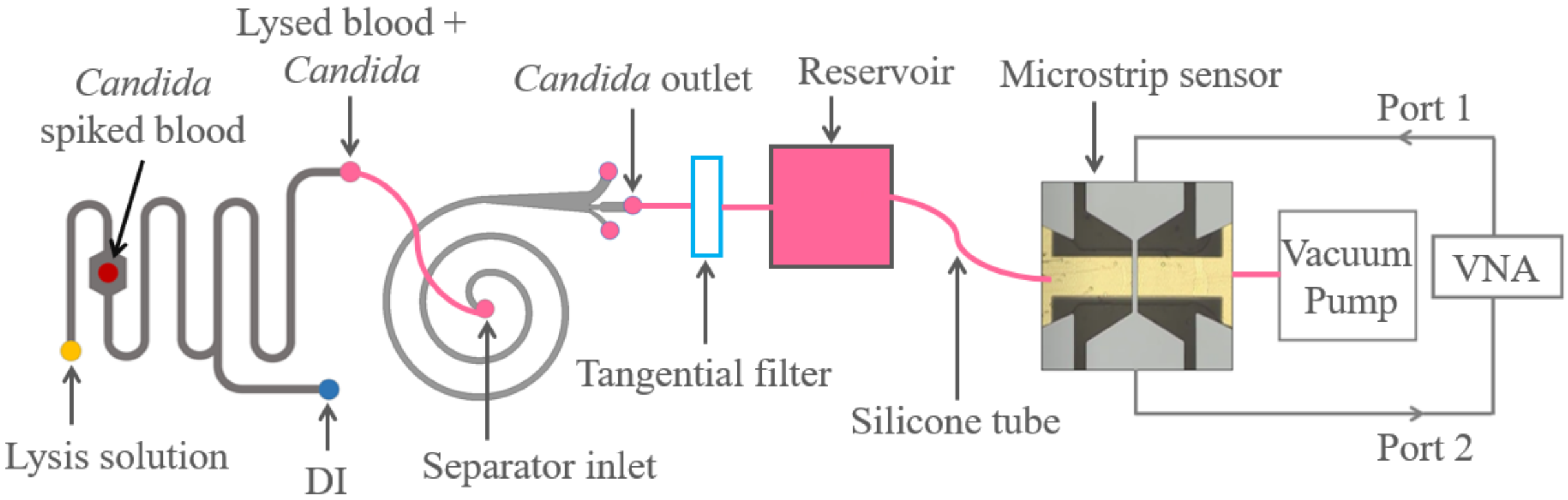
Cell Size: *C. tropicalis*



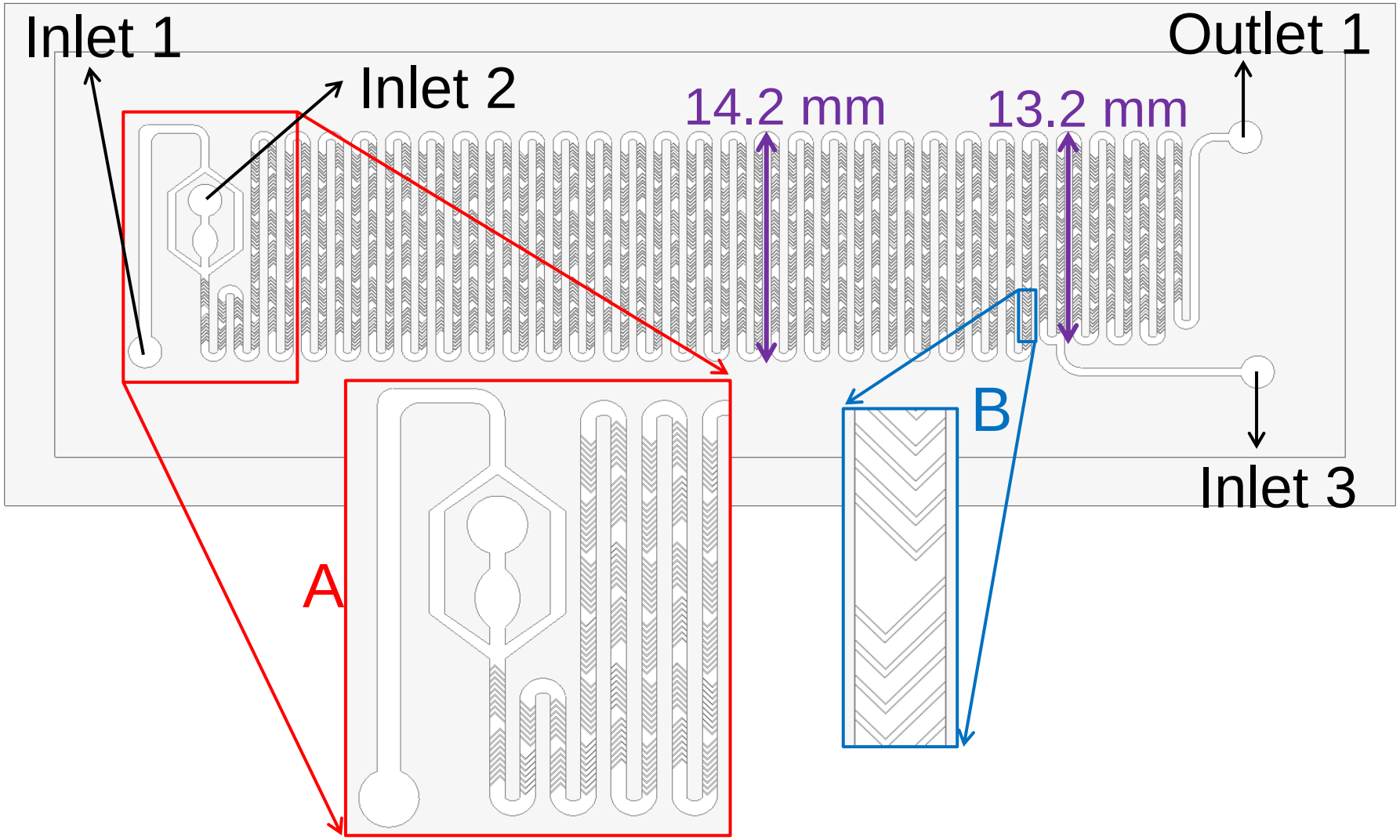
Cell Heterogeneity: *C. tropicalis*



Microwave System for Selective Sensing of Candida cells in Blood



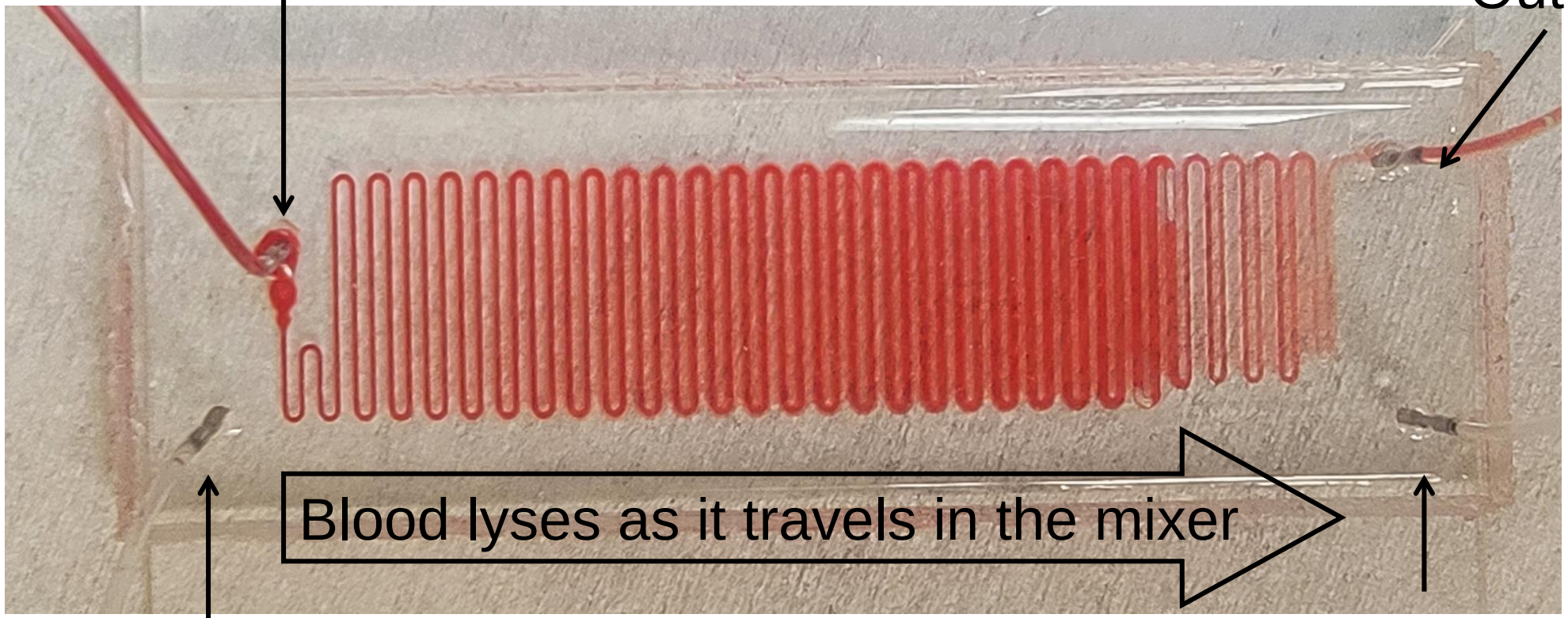
Microfluidic Mixer



Mixer Operation

Candida spiked bovine blood

Outlet

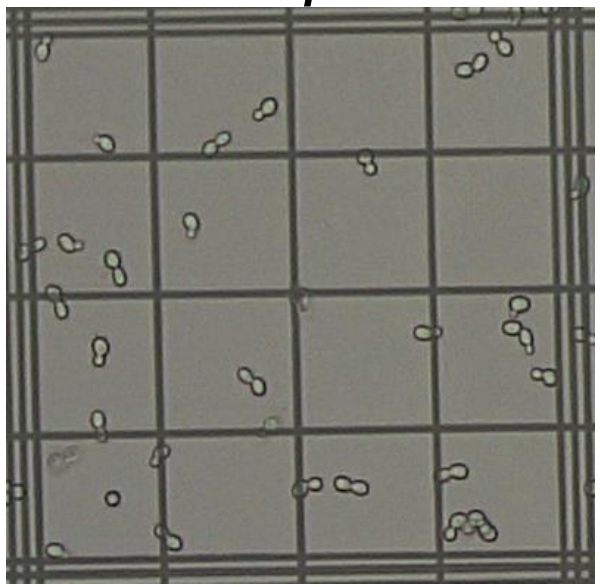


Blood lysis solution

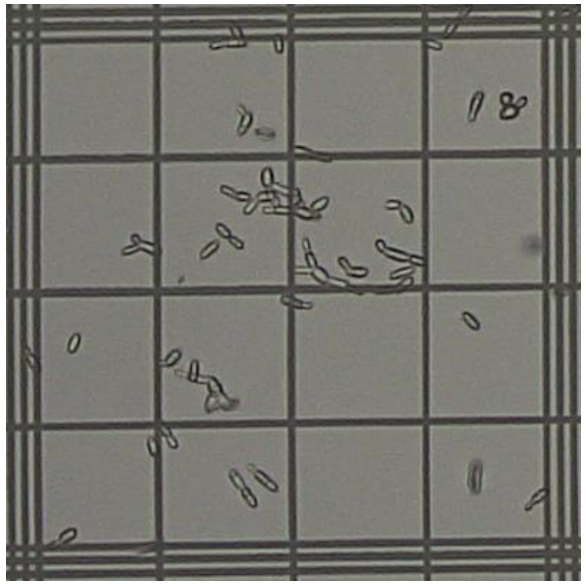
Recovery rate ~ 42%

De-ionized water

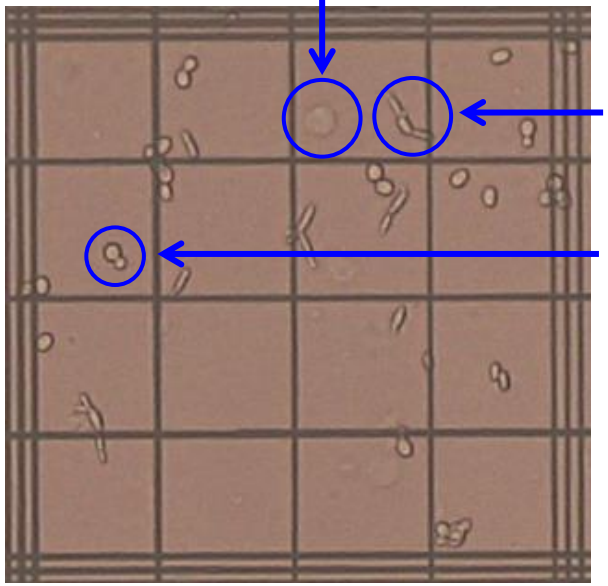
C. tropicalis



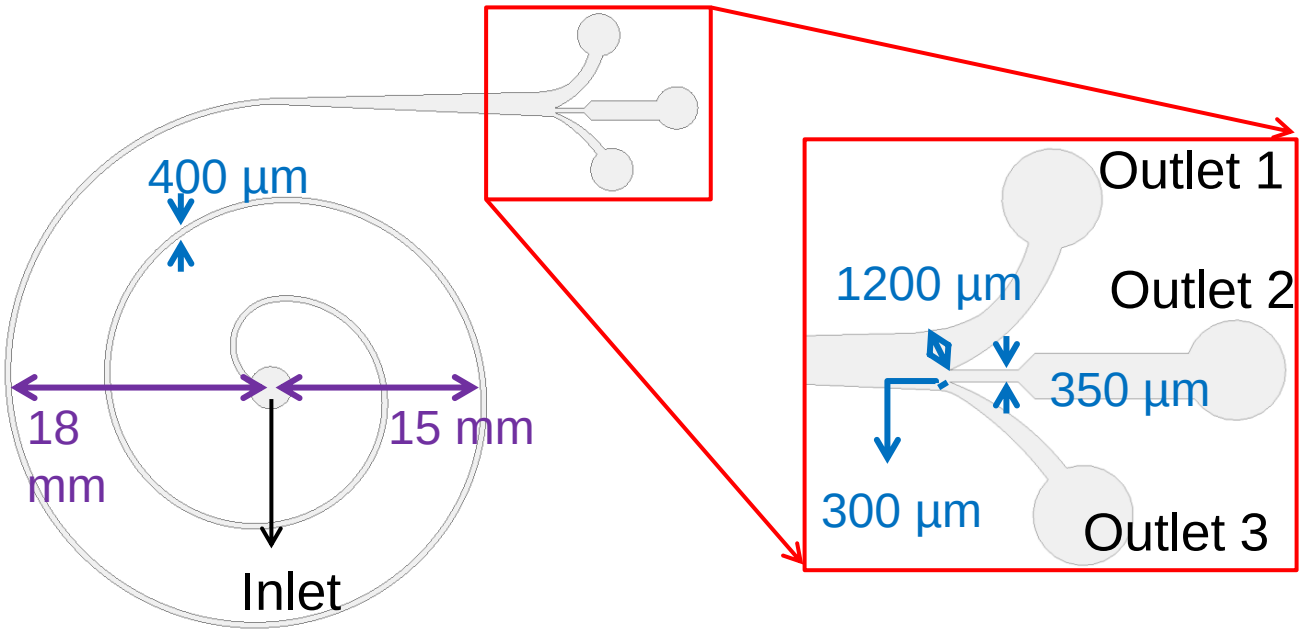
C. krusei

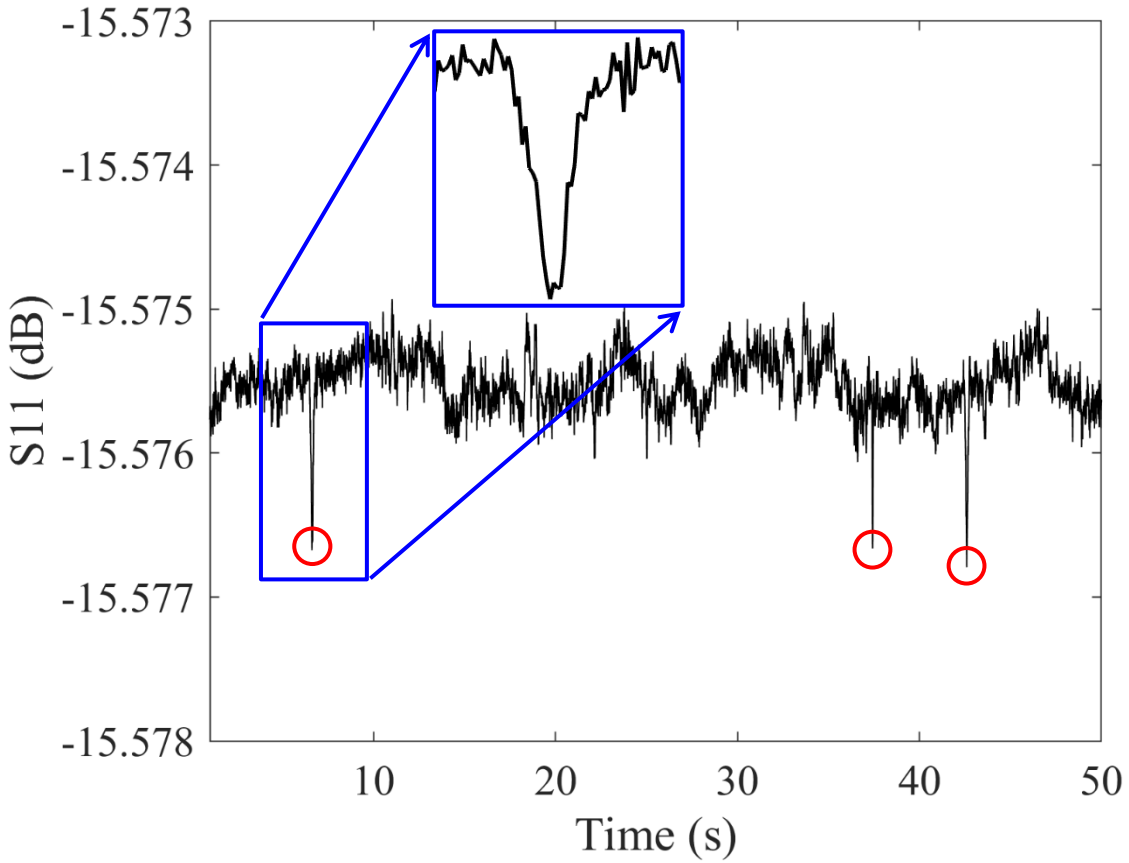


Blood cell

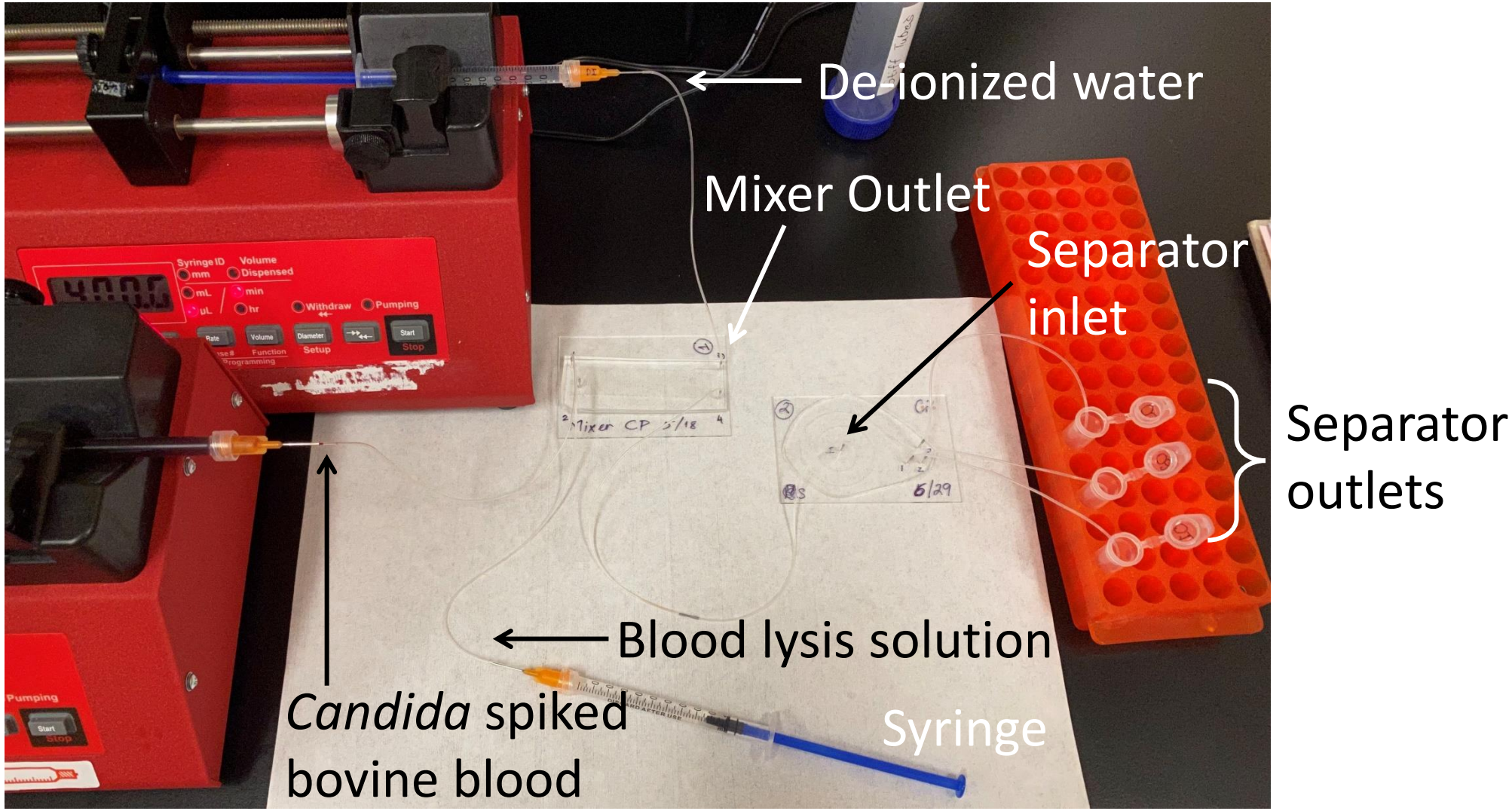


Microfluidic Separator

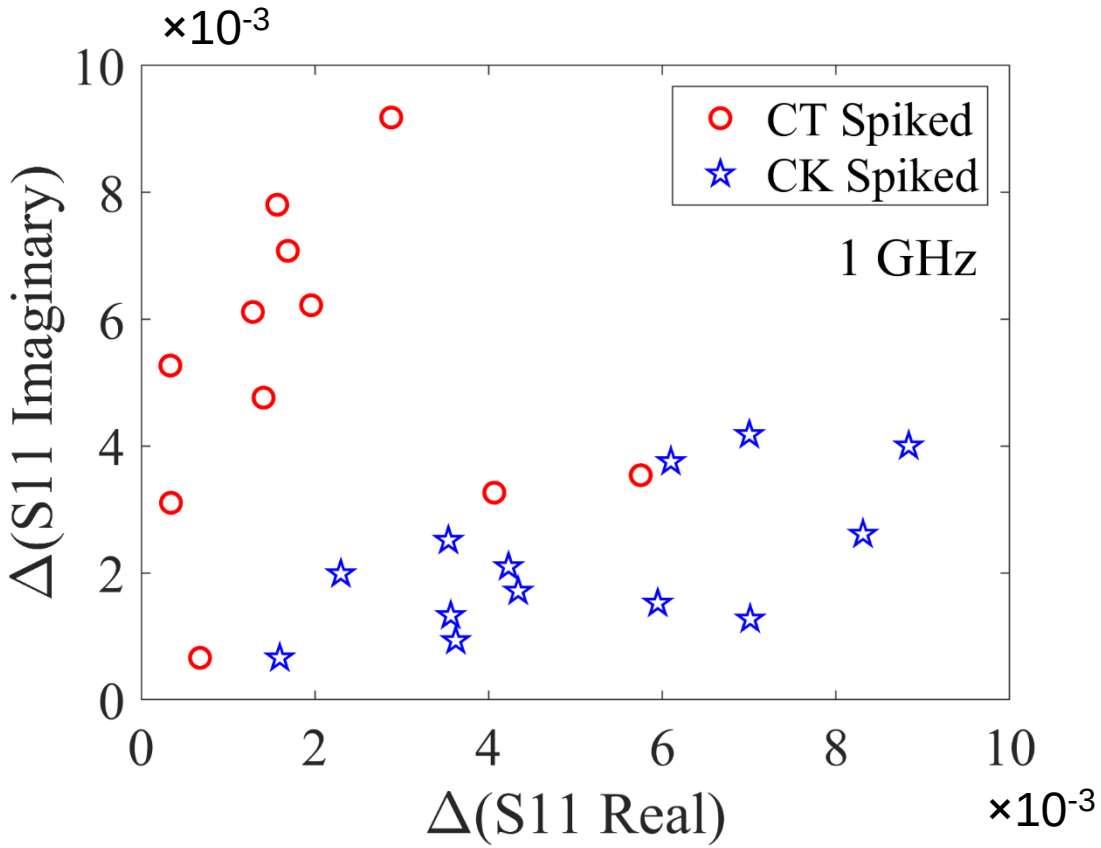


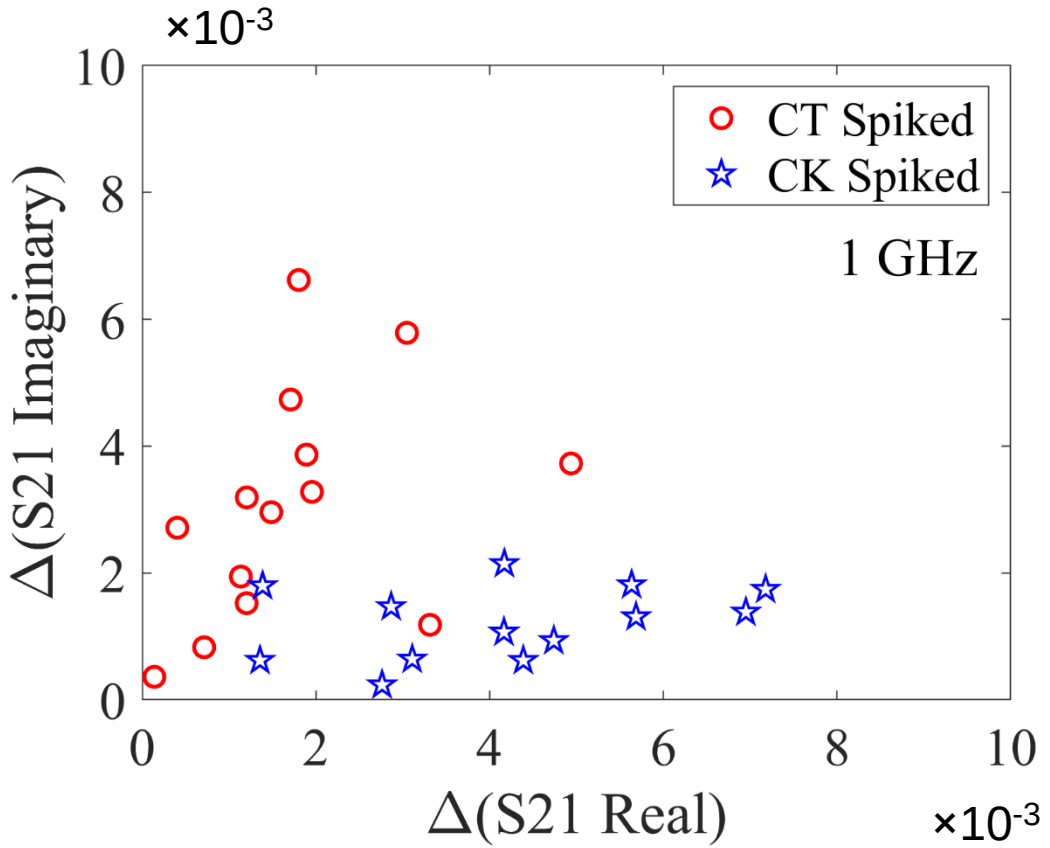


System Operation



Candida Cells: Direct VNA Measurement





Summary

A microwave system is developed and operated to lyse bovine blood, extract spiked *Candida* cells, and measure single *Candida* cells. Measured *C. tropicalis* and *C. krusei* have significantly different microwave properties. Nevertheless, cell properties depend on cell shape and size, in addition to cell molecular composition profile. Further investigation is needed for selective microwave cell detection. Microfluidic channels-based sample processing also need further development to improve system performance, including cell recovery rate.

Thank you!

Acknowledgment

This work is supported in part by the US Army Office of Research, contract W911NF2210044. Ralu Divan is supported by the U.S. Department of Energy, Office of Science, Office of Basic Energy Sciences, for the use of the Center for Nanoscale Materials, an Office of Science user facility, under Contract DE-AC02-06CH11357.