Development of an infrared-functionalized microbalance sensor for *Cyclospora* cayetanensis detection and differentiation

Contact

Jenny Maloney, PhD
USDA, ARS
jenny.maloney@usda.gov

Authors

Monica Santin (Co-PI), USDA, ARS, monica.santin-duran@usda.gov &
Laurene Tetard (Co-PI), University of Central Florida,
laurene.tetard@ucf.edu

Project funding dates

January 1, 2023 – December 31, 2023

Acknowledgements

Special thanks to Liza Lutter and EMFSL support scientists Nadja George and Aleksey Molokin.

Summary

The purpose of our project is to use multi-modal sensing technology to develop an accurate, rapid, and scalable tool to detect *Cyclospora cayetanensis*. Current procedures for *Cyclospora* detection are expensive and time consuming. This project will test and develop a novel detection system for *Cyclospora* by pairing infrared microscopy with cantilever-based microsensor technology. The sensing system will be developed using commercially available parasites, and final testing stages will use oocysts of *Cyclospora*. To determine if the sensing system has the sensitivity needed for testing produce and water samples, it will be compared to methods currently used for *Cyclospora* detection. This project represents the first step toward producing a new tool to detect and quantify *Cyclospora* quickly and cost-effectively.

Objectives

1. Determine the physiochemical signatures of model organisms using a cantilever-based microbalance sensing system.
2. Test the ability of the cantilever-based microbalance sensing system to accurately distinguish between the physiochemical signatures of multiple model organisms.
3. Determine the physiochemical signature of *Cyclospora* on the cantilever-based microbalance sensing system.
4. Test the ability of the cantilever-based microbalance sensing system to accurately distinguish the physiochemical signatures of *Cyclospora* from other protozoan parasites.

Methods

The first version of the sensing platform uses commercially available cantilevers and a micropipette delivery system aligned with controlled drop-by-drop delivery. The platform collects orthogonal signals: 1) cantilever deflection (bending), 2) shift in resonance frequency due to selective adsorption, 3) fluorescence, and 4) calorimetric infrared spectroscopic signal (**Figure 1**). Microscopic polystyrene beads were used for initial design and validation. Commercially available parasites are being used to test detection and differentiation abilities of the platform including the ability to distinguish live from dead cells. Following validation, the ability of the platform to detect *Cyclospora* and differentiate *Cyclospora* developmental stages and live/dead status will be assessed. Comparisons between the platform and traditional detection methods such as microscopy and PCR will be performed to assess sensitivity.

Results to Date

The initial development and testing of the cantilever-based sensing system was successfully completed using microscopic polystyrene beads. Data collected during this phase demonstrated that the sensing system can provide readings including mass, infrared fingerprint, and fluorescence signatures that allow for differentiation of microscopic particles (**Figure 2**). The sensor is currently being used to collect data from parasite cells, including *Cryptosporidium muris* oocysts and *Giardia muris* cysts (**Figure 3A**), which are commercially available and serve as model organisms for experimentation. Infrared spectra of the two parasites acquired with the cantilever-based scheme show some distinctive signatures (**Figure 3B** and **3C**). The findings from these initial design stages provide data that will be used to further develop and refine the sensor for testing with *Cyclospora* oocysts.

Benefits to the Industry

This proof-of-concept project will provide foundational data needed to develop a sensing device for *Cyclospora* detection. Once fully developed and paired with microfluidics, this sensing system will provide a sensitive and accurate tool for screening food and water samples (**Figure 4**). The sensing system can be used to both detect *Cyclospora* and differentiate life stages and live/dead status to determine infection potential of positive samples. Such a tool would reduce the need for recalls through early detection and intervention. Sensitive and specific detection of *Cyclospora* will better identify the sources of contamination in the environment leading to food safety improvements. The sensing system could also be developed to clean and concentrate parasites from positive samples, benefiting research into outbreak tracing and source attribution.

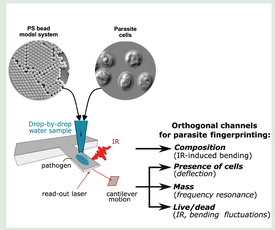

Figure 1. Model of the multimodal cantilever-based microbalance sensing system that will be used to determine the physiochemical signatures of *Cyclospora*. Microscopic polystyrene beads and parasite cells will be used to develop and validate the system.

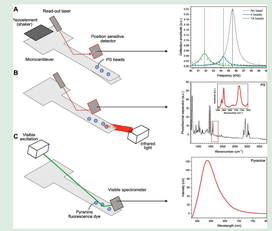

Figure 2. Data from the design and testing of the sensor using polystyrene microscopic beads. (**A**) Effect of added mass on the resonance frequency of the cantilever. (**B**) Assessment of bead composition by infrared spectroscopy showing a good match with conventional infrared spectroscopy. (**C**) Detection of the fluorescence of an added dye, pyranine, on the cantilever surface.

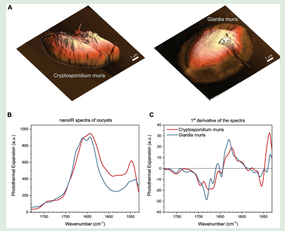

Figure 3. Differentiation of *Cryptosporidium muris* and *Giardia muris* with cantilever-based infrared spectroscopy. (**A**) Atomic force microscopy (AFM) images of the two types of parasite cells. (**B**) Infrared signature obtained with the cantilever-based measurement. (**C**) 1st derivative of the signal in (**B**) to identify the differences in the signals of the two parasite types.

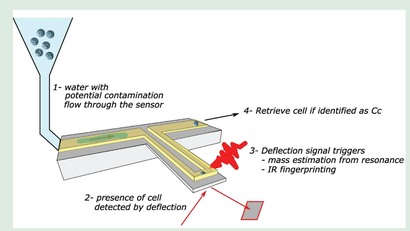

Figure 4. Diagram showing fully developed sensor.