TITLE

Enabling low-cost real-time microbial monitoring in the water industry.

RESEARCHERS

Awardee: Ameet J. Pinto

Trainees supported to date with these funds: PhD students: Benjamin Gincley, Katherine Vilardi, Solize Vosloo, Irmarie Cotto. Undergraduate students: Gabriel Goodman, Hannah Worden, Zach Flinkström, Kyle Miller, Rayne Skillin, Heloisa Tebaldi, Nikola Kapor, Tyler Gogal, and Roger Flores.

OVERVIEW

The central vision of the proposed research was to eliminate the time-gap between sample collection and microbial data acquisition by developing a technology platform that would, for the first time, empower the water industry to make real-time decisions to manage microbial communities. In doing so, the goal was to also ensure that tools for real-time monitoring become highly accessible - from large utilities to small laboratories - by developing low-cost methods that require minimal user intervention and operator expertise.

In 2019, I proposed to leverage the advances in Miniaturized Microscopy, Portable DNA Sequencing, and Deep Learning to develop a new modular platform for microbial monitoring for the water sector. The ambition was that a combination of images acquired from a low-cost optical device and low-cost real-time DNA sequencing can be used to train deep learning models that will predict the microbial concentration and composition of a sample by only analyzing images. The motivation for this work was that rapid and low-cost microbial characterization would empower the water industry to embark on a high-throughput and cost-effective exploration of the untapped microbial landscape. In turn, this would speed up biological process discovery and development in an industry where disruptive biotechnologies are infrequent to emerge and slow to be implemented.

The proposed research had three aims: (1) to develop a low-cost microscope for accurate cell counting and multi-spectral image acquisition; (2) to develop a low-cost DNA sequencing approach on a portable nanopore sequencing device using PCR-free target gene enrichment with the CRISPR/Cas system; and (3) to leverage advances in deep learning to blend the multi-spectral images with DNA sequencing data and train deep learning models to enable image-only based predictions of microbial community concentration and population abundance. The proposed design and development of the microbial monitoring system was intentionally modular, such that outcomes of the first two aims result in standalone low-cost technologies that can be used for cell counting and real-time DNA sequencing for microbial community characterization by practitioners and researchers in the water industry.

UPDATE ON RESEARCH

We have made substantial progress in development of an autonomous cell imaging system for rapid characterization of microbial communities. Specifically, we have now developed a system called the Autonomous Real-Time Microbial Scope (ARTiMiS) which combines advances in miniaturized microscopy (Aidukas et al., 2019) with flow imaging microscopy (FIM) which can automatically take in samples, capture, and process images (~20 seconds per image) using an embedded machine learning approach (**Figure 1**) (Aim 1). The ARTiMiS aspirates a liquid into a microfluidic chamber, uses CCTV camera lenses in a unique reversed lens configuration for imaging, and performs all necessary image processing on an onboard Raspberry Pi for cell counting. Microbial cell counts are consistent with expensive alternatives and morphological cell features of larger organisms like diatoms or cyanobacteria can be visualized in high resolution (**Figure 1**). The current device demonstrates high cell counting accuracy (~99.1%) as benchmarked against flow cytometry. To our knowledge, this is the first device of its kind for continuous microbial monitoring at a low price point (~$500/unit). We have filed one provisional patent for this platform and are in the process of filing a second provisional patent along with efforts to trademark the underlying bespoke software (i.e., PhycoSight) that utilizes state-of-the-art deep learning methods for image classification (Aim 3).

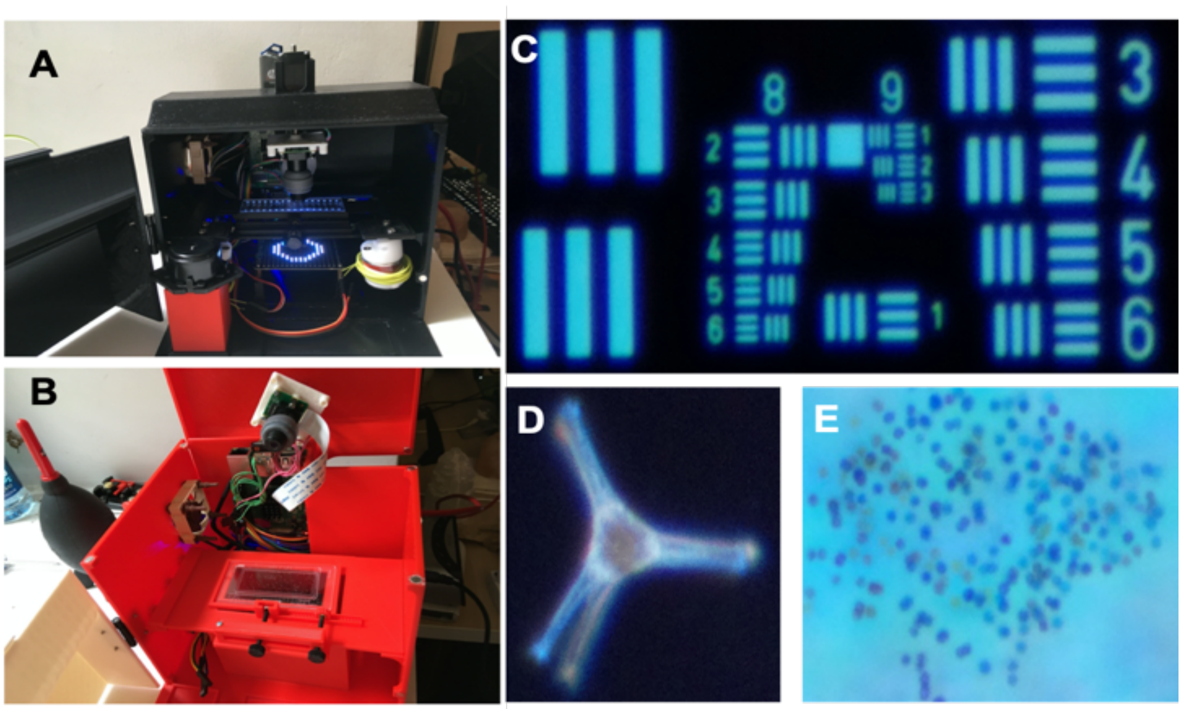

Figure 1: (A, B): Benchtop ARTiMiS prototypes designed and fabricated at Northeastern University. (C) USAF 1951 Resolving Power Test Target. Group 9, Element 3 Line Width: 0.78µm. (D) *Staurastrum* recovered from NJ lake (dark field). (E) *Microcystis* recovered from Charles River, MA (bright field).

APPLICATION UPDATE

We have leveraged this platform (ARTiMiS + PhycoSight) to develop collaborations with water utilities and vendors within the water sector. Specifically, we are currently working with the Department of Energy and Clearas Water Recovery Solutions to implement the ARTiMiS for the real-time monitoring of a full-scale advanced biological nutrient recovery (ABNR) system that utilizes microalgae for phosphorus recovery. Over the last year, we have sampled the full scale ABNR system daily using the ARTiMiS device (**Figure 2**) and have developed a highly curated image database of microalgal species of relevance for microalgal nutrient recovery systems.

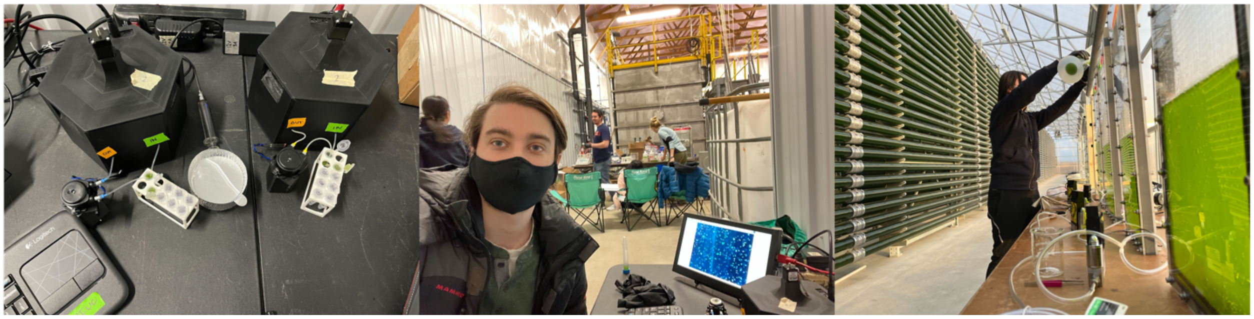

Figure 2: (Left to right) Two ARTiMiS devices (left panel) deployed by graduate student Benjamin Gincley (middle panel) at the full-scale microalgal nutrient recovery system operated by Clearas Water Recovery solutions (right panel).

Similarly, we are also collaborating with Great Lakes Water Authority, LimnoTech, and University of Windsor to develop our platform towards monitoring for harmful algal blooms in the Great Lakes region. This project is funded as a tailored collaboration by the Water Research Foundation. The goal of this project is to develop a dense data library to enable real-time species level classification of plankton in fresh waters to allow for rapid detection of harmful algal blooms (**Figure 3**). This library will significantly expand the impact of the ARTiMiS device in the environmental monitoring space.

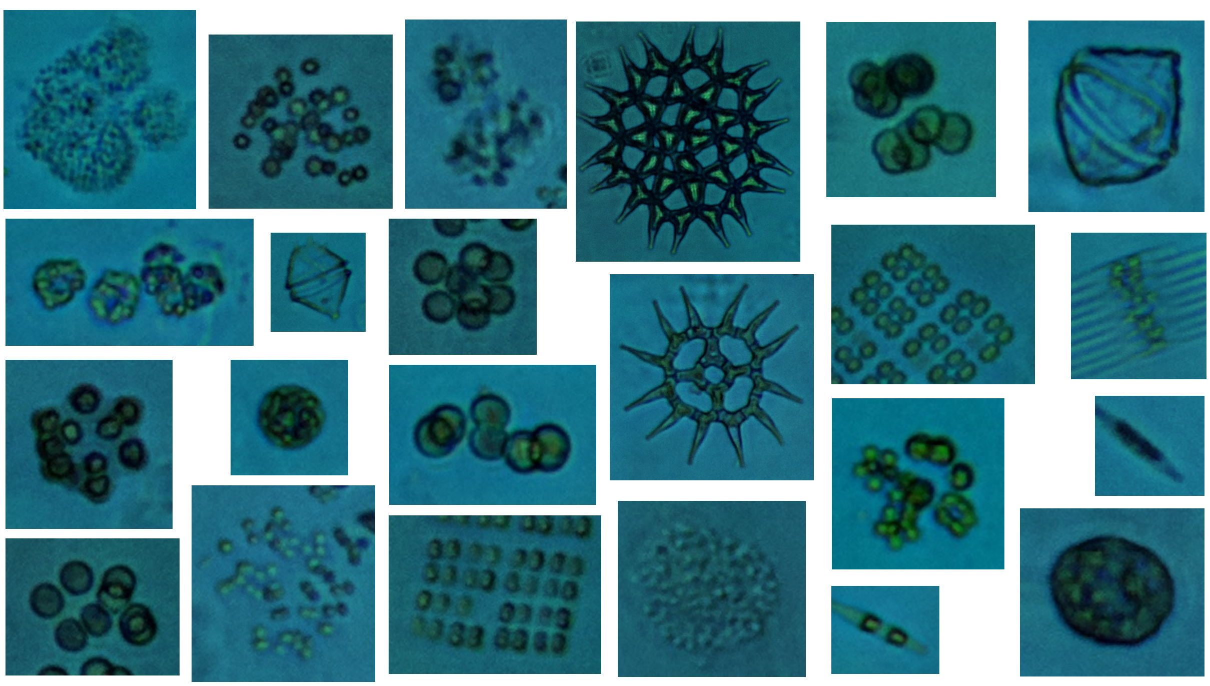

Figure 3: Example images of plankton species from the Great Lakes region. These images are being captures autonomously and are being annotated to generate high quality image database to develop an ARTiMiS enabled early warning system for harmful algal blooms.

AWARD FUNDS

Over the three years, the Paul L. Busch funds have helped support the research of four Ph.D. students, namely Benjamin Gincley, Katherine Vilardi, Solize Vosloo, and Irmarie Cotto. The funds have also helped support undergraduate students Gabriel Goodman, Hannah Worden, Zach Flinkström, Kyle Miller, Rayne Skillin, Heloisa Tebaldi, Nikola Kapor, Tyler Gogal, and Roger Flores. This student support and technology development has resulted in one student being awarded the NSF Graduate Research Fellowship, securing of a Department of Energy grant, as well as a WRF Tailored Collaboration grant to expand the impact of the Paul L Busch Award.

CONCLUSIONS

In summary, with the Paul L. Busch award, we have developed a novel low-cost autonomous platform that is beginning to have substantial impact in multiple sectors within the water industry, from nutrient recovery to harmful algal bloom monitoring. Further, the funds have allowed for sufficient technology development that the research team has filed one provision patent, is in the process of filing one more and intends to spin out a company in the next 12-24 months; this impact would not have been possible without the timely and generous support of the Paul L Busch Award.