National Aeronautics and Space Administration

Classification of bacterial life using spectral data from a multiwavelength digital holographic microscope

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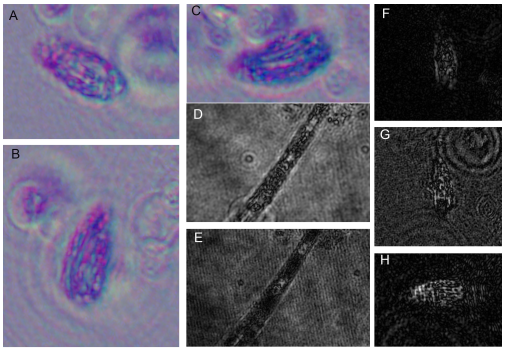
Objectives:

The objective of this project is to extend capabilities of the multiwavelength digital holographic microscope (DHM) for use in life detection and classification. We set out to use multi-wavelength DHM to make distinctions between various bacteria and algae based on the absorptions of chlorophyll. A stretch goal was to develop a submersible enclosure for the DHM that would allow for the collection of marine data sets in situ and down to 1000 meters in depth.

Approach and Results:

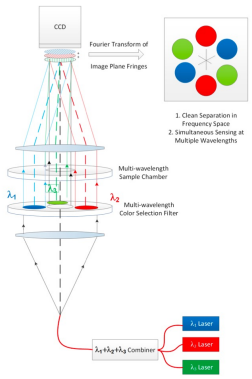
We began with an existing design that utilizes 3 color filters to separate light near the ranges of 440 nm, 520 nm, and 620 nm. This design was then retrofitted into an assembly that consisted of both lens tubes and cage-rod assemblies such that the DHM could be secured in a submersible housing. We then utilized an existing tunable fiber optic laser to acquire data from the DHM with real biological samples. Each sample underwent testing at 2 sets of 3 wavelengths. The first set consisted of wavelengths at 470 nm, 522 nm, and 640 nm. The second set contained wavelengths 10 nm apart from the first and were 480 nm, 532 nm, and 650 nm. The result was a data set of five data sets which included four separate biological samples and a single set where all four samples were mixed. In our analysis of this data, we could identify characteristics in the reconstructed data that were indicative of a relative sample containing chlorophyll. We have also begun working through the mixed cases of data to utilize these techniques and decipher between different bacteria by their absorptions across the wavelengths we have tested.



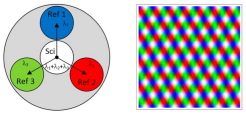
(A-C) RGB composites of *Paramecium bursaria* at 470, 522, and 640 nm. The Chlorella symbionts, which contain large amounts of chlorophyll, appear red. (D) A green algae cell in the green channel (522 nm). The lack of absorption makes the cells appear translucent, and intercellular structure can be seen. (E) The same cell in the blue channel (470 nm) appears dark due to chlorophyll. (F-H) Absolute differences between different channels for *P. bursaria*. (F) Difference between blue and green. (G) Difference between red and green (G) Difference between blue and red.

Background:

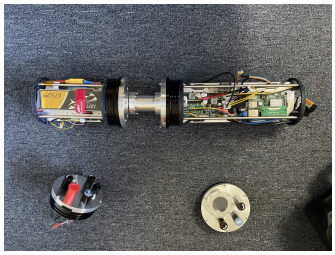
While we look for traces of life in distant soils and atmospheres, the first detection of a living extraterrestrial species is likely to come in the form of bacterial life. As a contribution to this effort, we have developed digital holographic microscopes (DHM) to detect life at the micron scale. Here we seek to improve the design of a multiwavelength microscope by fine-tuning the wavelengths such that they are strongly absorbed by known samples of bacteria. In this way, the differential spectral absorption between bacterial species may be discriminated. We will be specifically targeting the absorption wavelengths of chlorophyll A & B, commonly found in algae and plankton. We have tested our DHM on multiple species of algae utilizing 6 separate wavelengths. Alongside the development of this instrument was the development of a completely submersible enclosure that can take *in situ* live image captures with the multiwavelength DHM at depths down to 1000 meters.



Schematic of simultaneous multi-wavelength holographic microscope



Relationship of optical filters and fringes showing how the different wavelengths are separated.



Existing submersible system with housing removed to show internals. The lasers and filters can be readily exchanged to desired wavelengths.

The submersible instrument developed from this project has also undergone significant improvements from past designs. First, the enclosure has been upgraded to be suitable for extreme depths up to 1000 meters. This was done by upgrading the optical windows to sapphire ports and designing custom end caps made out of 7075 aluminum. Additionally, the previous generations of this submersible design included an open-water flow channel to view samples as they naturally flowed in front of the microscope. This design originally meant that the volume being viewed by the microscope was tens of millimeters deep which increased the amount of noise and floating particles beyond the refocusing depths of the microscope. This was solved by a specially designed extruded optical flange that allowed for the optical windows separating the collimated laser and the microscope to be only 1 millimeter apart from each other. Between the two windows is a resin-printed separator that includes a flow channel for water to freely cross the path of the DHM.

Significance/Benefits to JPL and NASA:

Our results show the capabilities of using the multiwavelength DHM to classify bacteria by the individual absorptions of chlorophyll alongside the tracking of their movements in XYZ and time. This has impacts for future instrument generations which can be fine-tuned to detect absorptions from various other organic signatures in the search for life on other worlds, including use of other wavelength combinations adjusted for available light in those locations. Additionally, the testing of this microscope at depths of 1000 meters in the ocean is a steppingstone toward the first set of holographic microscopes to take data *in situ* in ocean environments. This reduces the need to collect and transport biological samples from deep depths of the ocean for analyzing in a lab or in surface systems. There are potential collaborators with science and commercial applications that are interested in our deep-sea instrument innovations to study plankton and algae *in situ* at depths up to 6000 meters. This will significantly advance the research goals at NASA/JPL as the first steps toward instrumentation capable of analyzing bacteria on ocean worlds within our solar system.

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