Through the Looking Glass: Holographic and Molecular Detection of Zooplankton

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ABSTRACT

Monitoring of plankton communities is of increasing interest to marine scientists for reasons from the monitoring of harmful algal blooms (HABs) to understanding larval recruitment and the affects of climate change on oceanographic processes such as upwelling. The LISST-HOLO (laser in situ scattering and transmissivity holographic system) is a recently developed instrument that provides statistics on complex particles such as size, volume and counts, as well as detailed images of particles throughout the entire sample volume. This instrument has the potential to give highly detailed information about plankton communities and their taxonomic composition and could replace time consuming microscope identification in some cases. It is important to test and calibrate the LISST-HOLO for better understanding of its sampling abilities and data output. One useful comparison is with molecular data, such as the sandwich hybridization assay used in this study, as it can provide a ground truthing for the LISST-HOLO particle data. This study found strong correlations between LISST-HOLO data and molecular SHA signals for known numbers of

the copepod *Tigriopus californicus* (Order Harparticoida). While the LISST-HOLO produces useful and highly detailed images, it seems likely that *in situ* communities are being undersampled due to the fast flow rate and slow sampling time of the LISST-HOLO when used on the AUV Dorado platform. Further work is needed to develop a laboratory flow through system to better mimic LISST-HOLO sampling from AUV platforms, as well as better understand the LISST-HOLO's response to other zooplankton, phytoplankton, and mixed communities.

INTRODUCTION

Earth's oceans cover most of the planet's surface and feed more than fifty percent of the population, so it is crucial that we understand the complex web of interactions that exists in its depths. While charismatic megafauna capture the public's interest, one of the most important areas of research in marine science is the study of microscopic plants and animals- plankton. Our understanding of planktonic community is crucial in understanding harmful algal blooms (HABs), larval recruitment, and changes due to climate change and ocean acidification. Currently, much work is being done at MBARI to monitor the changing in communities along frontal systems in the Monterey Bay and California Current System.

In recent years, monitoring efforts have turned increasingly to remote sensing using satellites, buoys, and autonomous vehicles. MBARI's autonomous underwater vehicles (AUVs) are used for everything from monitoring harmful algae blooms to tracking upwelling plumes to high resolution seafloor mapping. The AUVs carry a wide range of sensors for biological, chemical, and physical parameters that can be adjusted to optimize capabilities for a given task. For biological studies, monitoring can involve various fluorometers, the LISST-100x, the LISST-HOLO, the laser optical plankton counter (LOPC), bioluminescence measures, and many other instruments. Many of these sensors measure a proxy of the organisms such as fluorescence, bioluminescence, or chlorophyll, knowing exactly what taxa are present is much more difficult. The LISST-HOLO is an instrument developed in the last decade that endeavors to give researchers an automated view of the particles *in situ*. On the Dorado class AUVs, whole water samples

are taken using “gulpers,” fast vacuum suctioning of approximately two liters of water, which is stored in the vehicle and analyzed in the laboratory after retrieval of the vehicle.

This study focuses on the LISST-HOLO as an imaging platform with a wide range of size detection including both phtyoplankton and zooplankton. The LISST-HOLO is mounted as a flow through system on the same channel as the LOPC. The sample then flows between the light source and a detector and is measures at 0.2 Hz. Particles are measured using a 658 nm (red) solid state laser diode over a sampling volume of 1.86 cm^3. While the flow rate should not exceed 0.4 m s^{-2}, the current speed of the AUVs is 1 to 2 m s^{-2}, the effect of which has yet to be explored. The LISST-HOLO can detect particles from 25 to 2500 µm, and bins them into the same 32 size classes as the LISST-100x, making comparison and condensation of data sets between the instruments efficient. The instrument has a depth rating of 300 meters, allowing the AUV to dive to significant depths to sample the thermocline and shallower sea bottoms. MBARI currently uses both the LISST-100x and the LISST-HOLO on its Dorado AUVs.

Data output for the LISST-HOLO is in the form of raw holographs, particle montages, as well as particle statistics for major and minor axes, area, volume, and numbers in size bins, and summary statistics for each holograph.

Copepods are one of the most common zooplankton groups in both marine and freshwater systems, making them highly relevant organisms for study. This study uses *Tigriopus californicus,* an intertidal Harpacticoid copepod, as the organism of focus due to its ease of culturing and the availability of existing cultures. These copepods are also quite large, which was a factor in ease of detection for the LISST-HOLO and molecular probes for this group are readily. While *in situ* measurements of plankton communities using the LISST-HOLO have been successful, it is unclear how representative the LISST-HOLO data is compared to the plankton community.

Molecular methods are commonly used to analyze whole water samples for understanding of plankton community composition and diversity. Sandwich hybridization assay (SHA) is a molecular technique used to detect specific taxa of interest and is proportional to the amount of RNA in the sample. In this study, SHA is used as comparative data set to the holographic particle data from the LISST-HOLO. SHA has

been used in previous studies (e.g. Harvey et al 2012) to study *T. californicus* RNA concentrations.

The purpose of this study is to examine the relationship between detection of *T. californicus* by the LISST-HOLO and the molecular signal obtained through sandwich hybridization assays (SHAs). With increasing numbers of copepods in a sample, the LISST-HOLO should detect more large particles and the signal produced with SHA should also increase proportionally.

METHODS

The *Tigriopus californicus* (Harpacticoida) cultures were kept at 15°C in a twelve hour dark-light cycle during the duration of this experiment. Samples were isolated from culture into batches of 1, 5, 10, 15, 20, 30, and 50 individuals and placed into 50 mL of filtered sea water (FSW).

LISST-HOLO

Each sample was placed into the LISST-HOLO sampling chamber for three minutes with holographs taken every five seconds (the maximum sampling rate possible for the instrument). The samples was then drained onto a 60 µm nylon mesh filter, and the LISST-HOLO sampling chamber rinsed three times with 50 mL of FSW to avoid cross-contamination between samples. Despite this rinsing, *T. californicus* is extremely persistent, and as such, samples were run from the controls to the highest concentrations to prevent minimize the effect of contamination of subsequent samples. The number of copepods on the filter was recorded to control for copepods remaining in the sample chamber. The filter was then placed in a 2 mL cryovial and stored in liquid nitrogen until molecular analysis.

Output from the LISST-HOLO includes particle statistics files and image montages of particles detected. Batch processing of holographs was done with research code provided by Alex Nimmo-Smith (University of Plymouth, UK). Particle statistics files were binned into particles with a minimum axis larger than 100 µm to exclude possible detrital particles from inclusion in analyses.

SANDWICH HYBRIDIZATION ASSAY (SHA)

Sandwich Hybridization Assay (SHA) is a molecular technique that uses taxon specific probes to identify organisms and provides a signal proportional to the amount of rRNA in a sample (See Harvey et al. in press Fig. 1, 4). The probes bind to specific sequences of 18s rRNA, and the podoplean copepod probe Pod1951 was used for this analysis. Plates were built according to Harvey et al. in press Fig. 4, with following solutions:

Substrate: Therrmo Scientific 1 step Ultra TMA Elisa (Prod. # 34028)
Conjugate: 1:400 conjugate from the Scholin lab
Signal Solution: Zoo Signal solution of EUK519, EUK915, EUK1194
Capture Probe: Pod1951

Samples were removed from liquid nitrogen storage dewars and defrosted slightly before analysis. 1.9 mL of 1.5 M lysis buffer was added to each sample tube, and the sample incubated in a water bath at 85°C for five minutes. Each sample was vortexed before incubation and at the 2.5 minute mark. After incubation, an additional 0.2 mL of lysis buffer added and the sample was syringe filtered through a 0.22 µm filter and loaded into 3 wells of the lysis row in the 96 well plate. Plates were run with polystyrene biotinated prongs on a Microprobe Affirm Processer. Plates were then read on a microplate reader at 650 nm, fixed with 10% sulfuric acid, and read again at 450 nm. Signals were averaged and standard error calculated from each sample of three replicates.

RESULTS

Particle detection was very low, with an average of 9.8% of copepods known in sample detected by the instrument (Fig. 1). There was a slight trend of increasing particles with increasing copepod numbers in sample, but it was a not a strong relationship ($R^2 = 0.459$).

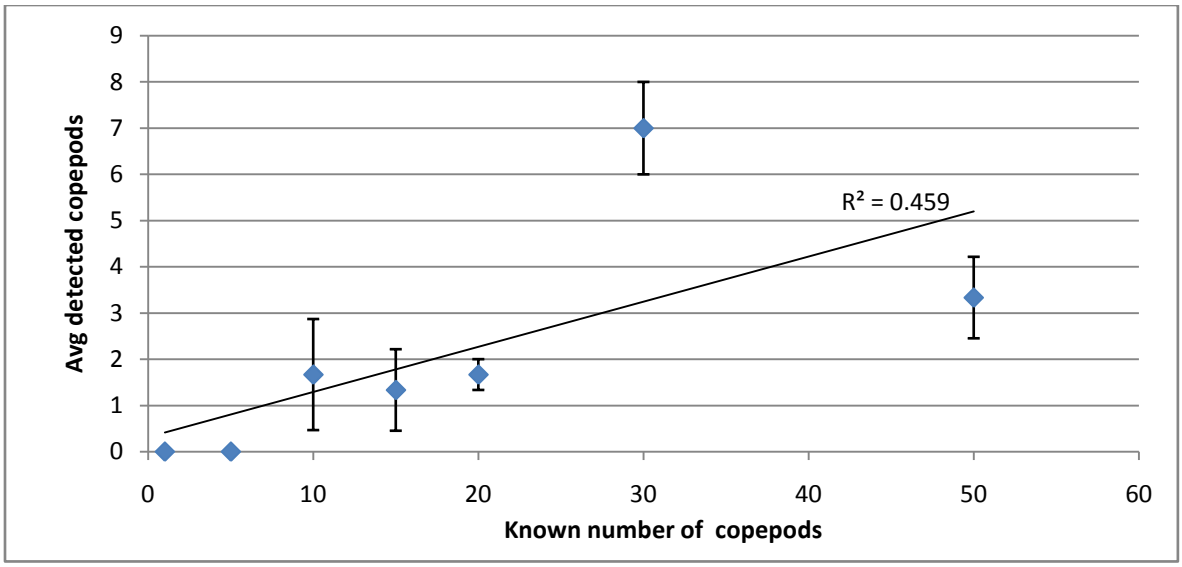

Figure 1 – The particle area measured by the LISST-HOLO increases with increasing numbers of copepods in the sample. ($R^2 = 0.459$)

SHA signal is representative of the amount of rRNA in a sample, and SHA signal was found to have a strong positive relationship to increasing numbers of copepods in sample (Fig.2).

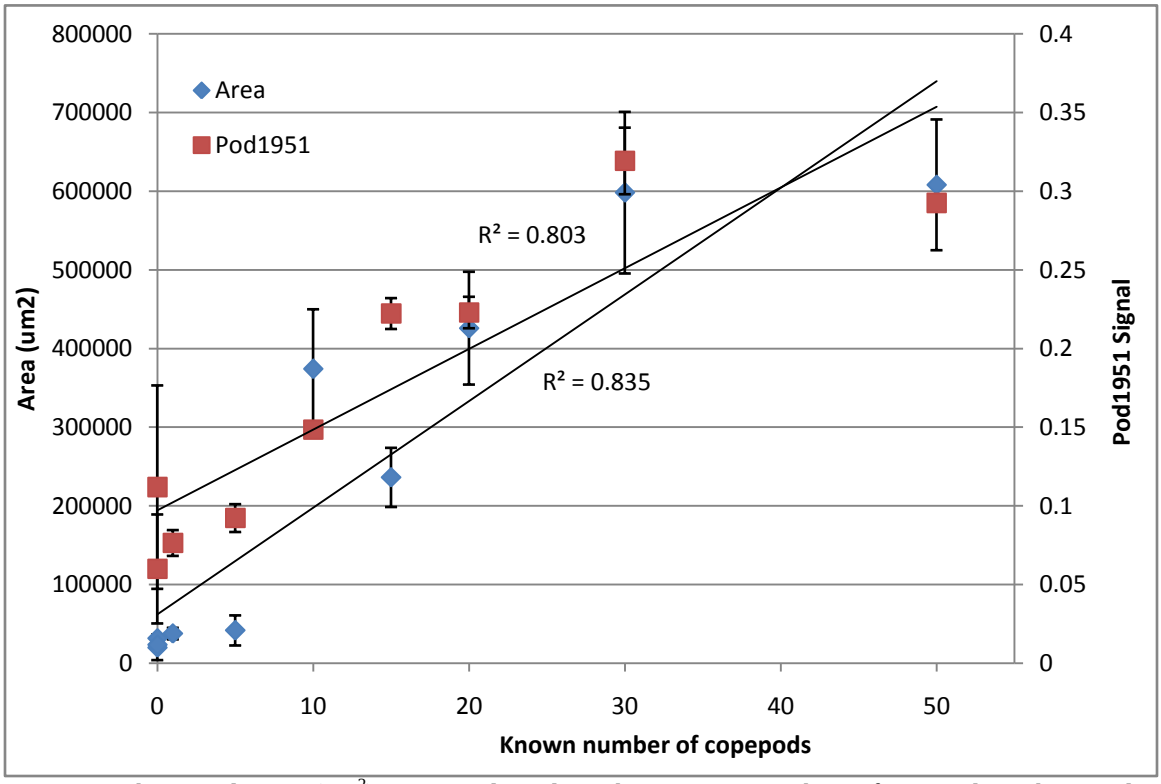

Figure 2 - The particle area (μm^2) increases linearly with increasing numbers of copepods in the sample. ($R^2 = 0.835$)

DISCUSSION AND RECOMMENDATIONS

There was a positive relationship between increasing copepods in a sample and increasing copepods detected, despite a weak linear correlation (Fig. 1). The strength of the relationship could likely be improved with increased experimental replicates. A very low percentage of copepods were detected (9.8%), however the laser optical sampling area makes up only 4% of the 50 mL volume and water motion was not likely enough to circulate the full volume through the optical detector. More information and study of the effect of flow on LISST-HOLO sampling would be required to get a better idea of the sampling rate *in situ.*

The strong correlation between particle area, SHA signal, and number of copepods in a sample is promising for the representativeness of LISST-HOLO detection. For this experiment, the instrument and software detection settings were optimized for the large copepods being tested, and work with other zooplankton, phytoplankton, and mixed communities would reveal more about the detection and limitation of the LISST-HOLO system.

Due to the complexity of the sample chamber and the tenacity of *T. californicus*, it was difficult to ensure the independence of samples, so the number of copepods on the filter was enumerated. When comparing the LISST-HOLO detection and SHA signals to this other number of copepods, the linear correlation was almost identical to the results above ($R^2 = 0.834$ and $R^2 = 0.83$ respectively).

This study concluded that the use of the LISST-HOLO is incredibly useful, and can be used to get an idea of taxonomic diversity in *in situ* communities. However, further analysis for the detection rates and sensitivity in particle statistics is needed. Using molecular analysis provided a strong basis for comparison with the holographic data and confirms that the LISST-HOLO particle detection is proportional to number of organisms. A flow through laboratory system would be crucial for further laboratory studies to better mimic the *in situ* conditions of AUV sampling. Further testing with other organisms, zooplankton, phytoplankton, and mixed communities would give a better idea of the detection limitations and parameters possible with the LISST-HOLO. The software

for processing holographs also require careful use of settings for accurate particle detection and statistics output.

The big picture goal of this project was to look at the feasibility of the LISST-HOLO as a substitute for time consuming microscopic or molecular analysis of whole water samples examining plankton communities. While there is still a ways to go in understanding the instrumental limitations, there is plenty of evidence for successful implementation of the LISST-HOLO for future taxonomic studies. In the context of the long-range AUV lab, the LISST-HOLO could be add a level of complexity to vehicles that are not large enough to carry whole water samples, but a reduction in size and weight of the instrument would be required for addition to the LR-AUV platform.

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