AN OPERATIONAL *IN SITU* ICHTHYOPLANKTON IMAGING SYSTEM (ISIIS)

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1. ABSTRACT

One driving factor improving the resolution of oceanographic sampling has been the observation of fine structure in the ocean. As oceanographers improve their sample resolution, the finer patterns that are discovered lead to a better understanding (and new questions) about dynamic processes in the ocean. To date, current technologies available for the study of many zooplankters remain limited in comparison to the spatialtemporal resolution and data acquisition rate available for physical oceanographic measurements, especially for the relatively rare meso-zooplankton. To overcome these challenges, we have built a towed, very high resolution digital imaging system capable of sampling water volumes sufficient for accurate quantification of mesozooplankton in situ. The images are high quality, enabling clear identification of meso-zooplankters (e.g. larvaceans, gelatinous zooplankters, chaetognaths, larval fish), often to family or genus level. However, the efforts directed toward high speed and high-resolution imaging have the potential to create a bottleneck in data analysis. To address this problem we also have developed efficient algorithms to detect multiple regions (organisms) of interest (ROI) automatically, while filtering out noise and out-of-focus organisms, and simultaneously classify the detected organisms into pre-defined categories using shape and texture information. Here we demonstrate the current design, image quality, image analysis approach and example data analyses as an overview of the system capabilities.

2. INTRODUCTION

Biological sensor development, especially for whole organisms, has lagged behind that of physical and chemical sensors. Yet, with advancing plans for integrated ocean observing systems (IOOS), there is a critical need for sensors capable of autonomously gathering biological data relevant to ecosystem health and resource assessment. This need is clearly outlined in the Ocean Research Priorities Plan (ORPP), and implementation planning of the Ocean Observation Initiative (OOI). Here we describe the development of a plankton sensing system capable of quantifying mesoplankton via very high resolution imagery, coupled with an advanced, *automated* image analysis system. This sensor system, *IS*IIS (*In Situ* Ichthyoplankton Imaging System) has the potential to significantly enhance the spatial and temporal resolution of plankton sampling, while reducing time and cost of biological data acquisition, processing and analysis [4].

We have built a *towed*, very high-resolution digital imaging system capable of sampling water volumes sufficient for accurate quantification of mesozooplankton *in situ*. We combined various state-of-theart digital imaging and computer technologies (e.g. incorporating machine vision technology) with a shadowgraph light scheme. The images are high quality, enabling clear identification of meso-zooplankters (e.g. larvaceans, gelatinous zooplankters, chaetognaths, larval fish), often to family or generic level. Simultaneously, we have initiated work on image analysis of the digital data from this system – developing automated extraction of Regions of Interest and recognition of the detected organisms using shape and texture information.

3. ISIIS DESCRIPTION

This camera system utilizes a high-resolution linescanning camera with a Light Emitting Diode (LED) light source, modified by plano-convex optics, to create a collimated light field to back-light a parcel of water (Fig. 1). The imaged parcel of water passes between the forward portions of two streamlined pods (UW housings), and thereby remains unaffected by turbulence. The resulting very high-resolution image is of plankton in their natural position and orientation (see Figure 2).



Figure 1. Light scheme using shadowgraph technique. Light passes through plano-convex lenses thereby establishing a pseudo-collimated light beam refocused by a second field lens before it impinges on an imaging lens. The advantages of this approach over other lighting techniques include: High depth of field (40+ cm), telecentric image (magnification level not affected by distance from object to the lens), and very sharp outlines of organisms and internal structures (facilitate automated recognition).



Fig 2. **ISHS example images**. Images taken from both low latitude (clear waters) and high latitude (highly productive waters). From left to right: Larval flatfish (~6 mm TL), pelagic polychaete (Tomopteris sp. ~ 6 mm; note also in this figure a small larvacean and multiple diatoms), ctenophore, (~ 20 mm) larval wrasse (Thalassoma bifasciatum; ~ 7 mm)). Next row: pelagic shrimp (~ 15 mm), larval flatfish (Bothus sp. ~ 6 mm), larvacean (appendicularian – Oikiopleura sp.; ~ 2 mm), urchin pluteus (~1 mm), copepod (~ 2 mm).

When a sufficient volume of water is imaged this way, quantification of density and fine scale distribution is possible.

3.1 Lighting - The focused shadowgraph technique (Fig. 1) allows for a long depth of field not achievable with other lighting techniques such as dark field or simple backlighting [1], [8]. Since the light rays are directed

toward the imaging sensor and not reflecting off the imaged subject, the intensity of light required is extremely low compared to any other lighting technique. This avoids the use of bright light sources that may deter organisms away from the imaging area.

3.2 Camera - For imaging, we used a line-scan camera (DALSA Piranha 2). These cameras create a continuous image, differing from sequential flash or video images that are successive and may have gaps or overlap. Hispeed scanning rates of the line-scan camera also allow for high-resolution images. The camera system used in our prototype had a vertical resolution of 2048 lines and a 36 KHz scanning rate. This combination provided for a continuous visual field that was approximately 14 cm tall with a 20-40 cm depth of field depending upon the size of the point source of light used. Thus, when towing the instrument at 5 knots (2.5 m s⁻¹), the volume of water imaged every second was ca. 70-140 liters (14 cm X 40 cm X 250 cm). As a typical 1 m² plankton net filters *ca*. $0.75 \text{ m}^3 \text{ s}^{-1}$ (at a tow speed of ~ $0.75 \text{ m} \text{ s}^{-1}$), our system images close to 10-15% of the volume filtered by a net, which is greater than an order of magnitude improvement over other imaging systems. Moreover, pixel resolution is approximately 68X68 µm, resulting in a very high-resolution image.

In collaboration with the ocean engineering firm, Bellamare, LLC, we designed and constructed a selfundulating, towed vehicle, including underwater housings for the camera and light system. We then utilized fiber-optic cable to carry the signal from the system to the surface enabling real-time storage and initial processing via a high-throughput computer system capable of handling the high data transfer rates (up to 140 MB s⁻¹).

4. IMAGE ANALYSIS

This imaging system produces very high-resolution imagery at very high data rates necessitating automated image analysis. As we are interested in the identification and quantification of a large number of organisms, sometimes morphologically similar to each other, we propose to develop an *automated* system for detection and recognition of organisms of interest using computer vision tools. The method aims to: 1) detect multiple regions (organisms) of interest (ROI) **automatically**, while filtering out noise and out-of-focus organisms, and 2) **simultaneously** classify the detected organisms into pre-defined categories using shape and texture information.

What differentiates our effort from published methods and publicly available software is that we aim at analyzing entire raw images as they are acquired by ISIIS, containing multiple candidate specimens, which makes our system fully automatic: from data capture to the storing of recognition results. In contrast, existing methods assume the specimens have already been *precisely* segmented, or aim at analyzing images containing single specimens (extraction of their features and/or recognition of specimens as single targets in-focus in small images. The term "precisely" is the key difference and the novelty of our overall approach. We start with the assumption that the typical scenario will be "imperfect segmentation" (i.e. either partial or oversegmentation).

This software implements a set of methodologies for detecting plankton objects in cluttered images and recognizing their types. The functionalities of the developed system are divided into three major procedures:

> 1) First, a low-level image segmentation algorithm is applied to extract salient objects from a set of input cluttered images. The employed methodologies are designed to effectively handle images containing multiple objects as well as significant levels of noise.

> 2) Subsequently, a set of low-level image descriptors (features) are computed, so that each extracted blob is represented as a feature vector of characteristic image features. Here, the

extracted feature vectors include shape histograms, blob solidity, Hu moments up to third order, Fourier descriptors, and the circular projection descriptors defined in [6].

3) Finally, a set of advanced machine learning methodologies are used to select those of the extracted objects that correspond to plankton images, and determine their most likely types, choosing from a set of predefined plankton categories previously learned from the system. The core functionality (of recognizing whether an extracted object corresponds to a plankton image or not, and what is the type of the detected plankton) of this final component of the developed system, is based on multiclass SVM classifiers [2].

A significant issue in object detection and recognition systems is the problem of over-segmentation, i.e. when a single object image is segmented into two or more fragments. To tackle this issue, on top of the employed GP-based (Genetic Programming) classifiers, we deploy an object over-segmentation rectification methodology based on conditional random fields (CRFs) [5]. Conditional random fields are graph-based discriminative classification models, with a wide range of applications in the computer vision domain. Here, CRFs are employed to detect which extracted objects



Figure 3. Example of steps taken during segmentation, extraction and eventual reconstruction of over-segmented objects and exclusion of noise. See [3], [7], [9]. [10], for details on the methods used in this software.

comprise segments of a single, over-segmented object, hence allowing for the model to attain a low final oversegmentation rate (see Fig. 3). In our system, the nodes of the CRF graph structure are taken as the extracted blobs in an input image. The unitary potentials of the model are based on the probabilities obtained by the trained GP multiclass classifiers for each blob, whereas the pairwise potentials are taken as the multiclass GPinduced probabilities of two concatenated "neighboring" objects. As neighboring objects in an image are regarded, objects with high similarity are joined, in the sense implied by application of a k-NN algorithm [2].

5. REFERENCES

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