RAPID
Research on Automated Plankton Identification

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When Victor Hensen deployed the first true plankton[1] net in 1887, he and his colleagues were attempting to answer three fundamental questions: What planktonic organisms are present in the ocean? How many of each type are present? How does the plankton's composition change over time? Although answering these questions has remained a central goal of oceanographers, the sophisticated tools available to enumerate planktonic organisms today offer capabilities that Hensen probably could never have imagined.

Nets still remain the central instrument in our plankton sampling toolbox. But at the present time, it is not uncommon to have computer-controlled underwater vehicles equipped with multiple nets or cod-ends that can be flown along precise trajectories while transmitting real-time environmental data and system telemetry to a surface ship (see Wiebe and Benfield, 2003). In addition to nets, pumping systems bring water to the surface, where plankton from different depth strata can be filtered out. The most dramatic development in plankton survey technology has been the emergence of cameras capable of imaging the contents of defined and generally undisturbed volumes of water. These imaging systems provide nearly continuous records of fine-scale distributions of plankton from centimeter- to basin-wide volumes.

Plankton-imaging systems pose new challenges to studies of aquatic biota. In this paper we summarize the development of plankton-imaging systems, advances in extracting useful information from image data sets in a timely manner, and the most pressing issues that must be resolved to further advance this field of study.

PLANKTON-IMAGING SYSTEMS

The development of plankton-imaging systems was not a simple response to the availability of compact cameras and associated electronic components. Their genesis reflects the influence of early attempts to accelerate processing of samples from plankton nets, the recognition that we needed instruments that could provide information on fine spatial and temporal scales, and interest in quantifying fragile marine aggregates.

Plankton-imaging-system development has been strongly influenced by the desire to reduce sample processing time. One thing that has not changed since the late 1800s is that the collection and enumeration of plankton samples remains a labor-intensive endeavor. Traditional microscopic analysis of preserved samples usually involves subsampling, counting, and sorting large numbers of individuals into taxonomic groups. Often, individuals are also measured using a calibrated ocular micrometer. Such activities are time consuming, resulting in a long lag between sample collection and data analysis and interpretation. Moreover, processing requires a well-trained human expert capable of frequently distinguishing subtle morphological features. Attempts to accelerate processing by extending the amount of time spent working with a microscope can lead to fatigue and increased error rates. Careful processing of samples, therefore, requires a patient and competent expert with ample time.

[1] The term plankton is used to include phytoplankton and zooplankton. While most of the current study on image classification has focused on mesozooplankton, the challenges involved are common to microzooplankton and phytoplankton.

Silhouette photography (Ortner et al., 1979) was the first attempt to create a permanent record of the contents of a plankton sample collected with a net in the form of a contact print on photographic emulsion. This print then could be examined, enumerated, and measured under a microscope or with a computer-aided system that tracked the coordinates of a cursor based on the times of arrival of a sound pulse emitted by the cursor (Davis and Wiebe, 1985). In addition to capturing silhouette images of plankton samples on photographic film, direct video imaging and digitization of plankton samples were developed together with early methods for automatic identification (Jeffries et al., 1980, 1984; Berman et al., 1990) and size-structure determination (Rolke and Lenz, 1984; Gorsky et al., 1989). More recently, silhouette photography has been modified by incorporating flatbed scanners to digitize photographic silhouettes. The resultant files can be enumerated, counted, and measured using a graphical user interface within a Matlab software package (Little and Copley, 2003).

One of the first in situ imaging systems was a direct extension of laboratory silhouette photography. Ortner et al. (1981) placed a camera in the cod-end of a plankton net and imaged plankton as they passed through the field of view. Still cameras were replaced with video cameras (Froese et al., 1990), and later the net was eliminated entirely. The Video Plankton Recorder (VPR) developed by Davis et al. (1992a) was the forerunner of a suite of modern, in situ plankton-imaging instruments. During the 1997 Global Ocean Ecosystems Dynamics (GLOBEC) Georges Bank field program, the VPR demonstrated the immense power of optical imaging systems. It was the first plankton sampling device to automatically identify and count phytoplankton and zooplankton taxa in situ and quantitatively map their abundance and distribution patterns with high resolution in real time (Davis et al., 2004).

Over the past decade there has been a proliferation of in situ imaging systems (Figure 1). Much of the focus has been on imaging mesoplankton (e.g., Gorsky et al., 2000a; Ashjian et al., 2001; Benfield et al., 2003; Davis et al., 2004; Remsen et al., 2004) and marine snow (e.g., Asper, 1987; Pilskaln et al., 1991, 1998, 2005; Gorsky et al., 1992; Diercks and Asper, 1997; Jackson et al., 1997; Gorsky et al., 2000b); however, there is increasing interest in quantifying nano- and microplankton particles (e.g., Sieracki et al., 1998; Olson and Sosik, in press; Sosik and Olson, in press) (Figure 2). Several systems utilizing holographic imaging[2] have been developed (Malkiel

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[2] Holographic Imaging: A holographic imaging system records interference fringes of light diffracted from objects and reference light on a hologram. It differs from a normal imaging system in four ways. First, it uses coherent light (i.e., laser). Second, it has no focus lenses (it is also called lensless imaging system). Third, the object image needs to be reconstructed either physically (by shining a reference light on the hologram) or numerically (by digital computer). Fourth, it has much larger depth of field and yields three-dimensional information on the spatial interrelationships among the potentially large number of objects in its greater volume.

et al., 1999; Katz et al., 1999; Hobson and Watson, 1999; Nebrensky et al., 2002) (Figure 3) and these may offer a means of imaging nano- to mesoplankton from larger volumes of water. Whether designed for small or large plankton, these instruments collect quantitative images of the contents of defined volumes of water, which provide unique

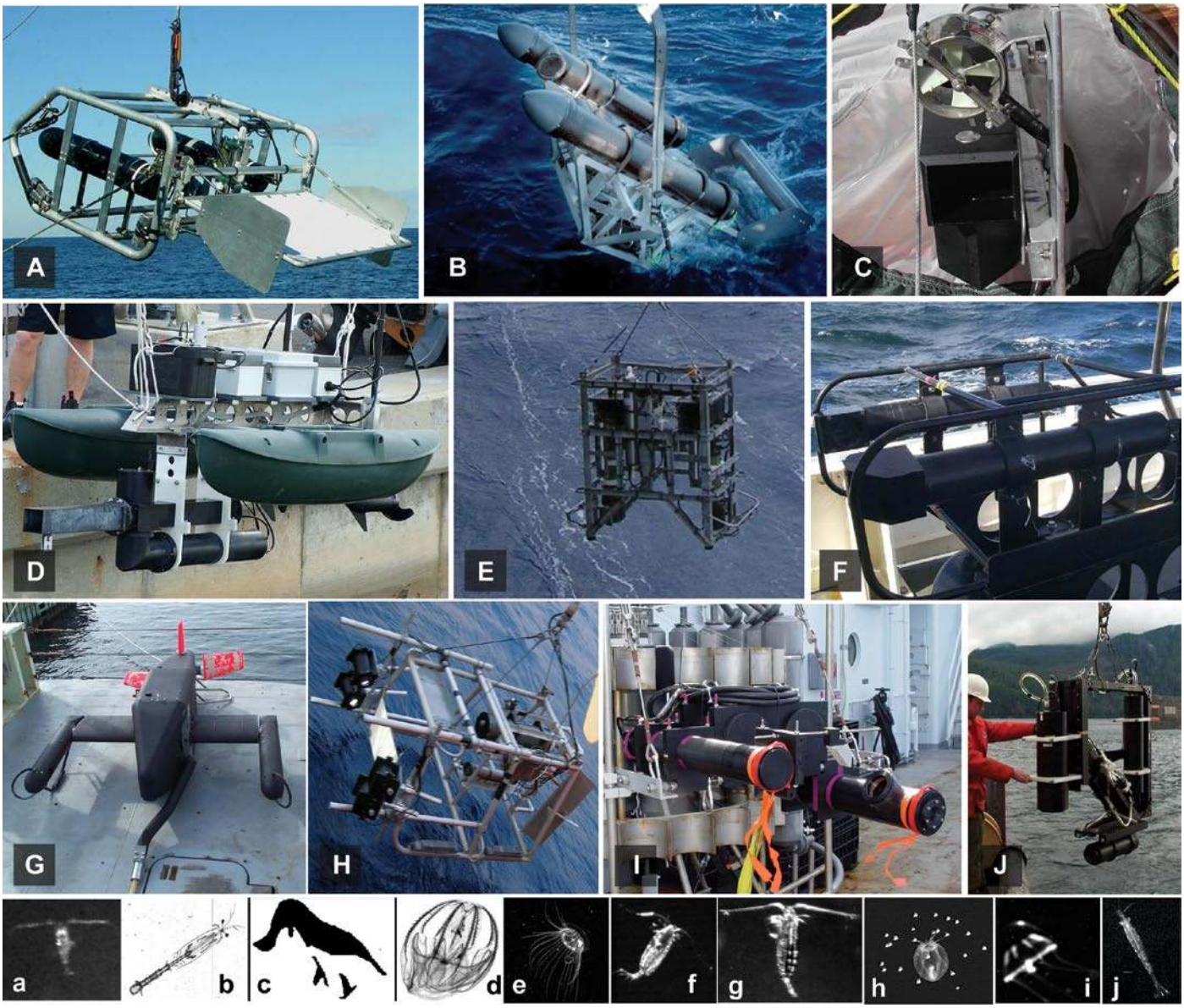

Figure 1. The number of in situ imaging systems is increasing rapidly. These are examples of some zooplankton and micronekton imaging systems (A-J) along with their corresponding (a-j) representative regions of interest (ROIs). Note that in most cases, the ROIs have been cropped from a larger image and have been resized to fit in the figure. None of the ROIs are to the same scale. A. Ocean DiVA: Digital Video Acquisition System. *Image: C. Pilskaln, SMAST* B. ISIIS: In Situ Ichthyoplankton Imaging System. *Image: R. Cowen, RSMAS* C. LOPC: Laser Optical Plankton Counter mounted in a ring net. *Image: A. Herman, DFO Canada* D. SIPPER: Shadowed Image Particle Profiler and Evaluation Recorder mounted below an autonomous pontoon vehicle. *Image: A. Remsen, USF* E. UVP: Underwater Video Profiler. *Image: G. Gorsky, Laboratoire Oceanography Villefranche sur mer* F. VPR: Video Plankton Recorder mounted on BIOMAPPER II vehicle. *Image: M. Benfield, LSU* G. VPR II: Video Plankton Recorder II mounted in the Flying Fish high-speed towbody. *Image C. Davis, WHOI* H. LAPIS: Large-Area Plankton Imaging System. *Image: E. Horgan, WHOI* I. ZOOVIS-SC: Self-Contained Zooplankton Visualization System. *Image: M. Sutor, LSU* J. ZOOVIS: Zooplankton Visualization System. *Image: M. Benfield, LSU*

information about the distribution, abundance, and behavior of plankton on scales that cannot be approached by conventional sampling systems such as nets and pumps.

One of the major advantages of imaging systems is their ability to collect information on distributions and abundances without physically contacting the target plankton. Because many taxa are quite fragile, cameras are particularly effective for studying gelatinous forms that would otherwise be destroyed or damaged in nets (e.g., Benfield et al., 2003; Remsen et al., 2004; Stemmann et al., in press). Small translucent objects such as fish eggs can be effectively imaged and counted using flow-through imaging systems adapted for shipboard use (e.g., Iwamoto et al., 2001). Most imaging systems are equipped with environmental sensors that measure hydrographic parameters on scales that can be directly related to the organisms imaged to provide insights into the subtle relationships between hydrography and species distributions (e.g., Ashjian et al., 2001, 2005; Davis et al., 2004). Cameras permit measurement of the orientations of zooplankton, which affect their acoustical scattering strength and may also be used to infer behavior (Benfield et al., 2000). Highly capable imaging systems can provide a near-continuous picture of the distributions of plankton on basin scales. Such deployments have recently revealed much deeper distributions of the cyanobacterium *Trichodesmium*, with implications for nitrogen-fixation rates and patterns (Pilskaln et al., 2005; Davis and McGillicuddy, 2006).

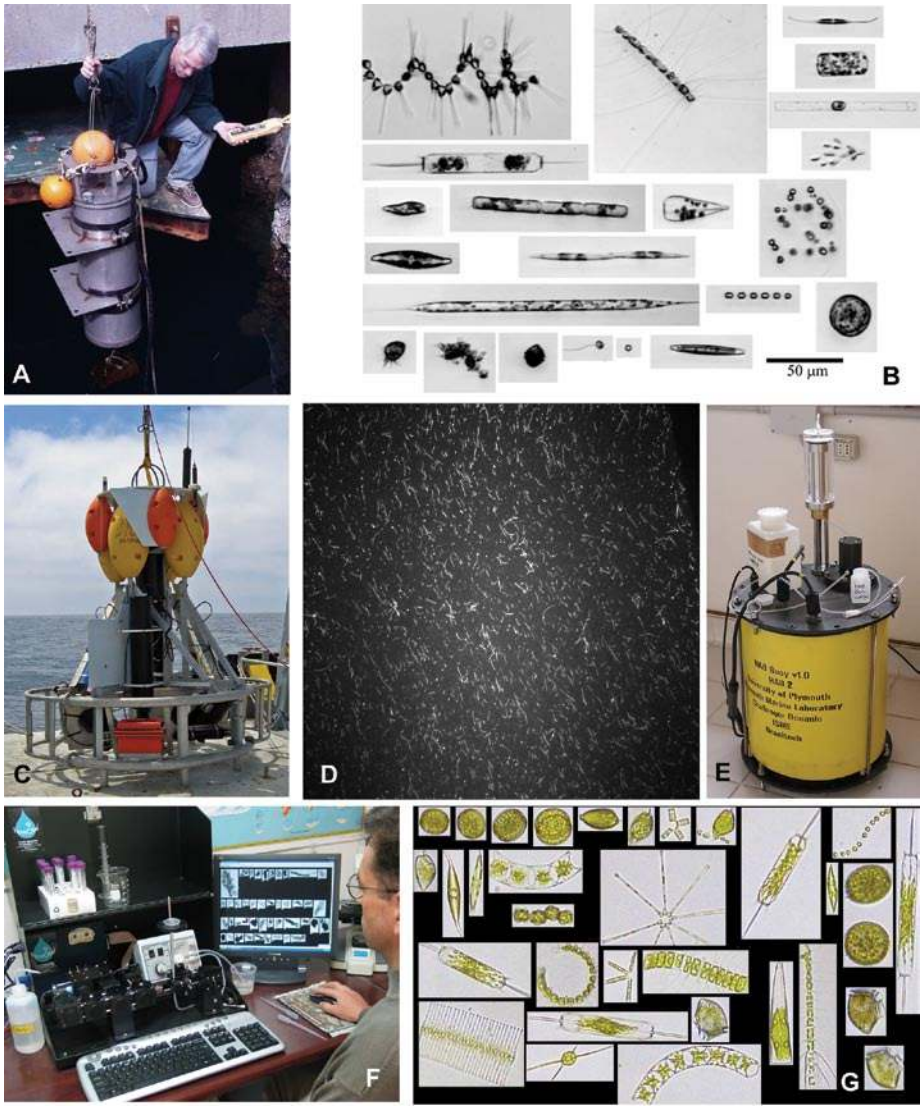

Figure 2. There is great interest in developing systems capable of quantifying phytoplankton-sized particles in situ. A. An in situ imaging flow cytometer called the FlowCytobot being deployed. B. A collage of images produced by the FlowCytobot. *Images: R. Olsen and H. Sosik, WHOI* C. Fido-ϕ is a free-falling imaging fluorometer that quantifies phytoplankton and other particle distributions within discrete slabs of water. D. Images of diatom chains from Fido-ϕ. *Images: P. Franks and J. Jaffe, Scripps Institution of Oceanography* E. The Harmful Algal Bloom (HAB) Buoy, an in situ phytoplankton and zooplankton-imaging system currently under development. F. FlowCAM is designed to image microzooplankton and phytoplankton. *Image: M. Sieracki, Bigelow Laboratory for Ocean Sciences* G. A collage of images from the new Color FlowCAM. *Image: M. Sieracki, Bigelow Laboratory for Ocean Sciences*

IMAGING PRESERVED PLANKTON SAMPLES

In situ instruments are not the only area where plankton imaging is making inroads. Direct digitization of plankton samples from nets and pumps is an increasingly popular method for pro-

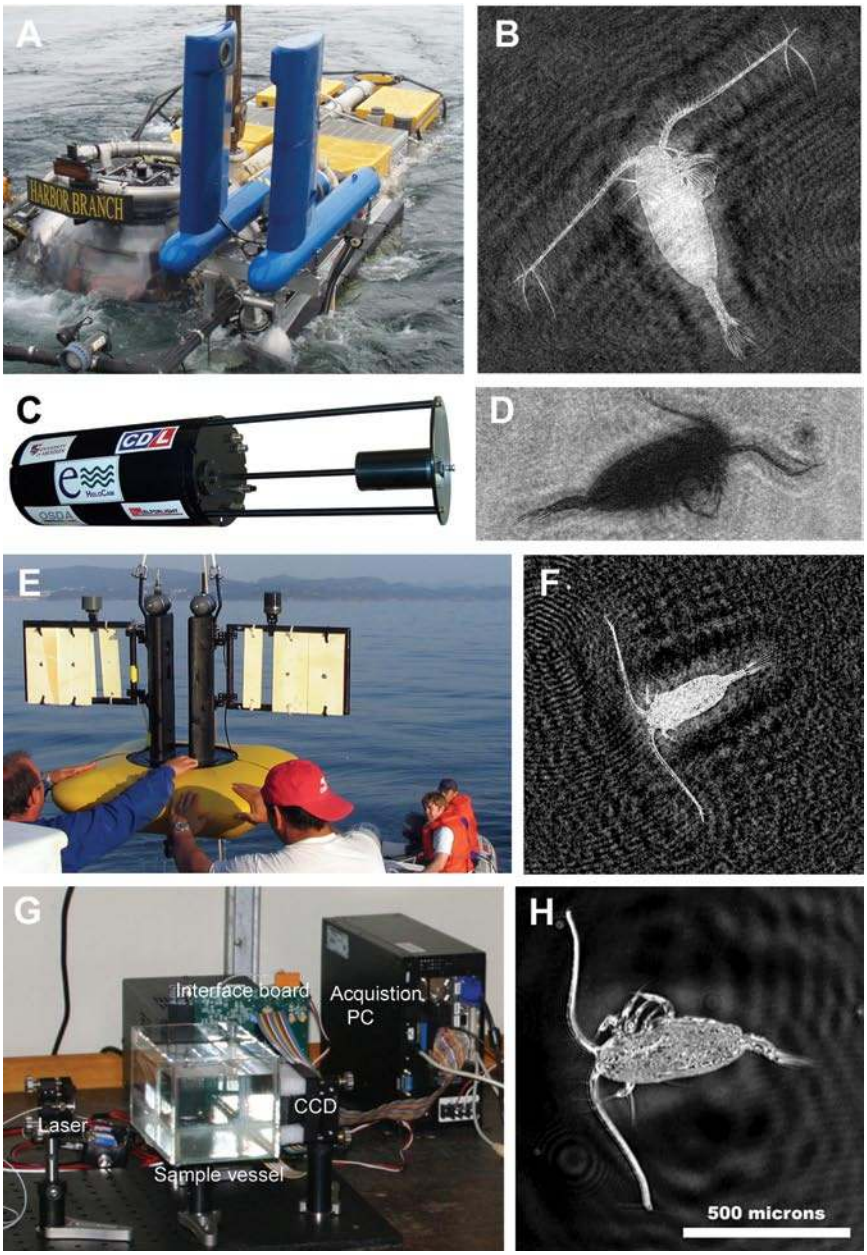

Figure 3. Holographic systems offer a means of imaging plankton over a broad range of sizes while preserving their spatial interrelationships. Examples of systems that are operational or under development along with example plankton images include: A–B. The Holocamera. *Image: J. Katz, Johns Hopkins University* C–D. The eHolocam. *Image: CDL Ltd., Aberdeen and University of Aberdeen* E–F. The Digital Holosubmersible. *Image: E. Malkiel, The Johns Hopkins University* G–H. The DHI: Digital Holographic Instrument. *Images: N. Loomis, MIT, C. Davis, WHOI*

cessing preserved plankton. Silhouette photography is effective because it produces a sharp image of plankton on high-resolution photographic emulsion. Early attempts to eliminate the need for darkroom techniques by directly scanning preserved zooplankton samples were unsuccessful. Vibrations from the scanner head introduced oscillations in the samples that resulted in blurred images. Advances in scanner technology now make direct scanning feasible, and many commercial scanners are capable of producing clear images of plankton (Figure 4). Dedicated instruments to perform this task have also been invented. ZOOSCAN (Grosjean et al., 2004) is an instrument that uses a scanner sensor with a custom-built lighting system and a watertight scanning chamber into which zooplankton samples can be poured, digitized at high resolution, and recovered without damage. The contents of these images then can be identified, enumerated, and measured by a human working with specialized software.

Silhouettes or scans of plankton samples offer the advantage of a permanent record of the samples' contents and provide improved means of measuring nonlinear objects using multisegment paths as well as options for randomly subsampling the images. The images in silhouettes generally suffer from reduced resolution relative to the original planktonic organisms, which may limit the level of taxonomic detail that can be obtained. Moreover, a single scan may contain many hundreds of individuals. Whether samples are examined under a microscope or on a computer screen, the time-consuming process of examining, identifying, and measuring large numbers of objects remains a challenge.

The end product of any plankton survey, whether conducted with nets, pumps, or cameras, is taxonomically explicit estimates of the distribution, abundance, and perhaps sizes or biomasses of the organisms of interest. Imaging systems excel at producing dis-

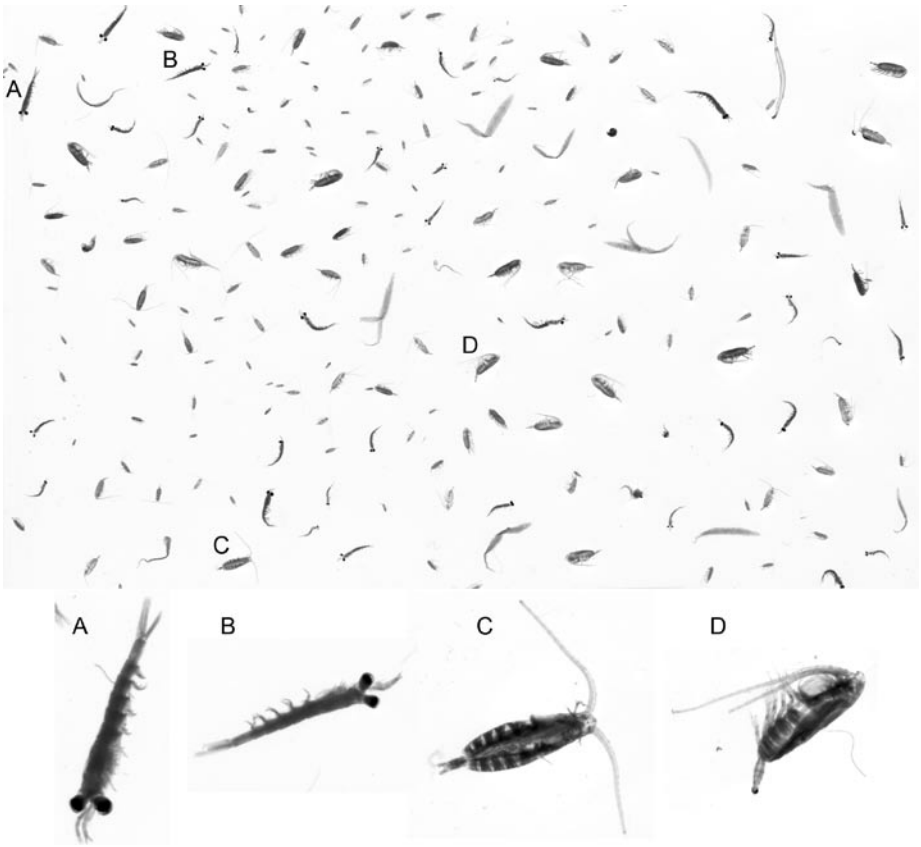

Figure 4. Advances in scanner technology make it possible to obtain high-resolution images of preserved plankton. These preserved zooplankters were scanned in a clear plastic tray at 2400 dpi with 16-bit gray-scale resolution. Four individual organisms (A–D) are presented at the bottom of the figure in larger scale to illustrate details present in the scan.

tributional data on fine horizontal and vertical scales (e.g., Davis et al., 1992b, 2004; Davis and McGillicuddy, 2006) (Figure 5). Image analysis of plankton samples can produce insights into distributions (e.g., Figure 5) in considerably less time than traditional plankton sample processing. The potential benefits from surveying plankton with in situ imaging systems, or analyzing net or pump samples via image-processing techniques, have led to a considerable body of research on the development of effective means of extracting useful information from the vast numbers of images both sampling approaches produce.

IMAGE CLASSIFICATION

The first attempts at taming the onslaught of images produced by in situ, digital imaging systems concentrated on automatically identifying images that contained valid, in-focus objects, and isolating these targets. Present limitations in size and resolution of imaging sensors (CCD and CMOS chips) mean that plankton imaging systems necessarily have much smaller sample volumes than plankton nets. Nonetheless, because plankton nets typically oversample the number of organisms required for abundance estimation (Cassie, 1968), imaging systems can provide an equivalent or better estimate of plankton abundance (see discussion of sampling volume in Davis et al., 2005). Some imaging systems such as the VPR have small image volumes of a few to tens of milliliters per image. Consequently, most images do not contain plankton large enough to be identified. Dedicated image processors scan each image for objects that are large enough for identification. These regions of interest (ROIs) then are isolated using binarization and segmentation routines, cropped, and written to disk as raster image files. This approach saves the user from visually inspecting thousands of images. The copious quantities of resulting ROIs then need to be classified, enumerated, and measured.

Although recognition and classification of zooplankton is a labor-intensive task for humans, we are extremely adept at visual identification, often integrating a large number of subtle features to arrive at an identification. As with manual sorting of plankton samples, however, we are easily fatigued and prone to bias, both of which can introduce errors (Culverhouse et al., 2003). The rate at which we can make correct identifications is eclipsed by the sheer number of images produced by scans of samples or in situ imaging collections. Whether images are produced from preserved plankton samples via silhouette photography or direct scanning, from a benchtop FlowCAM (Sieracki et al., 1998) or imaging flow cytometer, or from a towed camera system, obtaining useful, taxonomically explicit data from these images clearly requires an automated approach. Building upon advances made in machine vision, pattern recognition, and data mining, a number of research-

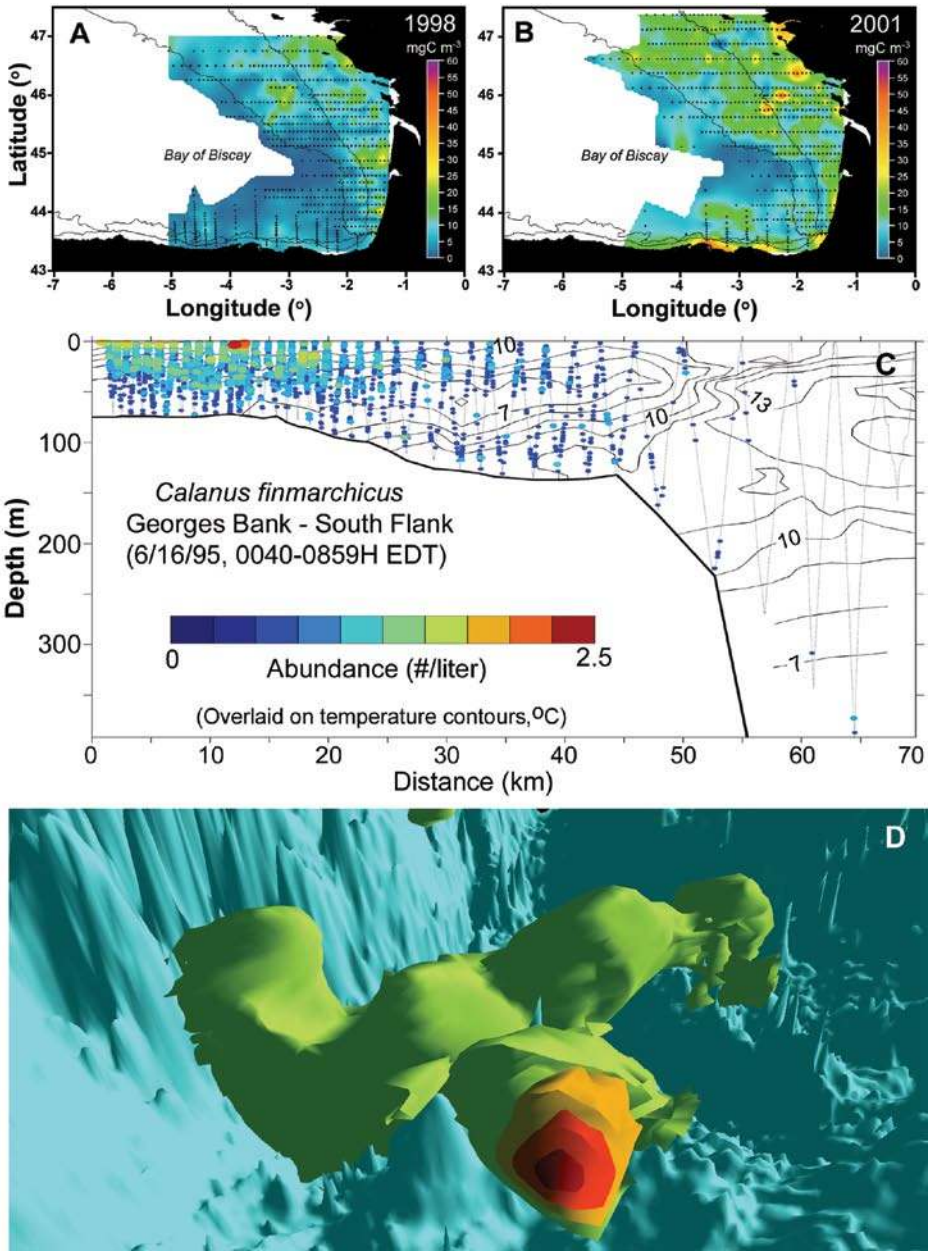

Figure 5. Plankton-imaging systems can collect distribution and abundance data from fine- to meso-scales. Examples of such data include the following: A-B. Distributions of mesozooplankton biomass (0.2–2 mm) equivalent spherical diameter collected from vertically integrated tows with a 150-µm parovet net in the Bay of Biscay during 1998 and 2001. Rapid processing of these preserved samples was accomplished using a flatbed scanner and software called Visual Plankton Analyzer, a precursor to ZooImage. *Images: X. Irigoien, AZTI* C.: Distribution of the copepod *Calanus finmarchicus* over a portion of Georges Bank determined in near-real time using the Video Plankton Recorder (VPR). The black lines indicate the trajectory of the VPR. *Image: C. Davis, WHOI* D: Three-dimensional distribution of *C. finmarchicus* during diapause in Wilkinson Basin, Gulf of Maine, as determined using a VPR. Isosurfaces correspond to abundances from 400–900 individuals m^{-3}. *Data: M. Benfield LSU, Image: W. Little, WHOI*

ers have developed specialized software that can classify plankton images. By turning the most laborious processing step over to computers, we can reduce the amount of time required to gain useful knowledge about plankton, thereby increasing our understanding of planktonic systems.

Plankton image classification is a highly challenging machine vision problem (Figure 6). Unlike the task of recognizing a defective circuit board on an assembly line containing thousands of similar circuit boards, or the more complex problem of matching a human face from a database, classification of plankton must contend with a series of challenges that can vary depending upon location, time, and the nature of the survey. Plankton constitute a morphologically heterogeneous group inhabiting a medium that also contains a variety of nonliving targets such as marine snow, sediment particles, and bubbles. Plankton vary in size by orders of magnitude, and some taxa undergo drastic changes in morphology during ontogenetic development. Definitive taxonomic features may not be visible in all images due to limits on the resolution or orientation of the organism. Some plankton, such as siphonophores and other gelatinous taxa, are large relative to the image volume. Their large size can result in images containing only a small portion of the total organism, with possible recognition problems. Planktonic objects imaged in situ are variously oriented in three dimensions relative to the imaging sensor. Even preserved or freshly collected specimens that are scanned may appear quite different depending on whether their dorsal or lateral aspect is presented. Thus, images of

the same individual may present dramatically different features for recognition depending upon its orientation relative to the camera. Several different organisms may be present in a single image, and symbiotic relationships can result in single images containing more than one species collocated in space.

Even though computerized identification of plankton is a very difficult problem for automated systems, a number of groups working in Europe, North America, and elsewhere have made substantial progress toward constructing useful plankton classifiers (Blaschko

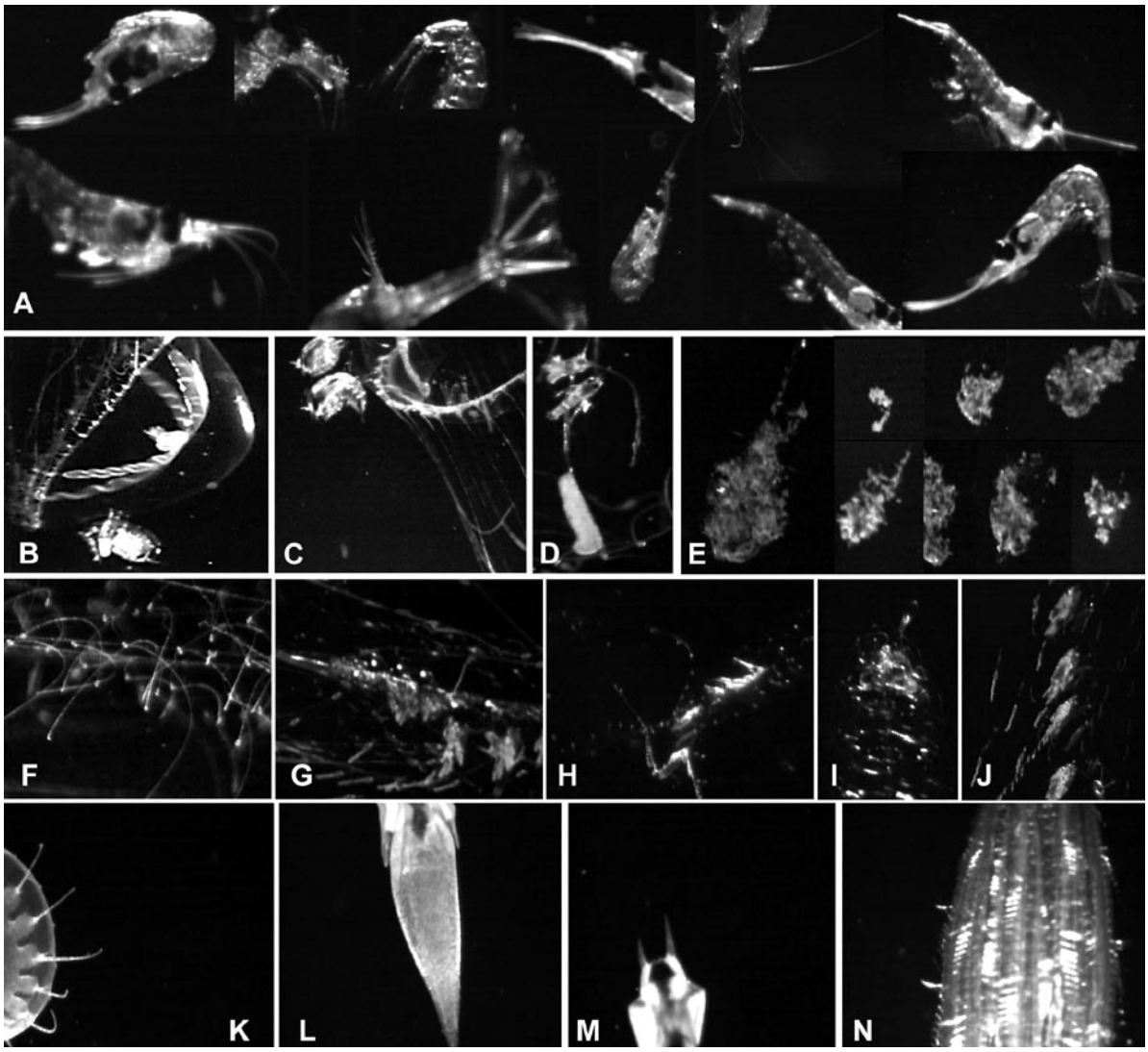

Figure 6. Some of the challenges facing those attempting to develop automated plankton classification systems are illustrated in these example ROIs from the Video Plankton Recorder. A. This collage of images of the euphausiid *Meganyctiphanes norvegica* shows how variable the same organism can appear to a camera because of rotational freedom in three dimensions and postural variability. B–D. Two different species can co-occur in a single image. These hyperiid amphipods are commonly associated with gelatinous organisms. E. Some groups may exhibit a great deal of morphological plasticity. This collage of marine snow particles shows how size and shape can vary among particles. F–J. Some large organisms have highly heterogeneous morphologies. Physonnect siphonophores appear very different depending upon which part of the colony is imaged. K–N. Partially imaged organisms can complicate their identification because features extracted from one part of an organism may be quite different from those extracted from another (K: medusa, L-M: *Clione limacina*, N: ctenophore). *Images: Mark Benfield, LSU*

et al., 2005; Culverhouse et al., 1996, 2003; Davis et al., 2004; Grosjean et al., 2004; Hu and Davis, 2005, 2006; Lisin et al., 2005; Luo et al., 2003, 2005; Tang et al., 1998; Sosik and Olson, in press). A recent GLOBEC workshop, "Image Analysis to Count and Identify Zooplankton," held in San Sebastian, Spain, during 2005 (Irigoien et al., 2006), brought imaging system users and developers together with classification software programmers to discuss the state of the art in this field. The results suggested that automated systems using texture, shape, and other image features are currently capable of correctly classifying plankton images with an accuracy of 70–80% for 10–20 taxonomic class problems using support vector machines, decision trees, and other supervised classifiers. When additional expert knowledge can be incorporated into classification algorithms, even higher accuracies may be possible.

Most of our progress in the field of plankton imaging has been the result of individuals or small groups working toward development of unique instruments suited to image specific groups of plankton or designed to address the research questions of the developers. The recognition that imaging hardware is of little use without image processing and classification software led to development of many different customized image-classification solutions. For example, the VPR employs a software package called Visual Plankton written for Matlab (Davis et al., 2005), the SIPPER has software called PICES (Luo et al., 2005), ZOOSCAN uses ZooProcess software in conjunction with Plankton Identifier (ZooProcess and Plankton Identifier manuals can be obtained at www.zooscan.com), and the HAB Buoy uses a neural network package called DICANN (Toth and Culverhouse, 1999). Classification software is highly specialized and difficult to develop. It occupies a completely different realm of science from the oceanographic engineering arena where imaging systems are designed and built. The effectiveness of existing imaging systems would be dramatically enhanced with access to readily adaptable classification software, and it is highly likely that many more imaging systems will be developed when flexible software toolboxes become available to the oceanographic community.

The considerable advances that have been made in the field of automated classification of plankton and other related image-classification fields are impressive. Moreover, they suggest that with appropriate support from the scientific community and funding agencies and increased collaboration among interested research groups, we are poised to develop a generic, operational, automated, plankton-identification toolbox. Collaboration is required because the current paradigm of individuals or research groups working in parallel to produce operational classifiers is likely to move the field forward more slowly than a collective initiative to develop a common set of effective classifiers capable of processing a broad range of image data sets. Unnecessary duplication of effort limits the broader utility of individual classification software and slows the rate at which systems evolve. What is needed is consensus on the characteristics and capabilities of a common classification system capable of:

- handling images from a variety of in situ and laboratory imaging systems,
- providing users with a broad selection of classification algorithms, and
- classifying images with a high degree of accuracy comparable to the performance of a human expert.

To achieve the latter requirement, we need to quantify human capacities for taxonomic classification of planktonic organisms represented in images.

SOFTWARE REQUIREMENTS

For any image-processing/classification software to be of broad utility to the research community, it must be capable of performing a series of tasks. There must be a means of importing the images into the system (*importation*, as discussed below). Next, valid targets of interest must be detected and separated from the background of the image (*segmentation*). Valid targets and their associated metadata must then be analyzed for features potentially useful for discriminating one kind of organism from another (*feature selection and extraction*). The software should provide an efficient means of visually sorting images to produce a classifier training set—essentially groups of images containing organisms of the same type whose identities are verified by a taxonomic expert (*training-set production*). The software must be capable of using a variety of different classifier induction algorithms (*classification*) that learn to classify unknown images by constructing decision mechanisms to associate features extracted from the images and metadata with the identification provided by the taxonomists (*training*). Any misclassification errors must be measurable using a con-

fusion matrix supported by appropriate statistical evaluations of correct classification.[3] Finally, the software should develop standards for representation of metadata and be capable of using metadata to produce useful *reports*, such as enumerations of abundances, size distributions, and biomass. Examples of existing software packages designed to accomplish these tasks are Visual Plankton (Davis et al., 2005), ZooProcess with Plankton Identifier (http://www.zooscan.com), and ZooImage (http://www.sciviews.org/zooimage). Each package differs in terms of the types of images it is designed to process, the degree to which image classification is integrated into the package, and its level of development.

Visual Plankton is specifically designed to work with the VPR system and is described in detail elsewhere (Davis et al., 2004, 2005; Hu and Davis, 2005, 2006). It includes a user-friendly Graphical User Interface (GUI) that presents five main steps: (1) calibration, (2) segmentation, (3) classifier training, (4) classification, and (5) data visualization. The program is now routinely used for real-time automated identification and visualization of plankton-abundance patterns at sea. The software is in the public domain, and it is available for download via the Internet (http://www.whoi.edu/instruments/vpr). It is written as Matlab m-files (ASCII files) and therefore requires a Matlab license to run. Visual Plankton is in the most advanced stage of development of the three example packages. Although it is specifically designed for the VPR system, its algorithms for segmentation, feature selection and extraction, and classification are generic and potentially available for incorporation into other image-analysis software packages.

ZooProcess is an open-source and free software package written in ImageJ macro language (http://rsb.info.nih.gov/ijl). It is designed specifically for the ZOOSCAN system and is described on the ZOOSCAN Web site (www.zooscan.com) where it is available for download. It calibrates the system, and acquires and saves images of zooplankton samples (containing about 1000 objects per scanned image) and metadata in a standardized way. ZooProcess allows image processing, analysis, and feature extraction. It isolates ROIs, permits manual classification, and supports the preparation of learning and testing data sets for classifiers. ZooProcess does not contain integrated classification routines. Image classification is accomplished using the Plankton Identifier software developed in Delphi 2005 PE (Borland), which runs the Tanagra (Rakotomalala, 2005), drag-and-drop data mining software in batch mode. The ZooProcess-Plankton Identifier package is freely available for download via the Internet and can be easily adapted to other image types and may also be combined with other classification software such as ZooImage.

ZooImage was developed as an integrated system that could handle importation, segmentation, feature extraction, training-set production, classification, and reporting (Figure 7). It has an intuitive GUI front end that makes calls to inexpensive or open-source software to perform specialized tasks. ZooImage was designed to work with a variety of image data sets, such as scanned images of preserved plankton and micro- and macrophotographic images, and has been modified to process FlowCam images. It is anticipated that with the addition of custom acquisition modules, it will be capable of processing a wide range of image sources. It is capable of running on Windows with future cross-platform capability anticipated. Cross-platform capability combined with open-source code is an important feature in establishing a broad user constituency. ZooImage currently includes Java-based ImageJ image-processing routines that perform segmentation, and image classification modules written for the R statistical package. Importation/exportation, image analysis, and classification routines are fully customizable and expandable through a *plugin* mechanism, so that the software can be tailored to particular needs and applications. ZooImage is operational as an advanced beta release and is continually being enhanced. ZooImage can be viewed as a toolbox that uses images of plankton (or other objects) as inputs and produces taxonomically classified groups of organisms that are measured and converted to estimates of abundance, size, and biomass as outputs. The real strength of the ZooImage model is that it provides

[3] Confusion Matrix: A confusion matrix is a contingency table that compares identification done by two independent classifiers (usually, human versus machine). It is, thus, a square matrix with the number of rows or columns being the number of groups in the classification. Each cell contains the number of classified items, with the diagonal counting correctly classified items in each group (both classifiers are in agreement). The sum of items in the diagonal divided by the total number of classified objects thus equals the overall correct identification rate.

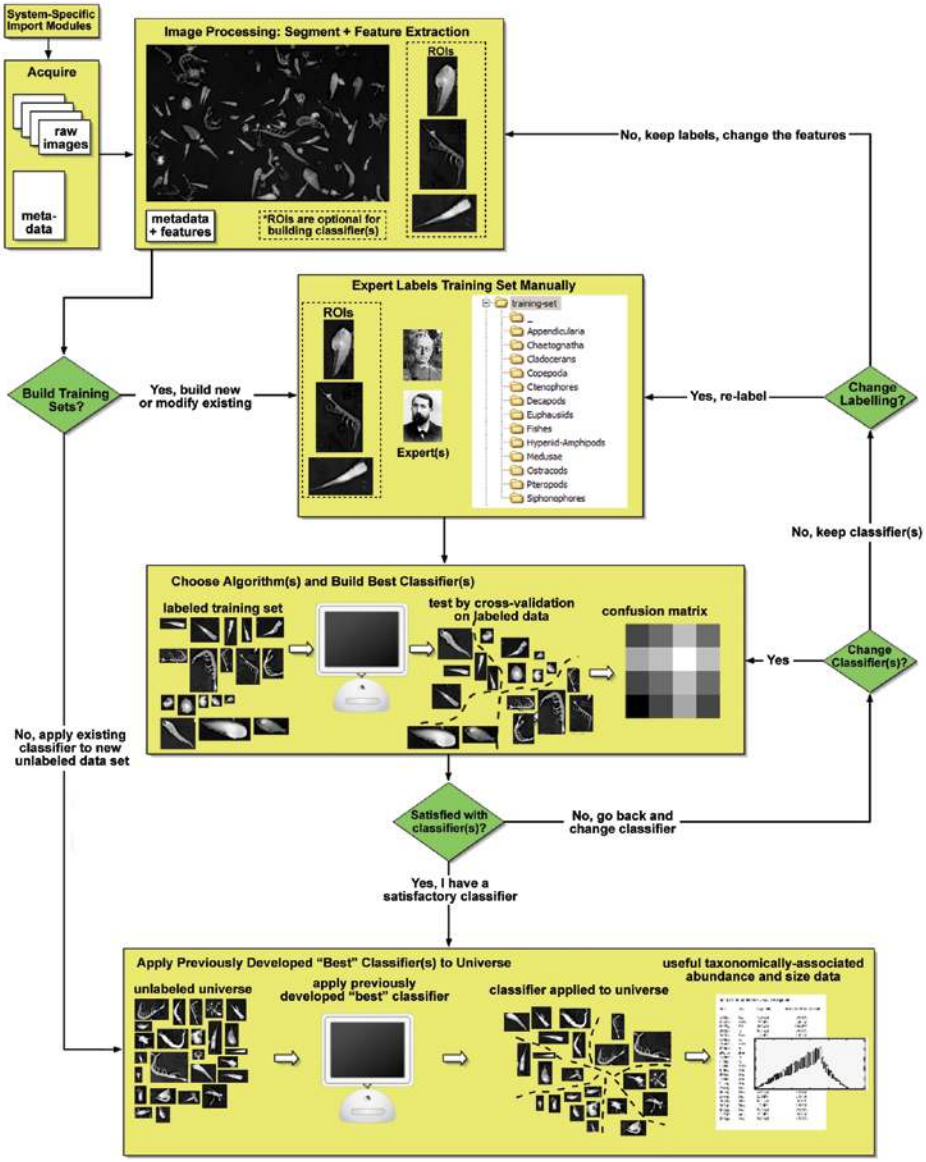

Figure 7. A conceptual diagram of how plankton classification occurs within a ZooImage-type model. Our experts are the famous taxonomists Georg Ossian Sars (top) and Franz Otto Schmeil (bottom). *Image modified from original by Ben Tupper, Bigelow Laboratory for Ocean Sciences*

a useful example of what an ideal plankton image-processing package should be able to do via clear identification of the sequence of events required to obtain useful data from plankton image data sets. This software currently provides the oceanographic community with a sound generic example of how to deal with a deluge of images.

Each of the example software packages is designed to handle images from different sources: the VPR (Visual Plankton), the ZOOSCAN (ZooProcess-Plankton Identifier), flatbed scanners, micro- and macrophotography, and FlowCam (ZooImage). Building upon their relative strengths, increasing capabilities for importing different types of images, segmentation, feature extraction, automatic classification, correction of systematic errors, and development of a common data standard are all improvements that need to be undertaken. These are precisely the types of issues that can be resolved through community-based initiatives to both enhance the capabilities of these software packages and potentially develop something new that incorporates the best characteristics from each.

RAPID

Access to software environments such as Visual Plankton, ZooProcess-Plankton Identifier, and ZooImage affords a means of accessing information contained in plankton image data sets that will lead to exciting new advances in zooplankton ecology. Research on Automated Plankton Identification (RAPID) is envisioned as a new initiative arising from the 2005 workshop entitled *Image Analysis to Count and Identify Zooplankton*. The purpose of RAPID will be to advance optical imaging of plankton to the point where useful data can be extracted in a timely manner from virtually any plankton image data set. This initiative will include software development and integration of existing image-analysis packages; hardware development; production of high-quality, taxonomically verified training sets that can be used for evaluating existing or new classifiers; and psychological studies designed to optimize how humans and computers classify images. Based on the highly positive atmosphere that emerged during our 2005 workshop as well as follow-up discussions, the time is right to bring the community of hardware and software developers and users together

to share information and strategies to advance our field.

The central challenge in an endeavor that pairs human and machine is to take maximum advantage of each partner's strengths while minimizing their biases and weaknesses. Although humans possess astonishing aptitude for image recognition, we are still highly flawed image processors. An expert can make mistakes (Culverhouse et al., 2003). Accuracy in classifying images can be influenced by one's mental state, exposure to prior images, bias associated with metadata such as where the images were collected, and taxonomic composition of associated net samples. Different experts and personnel from different laboratories can classify images differently. Machines can integrate subtle features that are recognizable to humans as well as subtle image attributes that may not be obvious to a human, and use these data to rapidly classify unknown images. The accuracy of a classifier is, in large part, only as good as the training data that are used to build the classifier. The saying "garbage in, garbage out" could well have been coined from the challenge of developing appropriate training sets for computer-image classifiers!

As the systems in Figures 1–3 illustrate, there is a great deal of interest in developing new imaging hardware. At present, with the exception of the VPR, ZOOSCAN, and FlowCAM, these instruments are prototypes or one-of-a-kind systems. Prototypes are expensive, and commercialization of any imaging system depends on volume production to reduce per-unit costs and availability of powerful image-processing software so that the instruments can produce useful data in a reasonable time. If RAPID emerges as an effective advocate of the development of efficient, flexible, freely available image-processing and classification software, then availability of useful software will not be an issue for users. A poll of plankton ecologists and taxonomists at the 2005 workshop indicated that many would consider adopting imaging systems as part of their sampling toolbox when the price of such systems dropped and user-friendly software was available to make them more useful. If this informal poll is accurate, development of useful software will likely make imaging systems more attractive, which will stimulate their commercialization. Economies of scale suggest that as demand for imaging systems increases, per-unit prices will decline, further accelerating the widespread adoption of these instruments.

Development of new imaging systems has been reasonably well funded. Most of the systems shown in Figures 2–4 have been funded by agencies such as the National Science Foundation (NSF), the Office of Naval Research (ONR), and the European Framework Programs. Support for software has lagged behind funding for hardware. In part, this may reflect a preponderance of hardware-development proposals. Earlier software development was funded by the National Oceanic and Atmospheric Administration National Marine Fisheries Service (NOAA NMFS) (Jefferies et al., 1980, 1984; Berman et al., 1990) and ONR (Tang et al., 1998; Davis et al., 1996), and later by NSF (Davis et al., 2004, Hu and Davis, 2005, 2006). In 2003, NSF's Information Technology Research (ITR) program funded a collaboration that brought hardware developers, machine vision researchers, software developers, and plankton ecologists together to try to develop software to advance plankton image classification. That team (consisting of Bigelow Laboratory for Ocean Sciences; University of Massachusetts, Amherst and Dartmouth; and Louisiana State University) has joined the developer of ZooImage (Numerical Ecology of Aquatic Systems, Mons-Hainaut University) and others in the community to develop useful tools to process plankton images. Although this project has made considerable advances, it is clear that the diversity of imaging systems

> If RAPID is to be successful as a catalyst in advancing our understanding of planktonic ecosystems, it will require participation and collaboration among researchers from diverse academic fields...

being developed, coupled with the complexity of plankton recognition and the challenges of developing unbiased training sets, will require additional resources and researchers from the global scientific community. RAPID is envisioned as the mechanism that will enable currently funded studies and future proposals to identify data needs that will bring about advances in plankton classification.

We recognize that a key to the success of RAPID will be long-term support of the software suite that is developed for, and accepted by, the community. When the open-source user community becomes large enough, then this support will happen naturally in a shared way. However, it is likely that there will be a critical period of time during which some form of external funding will be necessary to ensure that the software is supported and continues to be refined. For example, within the Linux community, the GNU open-source software has a group of organizers who work very hard to ensure standards, accessibility, and documentation. A project such as RAPID will require varying levels of support for an extended period to ensure that the software remains accessible and adaptable to an expanding plankton image classification community.

If RAPID is to be successful as a catalyst in advancing our understanding of planktonic ecosystems, it will require participation and collaboration among researchers from diverse academic fields such as engineering, computer science, biological oceanography, and psychology. Several steps have already been undertaken to ensure that RAPID becomes a formal initiative. A new Scientific Committee on Oceanic Research (SCOR) working group (WG130) on Automatic Visual Plankton Identification was established at the beginning of 2007. SCOR working groups are established to promote international cooperation in planning and conducting oceanographic research and in solving methodological and conceptual problems that hinder research. This working group will: (1) encourage the international cooperation of computer scientists, engineers, and marine scientists to use and enhance the open-source development platform so that a common tool set of value to the community can be built up over time; (2) evaluate the limits of taxonomic resolution possible from image-based classifiers and develop means of improving the taxonomic resolution that can be achieved from plankton images; (3) review existing practices and establish standards in the use of reference image data used for training machines and people; and (4) establish a methodology for intercomparison and intercalibration of different visual analysis systems. We envision the SCOR working group as a powerful mechanism for stimulating international and interdisciplinary collaboration within RAPID. A special session on "advances in imaging technologies and the application of image analysis to count and identify plankton" was included as part of the program for the 4th International Zooplankton Production Meeting in Hiroshima, Japan, May 28–June 1, 2007. This session was designed to encourage dialogue and information exchange among investigators working on RAPID-related studies.

RAPID will be committed to the development of effective software tools for plankton classification. It is still too early to say what form that software will take, although we have a good idea of what it needs to be able to do. ZooImage, ZooProcess-Plankton Identifier, and Visual Plankton each offer certain advantages, and there are undoubtedly other software tools in the community with desirable attributes. Ultimately, the success of RAPID will depend on establishing a spirit and practice of cooperation, communication, and collaboration among the diverse groups who are advancing the field of plankton imaging and recognition.

The study of plankton requires a triad of sampling and sensing tools. Nets, pumps, and other collecting devices remain a core constituent of field studies because they provide a physical sample of the organisms. The physical samples they provide enable detailed taxonomy and permit genetic sequencing leading to unambiguous identification. Imaging systems are capable of documenting the fine-scale distributions of plankton while frequently overcoming the limitations of nets, such as damage to fragile organisms and avoidance bias. Acoustic systems provide a quasi-synoptic, albeit taxonomically ambiguous, picture of the pattern of plankton distributions in the water column. It would not be the intention of RAPID to try to supplant nets, pumps, or acoustics with cameras. Rather, we foresee RAPID as a means of enhancing the capabilities of imaging systems to provide a more timely and accurate picture of the distributions and abundances of plankton. Moreover, the adoption of laboratory imaging systems, such as scanners to analyze net or pump samples, will only occur when image-

processing tools, of the type that the RAPID community will be committed to developing, become available.

Imaging systems have been criticized for their lack of taxonomic resolution. It is true that the level of detail present in images from underwater cameras is generally too limited to permit identification to the species level unless organisms possess distinctive morphological features. What has not been fully explored is how much taxonomically useful information may be contained in feature sets that can be extracted by image-processing systems. It is possible that features such as granularity, texture, color, grayscale distributions, and other parameters intrinsic to images may enhance our ability to identify the constituent organisms. Such features may become even more useful as new higher-resolution, multi-spectral imaging systems become available.

SUMMARY

At no time has there been a clearer need to obtain information on the taxonomic composition and size distribution of plankton. Phytoplankton mediate carbon flux from the atmosphere to the oceans, transfer energy into marine food webs, and contain taxa responsible for harmful algal blooms. Zooplankton, in turn, function as consumers, producers, and prey in food webs, and influence biogeochemical cycling in aquatic systems (Marine Zooplankton Colloquium 2, 2001). Furthermore, the zooplankton provide essential prey for most fish larvae and many adult fishes. They serve as sentinel organisms that provide information on changes in their physical and chemical environments through rapid changes in their abundance, taxonomic composition, and size distribution. As our ocean responds to global change, zooplankton populations provide a record of how such changes affect marine communities (see a recent review by Hays et al., 2005). The importance of zooplankton (and phytoplankton) is well recognized, and regular collections using nets and other samplers are components of almost all long-term monitoring programs.

Advances in zooplankton-collecting technology in the form of nets was generally due to the development of critical enabling technologies rather than to improved capabilities of the nets themselves (Wiebe and Benfield, 2003, Table I). Examples of such enabling technologies included developments of wire rope and powered winches, the ability to transmit electrical current through cables to underwater instruments, transistorized electronics, and others. Plankton imaging systems are at a similar juncture. The technology to produce high-resolution imaging systems is well developed and will advance at a rate limited by imaging sensor technology. What is holding back the widespread adoption of plankton imagers is the lack of low-cost or free image-processing software. Such software could be a refined version of ZooImage, ZooProcess-Plankton Identifier, or Visual Plankton; an amalgam of the best features of these packages; or something entirely new with enhanced capabilities. Whatever suite of software the community ultimately embraces, once it is widely adopted, we predict that the number of operational planktonic imaging systems will proliferate and the cost of their acquisition will drop. This will open a true window into the ocean, and through that window will come unparalleled insights into the ecology of marine plankton.

ACKNOWLEDGEMENTS

The authors wish to acknowledge the support of GLOBEC, SPACC, the Department of Agriculture and Fisheries of the Basque Country Government and Ministry of Education and Science of Spain for funding the 2005 Workshop on Image Analysis to Count and Identify Zooplankton, which stimulated interest in the RAPID initiative. This material is based upon work supported by the National Science Foundation under Grants OCE-0325018, OCE-0324937, OCE-0325167 and OCE-9423471, and the European Union under grants Q5CR-2002-71699, MAS3-ct98-0188, and MAS2-ct92-0015. This paper benefited from the constructive criticism of an anonymous reviewer and the editor. This paper is dedicated to the memory of our co-author Professor Edward M. Riseman (1943–2007).

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