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**Project Number:** 901303

**Project Start: Jan. 1, 2014** **Project Duration (in years): 1**

**Please indicate what type of project is being proposed (mark only one).**

| [X] | [ ] | [ ] | [ ] | [ ] |
|---|---|---|---|---|
| **Feasibility/Scoping** [Less than 100 days effort] | **Development[1]** | **Research** | **Infrastructure[2]** | **Education** |

| [ ] | [ ] |
|---|---|
| **Time Series** | **Institute Initiative** |

Project Manager[2]: Thomas C O'Reilly

Principal Investigator:

Lead Scientist: Francisco Chavez

Lead Engineer: Denis Klimov

# Project Title: Cytometer technology for autonomous platforms

[1] Development and infrastructure projects must complete the risk matrix (see item #5 at the end of the proposal form).
[2] Not all projects will require all four titles to be filled in. Please indicate individuals only for those titles that are appropriate for your project.

## 1) ABSTRACT (new projects only)

[Paste abstract here]

## 2) PROPOSAL

# a) New Projects

**Statement of the problem, why it is important**

[Click here to enter text]

**How does it relate/contribute to MBARI's mission and strategic plan?**

[Click here to enter text]

**How does it relate/contribute to the Packard Foundation's Ocean Visions Grantmaking policies?**
(See http://mww.mbari.org/oed/OED_Documents3.htm#Packard for documents.)

[Click here to enter text]

**Approach to the problem**

[Click here to enter text]

**Publications (from other projects over the last 5 years)**

[Click here to enter text]

# b) Continuing Projects

If the proposed project includes multiple individual projects, please list each of these projects as a subheading in the proposal. For each subheading, please address the following:

**Current status of project and results to date**

All of our work is documented on the MBARI Confluence wiki at https://oceana.mbari.org/confluence/display/CytometerTech/Home

We have reviewed and compared characteristics and capabilities of existing oceanographic cytometers that have been or could potentially be integrated with Dorado or LRAUV [table], based on size, complexity and power requirements. Our investigation has clarified that flow cytometers are most appropriate to small (< 10 micron) spherical particles, while imaging cytometers are best suited to larger, non-spherical particles with more complex morphologies. Some cytometers such as Imaging

Flow CytoBot, FlowCam and CytoSub include both pulse and imaging capabilities.

We have reviewed some techniques beyond flow and imaging cytometry that are at various maturity stages, including Coulter counters, holographic imagers, bulk optical techniques, mass spectrometric cytometry and microfluidic cytometry. We are arranging these in a matrix to evaluate applicability and potential adaptability to AUVs.

In addition to reviewing the 2010 CANON Sampling workshop report, we conducted interviews with Worden, Chavez, and Ryan to better understand MBARI science requirements. We held discussions with Jared Swalwell for updates on SeaFlow and SeaLabel development plans. Bellingham and Chavez initiated discussion with Fluid Imaging, and may procure a FlowCam instrument this year for evaluation during CANON. Chavez plans to request a CytoBuoy instrument owned by Dick Dugdale's lab at San Francisco State University, for use during CANON. We hope to invite a guest seminar speaker this year - possibly Heidi Sosik or Jared Swalwell. We held a telecon with Holomic LLC, who are designing low-cost cytometric blood counters for medical use; we learned that this technology is still in an early development stage, and it is not clear if it can be adapted to oceanographic use.

We have learned that cytometers are somewhat limited in the size range of particles that can be detected and measured, especially flow cytometers, and we have not identified a specific cytometer or technology that can measure particles across the entire range of interest to CANON scientists (0.5 microns to millimeters in size). Given the fluidics design of many of these instruments, it's now clear that cytometer integration times of many minutes or even hours may be needed to acquire statistically significant cell counts, depending on number density of the target organism. These long integration times are problematic on a moving AUV that may move into completely different populations of organisms within a time span of seconds. Of these potentially AUV-compatible instruments, we've determined that only SeaFlow has the required sensitivity (~0.5 micron) to address research interests of the Worden lab; however Jarred Swalwell indicated that miniaturized version of a SeaFlow would most likely be even more limited in a particle size range (~ 2-20 um) due to reduced size and laser power.

We have learned that imaging cytometers are able to provide wider size detection range and volumetric pumping rates than flow cytometers, thus reducing time required to acquire data with acceptable statistical errors. However, we also learned that processing of the image data typically requires building a custom organism identification or image database, which is labor-intensive time and hard to automate.

Moreover as this and other instrumentation projects have progressed in 2013, we now increasingly understand that the suite of in situ instruments currently in use at MBARI, even on the very well-equipped Dorado vehicle, do not yet provide an integrated characterization of particles and organisms across the size scales of interest. In many cases users do not fully understand the capabilities and limitations of these instruments, and in some cases the instruments are not sufficiently calibrated. Thus Bellingham is spinning off a project in his lab that will characterize the capabilities and characteristics of those instruments, utilizing standard samples that may include known monocultures, "wild" samples and calibration beads. Our Cytometer Feasibility project will have access to those standard samples for cross-testing against fluorescent microscope / particle counter test-bed we are proposing to construct and evaluate in 2014, and we also propose to explore approaches to analysis of the images produced by that instrument. Thus we expect considerable synergy between these two projects.

This year (2013) we will continue to evaluate existing instruments / technologies, assist in procuring and operating "loaner" cytometers for CANON if necessary, interview potential users, and provide an annual project report. Our original schedule called for construction of a lab test-bed this year, but that effort has been deferred by the findings described above, as well as schedule slip. We now have a more definite concept of the hardware test-bed, and propose to build that during 2014 (see below).

**Significant changes from previous project proposal**

**Publications based on the results of this project only**

[Click here to enter text]

# c) New and Continuing Projects: Plans for 2014

**Research Activities**

None

**Engineering/technology development**

During 2013 project activities, we realized limitations of flow cytometry; limited particle size range, and limited volumetric pumping rates. We also realized that imaging cytometry is able to partially overcome those problems (which may explain relative popularity of imaging cytometers for marine applications), at the expense of added complexity of image analysis and target identification. We also realized the volumetric rate advantage of bulk optical sensors such as ECOPUCK and LISST-100 for determining particle size distributions, but at the cost of quite demanding data processing requiring significant CPU power, and the need to assume certain particle properties such as shape. These issues of bulk methods will be further investigated in Bellingham's project referenced above. In mean time, we believe a method of direct image processing with some modifications might be an effective middle ground.

Therefore, for 2014 we are proposing to evaluate the concept of an Imaging Fluorescent Particle Counter with the following key characteristics:

- Fluorescent imaging to distinguish particles that contain chlorophyll-containing from particles that do not. In this approach, 2 images of 2-D flow cell frame would be captured, one of scattered light, and a fluorescent image.
- Simplified algorithm of image processing, allowing real time image processing without human intervention. Only a few parameters would be calculated automatically, no image database required:
    - With raster image processing, a "containment box" having each organism inside is identified.
    - For each "containment box", two dimensions are extracted: width, and length
    - For each "containment box", optical signal is integrated pixel-by-pixel, thus providing a proxy to the organism biomass.

- For each "containment box", fluorescent signal is integrated pixel-by-pixel, thus providing a proxy to the chlorophyll biomass.

The simple algorithm described could likely yield biomass, chlorophyll and size distribution data in real time on a reasonably low-powered computer consistent with autonomous in situ processing. While we are proposing a test-bed in 2014, future development could potentially result in a relatively simple, small instrument.

Lead scientist Chavez believes that the particle size distribution and biomass information provided by this method could be quite useful scientifically.

We propose the following scope of efforts and team member roles:

- Denis Klimov will build, test and evaluate fluorescence microscopy test-bed / prototype based on principles outlined above, with the following goals:
  - Evaluate physical limitations and trade space of the concept.
  - Investigate optimization of optics in terms of size vs. performance.
  - Evaluate sensitivity in terms of equivalent chlorophyll biomass per particle
  - Evaluate particle size limit.
- Tom O'Reilly will implement processing software for fluorescence microscopy test-bed; this software will likely run on a laptop computer during the proof-of-concept phase in 2014. O'Reilly will also investigate candidate CPUs for in situ processing by a future instrument, in collaboration with the HIPP project within CANON. HIPP engineer Thom Maughan has been researching hardware that could potentially host AVED software, and we believe that both projects could benefit from discussions. O'Reilly will also evaluate the accuracy of FlowCam particle identification software, using standard samples provided by the Bellingham lab.
- Francisco Chavez will evaluate potential applications and usability of proposed methods
- All will continue survey of particle characterization methods

Additionally, Alex Worden will be available in an advisory role for up to 12 hours next year.
As noted earlier, our project is closely related to Bellingham's plans to characterize the current suite of AUV instrumentation, and there is extensive mutual interest in the progress of both projects.

**Field programs (detail ship requirements/deployments/diver requirements)**

None

**Specific deliverables**

- Results of concept evaluation: energy analysis, test results, trade space, ~ size, ~ power, ~ cost.
- Algorithms of operation of biomass calculation/ prototype software; scale processing power required for such instrument
- Final Report

**Milestones (see personnel classification list)**

[Click here to enter text]

| **2014 Project Start Date:** MM/DD/2014 | **Milestone 1** | **Milestone 3** | **Milestone 4** | **Milestone 5** | **Total Resources[3]** for each resource category for all milestones listed here |
|---|---|---|---|---|---|
| **Milestone Date** | **3/1/2014** | **6/1/2014** | **9/1/2014** | **12/15/2014** | |
| **Milestone Description** | **Evaluate FlowCam processing software;** | **Demonstrate fluorescent microscope test-bed** | **Test bed results of evaluation** | **Year-end report** | |
| **EE** | **120** | **120** | **60** | **20** | **320** |
| **SE** | **80** | **40** | **40** | **20** | **180** |
| **ME** | **10** | **10** | | | **20** |
| **EE Tech** | | | | | |
| **SE Tech** | | | | | |
| **ME Tech** | | | | | |
| **Machine Shop** | **20** | **20** | | | **40** |
| **Divers** | | | | | |

## Budget justification

| Item | Description | Account | Cost |
|---|---|---|---|
| 1 | Books on a subject | 5020-000 - Books | $100 |
| 2 | Regional travel for a purpose of visiting collaborators, or collaborators visiting MBARI | 5500-000 - Travel - Domestic | $1,200 |
| 3 | OEM components for proof-of-concept and prototyping | 5320-000 - Supplies - Eng Lab | $4,600 |
| 4 | Miscellaneous supplies | 5360-000 - Supplies - General | $900 |
| | | Total | $6,800 |

## Project team

Francisco Chavez - Lead scientist
Denis Klimov - Lead engineer

[3] This number should be the same number you entered on the resource requests for this particular resource.

Tom O'Reilly - Project manager

**Project lab space**

Lab space in the B232 is well equipped with electrical and opto-electronic test equipment and work space; and should be sufficient for proposed activity. Any LRAUV-specific test can be performed in the LRAUV lab.

**Data availability outside project team, uniqueness**

No data outside project team will be made available.
Proposed concept could potentially enable a compact sensor suitable for autonomous platform integration, allowing measurement of size distributed biomass/ fluorescent particle counter.

**Export Regulation**
Does the project team anticipate any foreign collaborations or international travel? If so, please identify collaborators by name, institution, and country.

No.

Does the project team plan or anticipate export controlled purchases, shipments, or transfers of items (e.g., research instruments, proprietary data, hand-carried tools or samples) outside the U.S. temporarily or permanently? If yes, please identify country or countries and indicate whether these items will remain under MBARI control while abroad or controlled by others. Will the items be exported to more than one country? Please include foreign collaborator information as described above.

No.

**Monterey Bay National Marine Sanctuary SESAs** (General map)
Projects will need to indicate whether or not work will be conducted within one of the Monterey Bay National Marine Sanctuary's Ecologically Significant Areas (SESAs). An interactive map can be found on the Sanctuary's SIMoN webpages at http://sanctuarysimon.org/maps/sesa/. (*Note: It may take a moment for the page to load, so please be patient.*) To determine if your work will occur in a SESA, specific coordinates can be entered using the locator function (click on the red "target" button in the toolbar at the top of the map). If your work will be conducted in one of these areas, please indicate which area, by number.

None.

**Risk matrix (development and infrastructure projects only)**

| | Technical Risk | Organizational Risk | Tech Transfer Risk | Labor Estimate Risk |
|---|---|---|---|---|
| High | | | | |
| Medium | | | | |

Low