Space Details

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Name:	901303 Cytometer Technologies for Autonomous Platforms
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  - Visit by Peter Lopez 9-10-2014

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Home

This page last changed on Sep 11, 2014 by oreilly.

This is the home page for the 901303 Cytometer Technologies for Autonomous Platforms space.

Background

Scientific motivation for *in situ* autonomous cytometry was described at the 2010 CANON sampling workshop (web page, final report, engineering report), attended by scientists and engineers from multiple institutions. Heidi Sosik from WHOI presented an overview of the Imaging Cytobot instrument and its application for HAB detection. Several breakout groups worked to identify compelling science problems and potential technical approaches to address them. Autonomous cytometry was identified as a key technology by the following breakout groups: Harmful algal blooms and phytoplankton, Zooplankton, Thin layers, and Mesoscale eddies. As a result of this workshop, Worden, Bellingham, Swalwell (UW) and O'Reilly began discussing potential integration of Swalwell's SeaFlow cytometer with LRAUV.

MBARI collaborated with UW's Armbrust, Swalwell and others to submit a NASA ASTEP proposal in 2011. We proposed to miniaturize SeaFlow, integrate it with LRAUV, and deploy the system in a Greenland fjord. NASA rated the proposal highly, but was unable to fund it.

In 2012, we submitted an internal MBARI proposal that would miniaturize SeaFlow, integrate it with LRAUV, and test the system in Monterey Bay. We proposed that SeaFlow inventor Swalwell participate in a consulting role, providing guidance on approaches to minimize instrument size and power consumption, improve shock protection, etc. Although rated as "likely to be approved" by MBARI management, we were unable to reach agreement with Swalwell on his technical and scientific role. In response we submitted an internal MBARI feasibility study to look at a broad range of commercial and research cytometers which could potentially be integrated with LRAUV and other MBARI autonomous platforms.

Science applications and requirements

Taxa tracked in MBARI BOG database (D. Kolber)

Potential AUV applications

Potential platforms

Existing instruments

SeaFlow, SeaLabel

CytoSense, CytoBuoy, CytoSub

Imaging Flow Cytobot

FlowCam

Digital Holographic Microscopes

Oceanographic cytometers compared with one another and to other methods

Some non-oceanographic instruments

Technologies

Coulter Counter

Cell phone cytometer

Cell-substrate Impedance Sensing, Electroporation

Optical Detectors

Technologies overview - 1

Technologies overview - 2

Technologies overview - 3 (Specifications and parameters))

Meeting notes

Meeting notes

Ken Johnson and Gene Massion - Coulter Counter, 9/12/2013

Visit by Heidi Sosik, 9/11/2013

Telecon with Fluid Imaging, 6/12/2013

Telecon with Fluid Imaging, 8/20/2013

Discussion with Jared Swalwell on 6/6/2013

Visit by Jarred Swalwell, seminar speaker on 2/5/2014

Visit by Peter Lopez, 9/10/2014

Useful links

Practical Flow Cytometry (H. Shapiro - the standard text.)

Howard Shapiro's web page

Flow Cytometry - A Basic Introduction

Flow cytometry: retrospective, fundamentals, and recent instrumentation (Cytotechnology, 2012); includes discussion of emerging cytometry technologies

Imaging versus flow cytometers (Webinar)

http://plankt.oxfordjournals.org/content/30/3/333.fullThe emergence of automated high-frequency flow cytometryâ?? (Journal of Plankton Research, 2008)

Cellular Astronomy: a Foreseeable Future in Cytometry (H. Shapiro, *Cytometry*, 2004)

A Hardware-accelerated Approach for Imaging Flow Cytometry (Lee, Meng, et al, 2013 International Conference on Field Programmable Logic and Applications)

MIT 3D Optical Systems Group

Potentially relevant workshops, conferences, trade shows

Cytometry Development Workshop, Asilomar - Next workshop is October 23-27, 2013
This is apparently a technology-oriented workshop for experts in the field - perhaps Alex Worden is the only one who would qualify.

American Society for cell Biology , conference and expo, mid December annually.

www.ascb.org

Cell phone cytometer

This page last changed on Jun 24, 2015 by oreilly.

Developed by Zhu, et al [2011]: *Optofluorescent imaging cytometry on a cell phone*, *Anal. Chem.*, 2011, 83 (17), pp 6641-6647 (technology being marketed by Holomic LLC)

Zhu et al 2013, Cost-effective and rapid blood analysis on a cell-phone, http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3594636/

Imaging flow cytometer

Low-cost materials

Phone provides camera, potential image processing capability

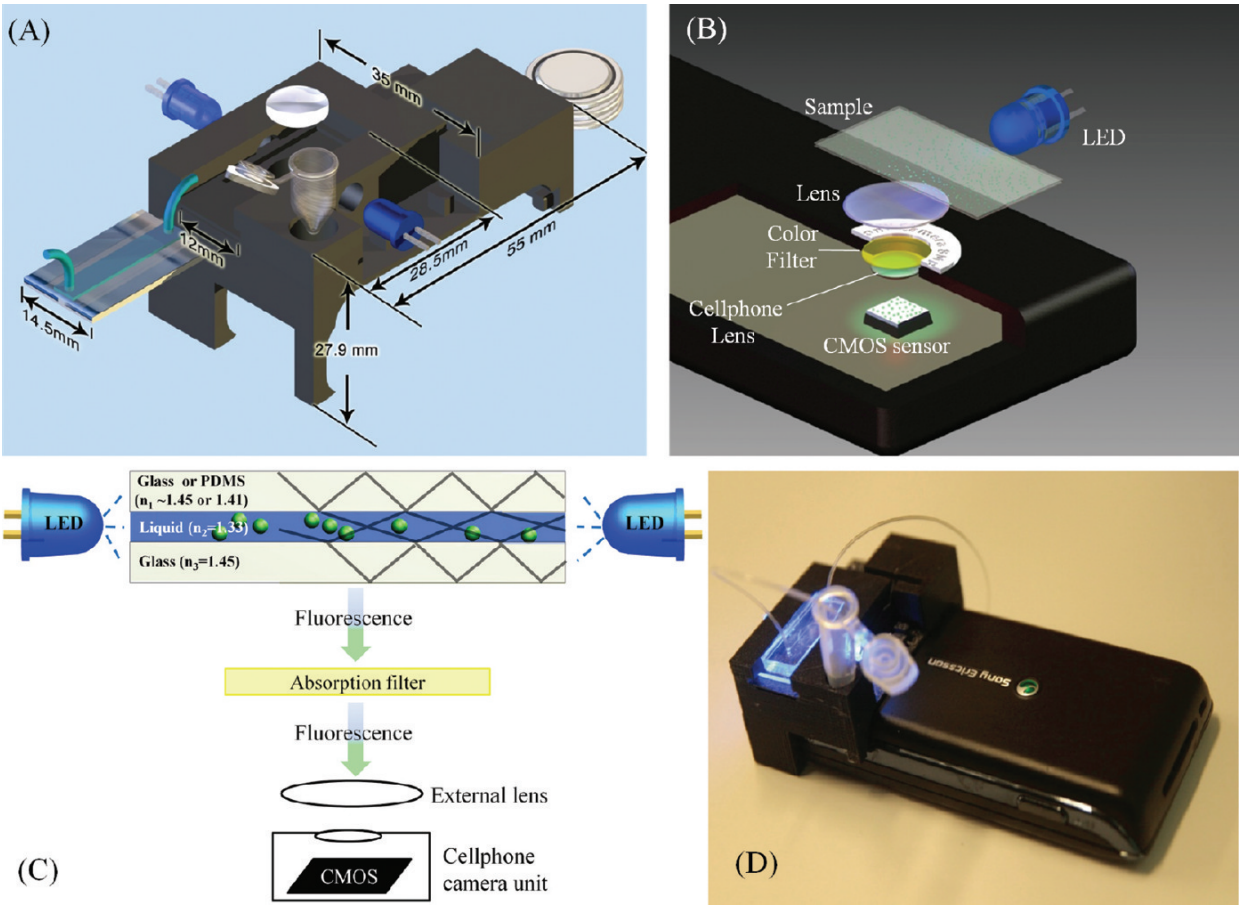
Spatial resolution: ~2 micron

Paper contains fairly detailed description of materials, design

Select filters and LED for target cell type

Image processing performed on laptop computer when this paper was published, algorithms implemented in OpenCV. Note that Android has OpenCV implementation.

Throughput largely limited by camera frame rate. Frame rate of 120 fps could result in whole-blood imaging time of 20 seconds.



Cytometer movie

Also see CellScope work, from Fletcher lab at UC Berkeley: http://cellscope.berkeley.edu/

Cell-Substrate Impedance Sensing Electroporation

This page last changed on Feb 26, 2013 by klimov.

Method of measuring cell properties and behavior (morphology changes, barrier function, cell motility, cell surface coverage, membrane capacitance, average spaces beneath the cells) by applying current and measuring voltage across small empty electrode, the impedance is calculated and then broken down to series resistance and capacitance

http://www.biophysics.com/

This page last changed on Sep 09, 2014 by oreilly.

Comparison between existing oceanographic cytometers

The below characteristic values are found in various sources, including peer-reviewed publications and manufacturers marketing literature (e.g. specification sheets)

	Imaging Flow CytoBot (Heidi Sosik, McLane Research)	**SeaLabel (Jarred Swalwell, UW)**	**CytoSub (CytoBuoy BV)**	**FlowCam (Fluid Imaging)**	**LISST 100X**	**LISST Holo (Sequoia Scientific)**	**LOPC**	**Submersible holographic microscope (Resolution Optics)**
type	imaging, pulse	pulse	imaging (optional), pulse	imaging (includes chl, phyco fluor channels)	nephelometer	holographic imaging	pulse	holographic imaging
cell size range	1-100 micron	0.5-20 micron (SeaFLow) 2-20 micron (SeaLabel)	0.4-700 micron ("pico" option)	10 - 600 micron		25 micron-2.5 mm	100 micron - 3.5 mm	1 micron-2 mm (depends on particle opacity, optical setup)
cell concentration range			10^3-10^9 cells/liter				< 10^3 particles/ liter	
instrument dimensions	26 x 102 cm	17 x 23 x 28 cm	37 x 75 cm	46 x 38 x 66 cm		76.7 x 13.3 cm		30 x 10 cm
instrument mass (air/ water)	32 kg		65 kg	18 kg (without housing?)		9.5/3.6 kg	11.4/6.0 kg	1.81/1.39 kg
power	35 W	35 W	sampling: 60 W (full options) idle: 20 W sleep: 2 W	40-60 W continuous		4.5 W sampling?	< 20 W	~5 W (not incl. computer)

max depth	40 meter	shipboard	200 meter	200 meter		300 meter	660, 3400, 6000 meter	100, 6000 meter
Fluor/ scatter excitation	635 nm laser	457 nm laser	various lasers	532 nm laser	670 nm laser	670 nm laser		N.A.
flow rate thru measurement volume	15 ml/hr	900 ml/ hr f(vcore) ~ 90 ml/ hr f(OPP) ~ .9 ml/ hr	0.25-64.8 ml/hr	With 4X Objective and 300µm Flow Cell: Up to 180 ml/ hour (50 mm^3/ s) WIth 10X Objective and 80µm Flow Cell: Up to 45 ml/hour (12.5 mm^3/ s)				
Detectors	2 PMTs: CHL (680), SSC (635)	5 PMTs: 2 position sensitive detect CHL1 CHL2 Phyco-erythrin	FSC Photodiode SSC PMT CHL PMT (up to 7 add. channels)					CCD
max throughput	? pulse/ sec 167 images/ min	24,000 pulse/ sec	5000 pulse/ sec 1500 images/ min	10,000 images/ min		2 Hz (analysis post-sample)		16 fps 2048x2048 50 fps 512x512
sheath fluid?	yes	no	yes	no		no	no	no
unattended duration	6 months "routinely"	?	?	?				
realtime data processing		yes				not implemented	yes	requires human operator,

								windows PC
cost								
Notes on measurement method	Olson and Sosik claim scatter/ fluorescence is usually not sufficient for genus/ species-level identification of nano- and micro-plankton.						Size histogram for particles 100-1500 micron; shape info for particles > 1.5 mm	
References	specification sheet Olson et al (2003) Olson and Sosik (2007)	CytoAUV 2013 proposal	Thyssen et al (2008) specification sheet	Web page specification sheet		specification sheet	Herman et al (2008) Web page specifications, integration guide	specification sheet
Event rate, time, energy to measure 1000 cells (100 cells/ ml)*	0.417 pulse/ sec 2400 sec 84 kJoule	2.5 pulse/ sec (detected) 40000 sec (OPP) 1400 kJoule	f=64.8 ml/hr: 1.8 pulse/ sec 555 sec 33 kJoule	5 - 20 particles/ sec 200 - 800 sec 12 - 48 kJoule (60 W)				
Event rate, time, energy to measure 1000 cells (10^5 cells/ ml)*	417 pulse/ sec 2.4 sec 84 Joule	2500 pulse/ sec (detected) 40 sec (OPP) 1400 Joule	f=64.8 ml/hr: 1800 pulse/ sec 0.55 sec 33.3 Joule	5000-20000 pulse/ sec 0.2 - 0.8 sec 12 - 48 Joule				
Notes						Has been integrated with Dorado	Has been integrated with Dorado AUV	Hobson investigating for possible AUV integration

* Time to measure N cells, where p is cell number density (i.e. concentration) and f is flow rate through measurement volume:

$$T = N / (f \rho)$$

If N = 1000, coefficient of variation = 3.1% and SNR = 32.

(See Bellingham's notes on cytometer performance.)

Comparison to other autonomous methods

	ESP	Fluorometry	LISST 100x	Cytometer
Advantages	Species ID	Fast sampling; bulk pigments		Species ID; straightforward data processing of pulse signals (image analysis more complex)
Drawbacks	Limited by onboard reagents; long sampling time	Doesn't discriminate species	Doesn't discriminate cells from other particles; complex data processing; must assume particle transmittance, shape; calibration problematic	Complex optics, hydraulics; precise optical alignment. Indirect cluster-based species ID for non-imaging cytometers
Sample rate	~hourly?	~1 Hz		KHz
Processing		simple	complex	Based on statistical clustering of light scatter/ fluorescence correlation
Species ID?	yes	no	no	yes
Samples per deployment	10's			

Cytometer performance

- Linear range (signal : number of photons)
- Detector efficiency (Qr) - determined by laser power, optical design, PMT sensitivity... photoelectrons detected per equivalent fluorochrome molecule
- Optical background (Br)
- Particle size range
- Signal processing speed (pulses/sec, images/sec)

Sampling protocols

This page last changed on Feb 27, 2014 by oreilly.

This page has moved. Please see https://docs.google.com/document/d/1OBrgAMAs3Zy2SfnxJySLtFnk3qF2xgU6pdAt2H8u4WM/edit

Coulter Counter

This page last changed on Feb 26, 2013 by oreilly.

A Coulter Counter counts and sizes particles suspended in an electrolyte fluid, based on changing electrical properties. The technique does not rely on optical sources or sensors.The particles are pumped through a narrow aperture separating two electrodes, between which a weak electric current flows. The voltage across the aperture is sensitive to changes in impedance, which is proportional to the volume of particles that pass through the gap. Variant techniques that utilize the Coulter principle include Scanning ION Occlusion Sensing and CASY.

The Ken Johnson lab is developing a small Coulter Counter for potential use on profiling floats, buoys, and other small platforms.

WHOI developed an in-situ Coulter Counter in the mid-1960s for use at-sea.

Marie et al have pointed out some drawbacks to the Coulter technique:

- Difficult to quantify particles less than 1-2 microns (i.e. cannot be used for picoplankton)
- Cannot distinguish between cells and other detritus or air bubbles
- Cannot discriminate between similarly-sized microbe species

What MBARI science applications could benefit even with the above limitations?

The Millipore Scepter hand-held Coulter counter for medical applications is available for $3.5K. Note that the channeled sampling tip apparently must be replaced after each sample. Here is a blog describing its use. (pointed out by Ken Johnson)

CytoSense, CytoBuoy, CytoSub

This page last changed on Apr 23, 2013 by oreilly.

Summary from http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3279584/ : CytoBuoy b.v. is a dutch company which manufactures particle analysis instruments and software for mainly aquatic and marine science. Since 2001, the bench top scanning flow cytometer CytoSense is designed for pico- , nano- and micro-plankton studies. It combines classical flow cytometry data with silico-images of the measured particles and targeted video imaging. This instrument is equipped with 2 lasers (460 or 488 or 532 or 561 nm and 445 or 635 or 640 or 660 nm) and up to 10 detectors. The CytoSense works with a hydrodynamic sheath fluid injection system with external and recirculating mode, and an auto-adaptive speed controlled from 0.2 to 20 µL/s. The CytoSense may be extended with a video imaging-in-flow at rates of up to 1,000 scans per second. The CytoSub module "Shallow" allows a submerged use in shallow waters (depth of 20 meter max.) and CytoSub module "Deep" transforms the CytoSense flow cytometer into a CytoSub instrument for submerged operation down to 200 meters. The Buoy module transforms the CytoSense into a CytoBuoy analyzer for moored operation, with 8 solar panels systems with rechargeable batteries and flashlight, telemetry and Argos transponder. CytoUSB software is used for instrument operation and storage of measured data files (8 bits---3.5 decades). CytoClus software is used for data analysis.

Thyssen et al(2008) evaluated CytoSub performance in the Bay of Marseille and the southern Indian Ocean; they compared CytoSub data to data acquired by "conventional" cytometers. They note that limitations in instrument memory and sampled volume can lead to undersampling, but describe a workaround to overcome this. (This problem might not apply to present-day CytoSub instruments).

The prototype CytoSub instrument was integrated with AutoSub by Alex Cunningham's team at the Environmental Optics Lab in Glasgow, in 2002 (presentation, paper1, paper2). The prototype has since been refined to today's commercial CytoSub, with smaller size, better performance, and imaging capability. But CytoSub hasn't been deployed on another AUV since AutoSub (according to CytoSense CEO George Dubelaar).

More technical info from CytoSense Dominic Stuart:

Flow Rates
Our sample pump has a range of 0.07ul/s up to 18ul/s or 0.1ul/s up to 30ul/s (depending on the tubing used in our sample pump). However the big speeds of 18 - 30ul/sec are usualy only used for flushing purposes or searching for very big organisms. The speed of the sample pump affects 2 things.

1. Particles/second
If you increase the sample pump speed, you will push more sample through the machine which means that the amount of particles per second increases. Our current system can process up to 5000 particles/second (small particles and a standard 3 detector system). If the concentration is too high, the sample pump speed needs to be lowered for the system to handle the datastream.

2. The Sample core
The sample pump pushes the sample into the CytoSense. When it reaches the injector, the sheath fluid "pulls" The sample fluid through the cuvette where the laser scans each particle one by one. The sample core is aligned by the sheath fluid. In the center of the sample core, the optics are the most sensitive and this is the "sweet-spot" where you would like the particles you are analyzing.

If you increase the sample pump speed, the sample core gets wider, this means that the position of the particles in the laser beam are not always optimal and you can notice shifts in your data for small particles (this is less important for big particles because a part of them will always be in the center).

3. Conclusion
It really comes down to:
a) What are the size ranges you are interested in? (bigger things, are generally less abundant and sample core width is also less important, and can be analyzed at higher flow speeds)
What we would suggest, if you would like to have a broad analysis of your sample, is to perform say 3 measurements at different speeds and trigger levels. This results in 3 data files where you get the best of both worlds (accurate data for your small particles and enough big particles). Roughly:

Sample speed 0.07 - 4 ul/sec - Picoplankton, bacteria
Sample speed 4 - 12 phytoplankton
Sample speed >12 big stuff (low abundant)

Digitizing and PMT Events
The electronics of the CytoSense digitize the data from the PMT's this works as follows:

1. Event
We specify an event as one single triggered data scan (across all channels) which is normally 1 particle unless the concentration is too high or the trigger level is too low. One event varies between 100 and 100K bytes depending on the configuration of the machine. Note that you get a datapoint every 0.5um on every channel.Examples for a 10 channel machine:
A particle of 2000um = 4000 bytes * 12 = 48000 bytes (46KB).
A particle of 10um = 20 bytes * 12 = 240 bytes.

2. Triggering
The trigger level and the trigger channel (PMT) can be set in software. The electronics will monitor the selected trigger channel and if the PMT output level is higher then the set trigger, the electronics will start to capture and digitize all channels (PMT's). The electronics capture the data at 4MHz, combined with the speed of the particles shooting through the laser (2m/s) this results in a datapoint for all channels every 0.5um.
Once the trigger level drops below the set trigger level, the electronics stop capturing and will consider that event as 1 particle.

3. Limits
The electronics can capture and process 5000 particles/second (small particles and a standard 3 detector system). If you have extra PMT's in your configuration, you generate more data with your machine and this number will be a bit less. However, we are currently working on new and quicker electronics! The electronic limit of 5K events/sec is not the only limitation of the system. Because of how our triggering system works, there needs to be a "gap" between particles. The electronics need to detect the end of 1 particles before it can detect the beginning of the next particle. If you pump to many particles/second into the machine, the system will eventually not be able to process all the particles because they are so packed together that the system will interpret it as one big particle.

4. Conclusion
The maximum amount of partciles (PMT events) the system can process is 5000 events/second.

SUB Power consumption
The amount of power varies depending on the configuration of your instrument (how many pmt's,how many lasers, high power laser, imaging in flow etc etc. To give you a general idea, if you have a full option machine during a measurement the instrument will peak at +/- 60W. When the system is powered but idle, the system will draw +/- 20W. Between measurements the system can entered a preprogrammed deep sleep, during this phase the system will draw +/- 2W.

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Digital holographic microscopy

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Digital holographic microscopy at Wikipedia

The LISST Holo has been integrated with Dorado

Jericho et al describe a submersible digital inline holographic microscope (SDIHM), with ~2 micron spatial resolution and subsecond temporal resolution. Rated to 20 meters depth. A deep version rated to 6000 meters, with ~5 micron resolution is described by Bochdansky et al. Spatial resolution is considerably higher than LISST Holo (~25 microns). Video presentation; describes lightweight 2 kg version for arctic app. A version of this instrument is sold by Resolution Optics.

- Simplicity: microscopy without lenses; laser + pinhole + CCD camera
- Maximum information: single hologram contains all 3D structural information
- Maximum resolution: "optimal" resolution "easily" attainable - down to level of bacteria
- Speed kinetics of motion/reactions can be followed at video rate in 3D

Resolution Optics might lend a Submersible Microscope to MBARI for evaluation.

Submersible Microscope

This page last changed on Feb 03, 2014 by oreilly.

Telecon with Resolution Optics, 1/31/2014

Further information provided by Resolution Optics (John Samson, 2/1/2014)

Submersible Microscope version 1.0.0 user guide (original version of the Submersible Microscope, which MBARI might borrow)

Conventional and holographic microscopy compared

Limitations

Flow Cytometer Performance for AUV Operations

This page last changed on Apr 24, 2013 by oreilly.

See attached notes.

FlowCam

This page last changed on Mar 28, 2013 by oreilly.

Technology presentation from Fluid Imaging

Note that Jim Bellingham has some connection at Fluid Imaging, may be able to borrow an instrument for Fall 2013 CANON provided there is MBARI science interest.

Submersible FlowCam page at FluidImaging.com

FlowCam telecon - August 20, 2013

This page last changed on Sep 09, 2013 by oreilly.

O'Reilly's notes:

Attendees: Jac Fought, Harry Nelson, Francisco Chavez, Denis Klimov, Tom O'Reilly

Action items
1. Chavez - compare Harry's FlowCam result to microscope analysis, sample 14213CO3
2. Nelson - work to get a FlowCam, training to MBARI for CANON (cruises on Sept 16-19, Sept 30, October 6)

Harry has done some analysis of samples provided by Francisco. Used 100x magnification - 25-30 micron image resolution. 100 micron pre-filter. Analyzed with Visual Spread Sheet.
Pseudo-nitzschia predominates.
Some image processing problems, due to "pilot error" by new technician.

Chavez processes a 25 ml sample thusly:
Fix, freeze sample
Operator counts cells under microscope - takes several hours
Operator enters data - takes about 2 hours
FlowCam processed Chavez 25 ml sample as follows:
Acquire images at 25 frames per second - actually images 3.189 ml of the total sample
About 1 hour to process 20 ml sample - automated aspect ratio computation
Can process large size fractions faster, about 1 minute per ml

How could analysis be done autonomously, e.g. on AUV?
Trigger imaging on fluorescence signal (chlorphyll or phycoerythrin), which is useful in oligotrophic water (too much signal in coastal water)
Will get one image frame per trigger
Note that laser excites entire camera fov

FC: We are thinking about a good MBARI FlowCam application
Replace microscope counts
Integrate with LRAUV for in situ analysis
Attach FlowCam to ship water intake, or Pennington's pumped profiler - autonomously id/count Pseudo N
Trigger ESP sample with FlowCam

Realtime image filtering
HN: Difficult to automatically ID all organisms; filtering helps. Alexandrium is difficult; small, spherical. Pseudo N is easier.
HN: Submersible FlowCam is designed for remote operation. Have run on cable for 56 days, ~10 minutes/hour duty cycle during dayl
JF: Submersible FlowCam memory is expandable. Expect to use 150 MByte per hour

Notes added by Klimov:

- 100um Nitex Mesh was used for pre-filtering
- Pseudo-nitzschia chains may stuck on filter and it explains why they were not visible in FlowCam data
- "Segmentation Threshold" (to discriminate between "dark" and "bright" pixels) was apparently set too high, this chops up long cells into separate images
- Fluorescence trigger mode, every frame is unique, 20 FPS
- Stats for 1H run:
  150 Mb file
  93k frames total, 3.6k frames used.
  1.64 parts/ image.
  19.97 FPS
  Intensity Mean: 179.90
  Intensity Min: 161.77
  Intensity Max: 183.05

FlowCam telecon - June 12, 2013

This page last changed on Jun 20, 2013 by oreilly.

Bellingham's notes

Notes from telecon with the FlowCAM folks:

Attendees: Bellingham, Chavez, Klimov, Jac Fought, Harry Nelson (Fluid Imaging)

Francisco:

160-170 codes for organisms quantified in 'standard' water analysis. larger by microscopy, smaller by flow cytometry, some ~um size in both.

Samples 250 ml in size.

Preserve material with glutaraldehyde, filter 25 ml of fluid.
- microscopy done from filter
- some fluid frozen w liquid N2 for cytometer (sent to U of Hawaii)

Catalog organisms to 200 um in size

Lots of small stuff - use staining to see bacteria, etc.

For the microscopy - use different magnifications and look over different areas

Harry:

We would probably use 10x objective for 100x magnification over 100um couvette w lower limit of about 10um critter.

4x objective has lower effective size resolution of 40um.

Can use 20x objective w 50um couvette but 'takes a while'

Harry will send us all some sample images of organisms at the various resolutions.

User builds their own library, does not come with library. Library is used for classification. Have taxonomic photos, but not a library.

How many entries does it need? Depends.

Three step process - build a library, build a filter, build a template from the filters. Is dynamic - can add and subtract from them. Use the filter iteratively (first pass for organism 1, then for organism 2, etc.).

Could build some libraries ahead of time.

Chavez:

Can we also get size distribution of what is in sample?

Harry:

Yes, that just comes from pixels. Easily done. Can also use fluorescence, but if the sample is really concentrated then several organisms might be in field of view.

1/4 ml/min volumetric flow rate for 100 um couvette.

Outcome of the meeting is that Francisco sent them some preserved material. They are running it this week. We are going to get a copy of their software and work on developing a library for the resulting images, and use it to develop a classifier for the remainder of the data....

Additional notes from Klimov:

10x objective, 100x magnification, 10-20um resolved microorganisms, 100um flow cell. This is a most common configuration.

4x objective, 40x magnification, 25-30um resolved microorganisms, 200um flow cell.
25x objective, 250x magnification, 50um flow cell. For highest resolution but also slowest pumping rate.

It was not clear what is the actual optical resolution of the system with say 10x objective. It should be higher then specified resolved microorganism size, perhaps 1-2 um.
Harry said "10 um" is "many pixels", as can be seen in "sample pictures".

Imaging Flow CytoBot

This page last changed on Sep 09, 2013 by oreilly.

Developed by Heidi Sosik and Robert Olson at Woods Hole.

HAB and Imaging Flow Cytobot case studies, Sosik's presentation from the CANON Sampling Workshop (2010)

Sold by McLane Reseach Labs (here is the McLane specification sheet)

Instrument emphasizes imaging - camera/flash-LED is triggered by CHL or SSC PMT pulse height (configurable threshold).

Performance notes

Core consists of seawater flowing at 15 ml/hr = 4.17 x 10^-3 ml/sec. Following PMT pulse, camera/LED may be triggered based on CHL or SSC pulse height and threshold settings. If camera/LED is triggered, subsequent pulses are ignored for at least 80 msec until camera is done (Olson and Sosik 2007). According to Mike Matthewson (McLane): "At 1000 cell/ml [...] IFCB looks at nearly all the sample (i.e., 15 ml/hour [sheath is not sample]); at 5000 cell/ml it looks at ~half of the water passing through the instrument. Reliable concentrations can be obtained up to at least 15,000 cell/ml. Again, the volume of sample actually examined is kept track of and used to calculate cell concentrations."

Compute time 'T' to measure N OPP cells at this flow rate 'f', for given values of number density 'p':

T = N / (f * p)

We assume that cells are not necessarily all the same size, but they are all detectable, and assume that each cell is individually detected (i.e. we don't see multiple cells counted as one).

	N=1000 (sigma=3.1%)	**N=500 (sigma=4.4%)**	**N=100 (sigma=10%)**
p=10^2 cell/ml	2400 s	1200 s	240 s
p=10^3 cell/ml	240 s	120 s	24 s
p=10^4 cell/ml			
p=10^5 cell/ml			

Johnson Lab Coulter Counter

This page last changed on Sep 13, 2013 by oreilly.

Attendees: Johnson, Massion, Klimov, O'Reilly

KJ: Want to keep cost cheap, less than $5K; want low power, suitable for profiling float deployment

No moving parts
Micro-electrode array exposed to external flow
Could have programmable electrode spacing to vary sensitivity
Probably don't need concurrent CTD
Shape of electric field is complex

TO: Would standard calibration beads with known size distribution help?
How about adaptively driving which electrodes are paired as particle moves across surface?

Hope for 5-200 um range - size spectrum
Impedance spectroscopy

Light sheet cytometry

This page last changed on Nov 30, 2014 by oreilly.

3D fluorescence imaging cytometer

2D fast fluorescence imaging cytometer

LISST Holo

This page last changed on Mar 27, 2013 by oreilly.

LISST Holo page at Sequoia

Meeting notes

This page last changed on May 06, 2013 by oreilly.

5/25/2013

Met with Alex Worden to discuss her cytometry applications, approaches

Attendees: Alex, Jim, Denis, Tom

Tom's notes

Jarred is willing to give a talk when he visits Alex' lab in May. Will keep us apprised of the schedule
Jarred has grant to develop staining capability for Seaflow/(Sealabel?) - measure grazing by diatoms, dinoflagellates, reproductive rates

Very important to engage UW collaborators at high intellectual level (as opposed to "contract labor"). UW has very extensive expertise in oligotrophic cytometry - informatics and biological. Too expensive/ daunting to attempt to replicate that...

Big science problems:
Oligotrophic ocean: what picoplankton organisms are out there? How are the populations changing on various spatial and time-scales? Why are they changing?
Only a few cytometers able to resolve picoplankton
Need 488 nm excitation for phyto pigments

Diurnal growth cycles can now be extracted from Seaflow data, based on new modeling approach.
AUV advantages
realtime survey capability to inform ship sampling plan
3D measurement capability
Long-duration measurements during extended missions
Vehicle sensor payload provides contextual data (e.g. nutrient measurements important!)

AW would use Cyto-AUV to write papers that utilizes the above advantages

Instrument features:
Need 488 nm excitation
Must resolve pico- and nanoplankton (0.2 - 20 micron)
imaging probably not useful - can't discriminate between very small (< 3 micron) organisms, e.g. grazers
staining is useful (e.g. for fungi, bacteria, DNA measurement) but not necessary
in-situ calibration w/ beads is critical

Measurement approaches:
Need 10% sigma or better on counting. Note that large counting errors can be amplified when deriving other quantities (e.g. growth rates). Note that medical applications usually require 1% sigma or better (10,000 count)
Always add calibration beads to sample to verify alignment, detection, etc (except for DNA samples)
Usually able to use single configuration for given seawater sample (detectors have 4-decade dynamic range). May need to change configuration for different water mass (e.g. open-ocean vs eddy?)
Not sure how large cells affect Seaflow measurements
BD Influx measurements are typically 10 minutes (25 microliters/minute)
AW lab does not "gate" on fluorescence or scatter - keep all raw data (may not be practical for coastal waters)
Current focus on cells smaller than 5 micron in open ocean, but including larger cells may be important in some environments
Prochlorococcus and synechococcus are readily identifiable - eukaryotes more problematic

Major challenges:
Time consuming, expensive to collect sparse 3D datasets from CTD casts. Seaflow is vast improvement, but surface water only.
Data analysis (see below)

Data analysis challenges:
Cytometer signatures depend on many factors; diverse organism populations, nutrients, depth, etc...

Lots of human judgement, calibration, tweaking required to analyze data - difficult to automate (look at Ribalet et al PNAS paper on bio-diversity index)
UW team has enormous experience and knowledge - biological combined with informatic

Potential alternatives to cytometry - Raman is talked about, no one appears to have used it yet.

Denis' notes

Application:
- What groups of organisms are over there? Cytometer on AUV could provide a yo-yo capability, 3D and so on, this means presence.
- Day-night dynamics.
- Characterize food web is good information is microbiology

Observational challenges:
- Total throughput from sampling to real data, is very low (presently), sometimes takes years if sample are analyzed in the lab. This also puts a limit on a numbers of samples.
- Presence is very low (presently), limited number of ship cruises

Target organisms and mixed populations
- Not really concerned with high dynamic range on sizes (<5um?).
- Discriminate between groups of organisms, using optical methods, Ch A and accessory pigments.

Features:
- Imaging. Value of imaging is limited, organisms below 5um appear as "blobs", can not distinguish. Low Value.
- Real time processing. Normally cytometer is used during cruises by Worden group. On ship, adaptive sampling strategy is used, and decisions on cruise plan are made based on CTD and cytometer data, if it possible at all, and a team have enough bandwidth to do so. High Value.
- Bacterial communities need staining.
- Population identification. Gating is not used, instead, trigger on scattering signal is used, and then accumulate data with other parameters. This is "see everything" approach. This works well in "blue water". Coastal zones with much more silt etc., is a different story.

Value of integration with mobile platform
- adaptive behavior, contextual data; real-time data, reconnaissance, surviving; persistent presence, high-res. All High Value.

Procedure:
- Typically, sample is split in 2, with calibration bids, and without, and both run thru cytometer.
- Beads are needed to calibrate optics for sensitivity and alignment.
- Typical sampling rage is 25uL/mil, so it takes 10 min to analyze 250 uL; ~ 1000 eukaryotes, number of cyano bacteria's ~ 100x higher.
- Statistical errors: <10% is good.
- As a "rule of thumb", about 10k cells needs to be accumulated in flow cytometry, in order to compute growth rate, which is difficult.
- Gain control: manual, interactive. Use to need gain control adjustment before running some samples, or to resolve different species in the same sample. Now, with 4 orders of dynamic range, only occasional adjustment for regions of ocean are being made.

Data analysis.
- Not a static process. Interactive process requiring involvement of skillful individual such as scientist.
- Signatures of groups of organisms are not static, that is why very helpful to use calibration beads.
- There is a lot of knowledge went into data processing in established groups, would be very hard to replicate that.
- Interdisciplinary field between biology and computation => high value.

Trends:
- Imaging cytometry
- Smaller particles
- Certain interest to Raman, but nobody did it yet.

3/27/2013

Sorry these notes are delayed. Denis have I missed anything important?

Attendees: Denis, Tom

Note that Dorota's "Taxa" table is now online.

Reviewed instrument comparison tables on project Confluence page.
Consider LISST Holo to be an imaging cytometer; should be included with other existing cytometers
Condense some table rows into a single row, with more descriptive text in each cell

Good start on science applications/requirements page

DK: We are a month behind schedule specified in our proposal - hoped to have "literature search" complete by March 1.
DK: As we read articles and attach them, it is useful to markup the PDF file with notes

Alex is available next week - review nature of her lab's work beforehand

Action items:
Denis to review new technologies described in "perspectives" section of Cytotechnology article.
Tom to continue instrument comparison tables, filling out science applications/requirements page
All: prepare to interview Worden by reading her lab's publications and posters (e.g. this one)

3/12/2013

Interview with John Ryan.

Ryan, Klimov, Bellingham, O'Reilly

Ryan is interested in particle measurements, going back to MUSE-2000 experiment looking at coupling of phytoplankton with re-suspended sediment. Has never used cytometry. Lots of work with LISST-100x and LISST-Holo.

LISST-100x yields particle size distributions (volume concentration, NOT particle counts) and shape info. Groups sizes into 32 bins between 1-250 microns.

LISST-Holo has been integrated with Dorado. Images particles in 25-2500 micron range. Images are identifiable at least to genus level.

Acquires 1 image per 5 seconds. Image generation (30 sec per image?) is currently very time-consuming, performed post-mission.

JGB notes that LISST Holo image processing could be much more efficient - e.g. see work by George Barbastathis of MIT

In situ Holo processing could enable HAB-seeking AUV

LIST 100x processing is also slow, non-realtime. No one has worked to make processing realtime.

Ryan, Maughan, Cline are working on processing algorithms (post-mission non-realtime for now) through CANON, contingency time

Must make several assumptions for LISST-100x processing; particle transparency, shape... there are also unexplained "blank" anomalies; must use "at sea" blank rather than "in lab" blank - why? (e.g. include dive to off-shelf deep clear water for "blank" sample)

LISST-Holo only costs $25K

JGB: LISST-100x is very difficult to work with, calibrate due to above problems. Cytometer approach is more direct, requires fewer assumptions.

JR: Imaging Flow Cytobot is very cool, not yet integrated with AUV though.

JGB: Check out FlowCam - some early problems (e.g. with biofouling) but making progress. Might be able to borrow one for CANON Fall 2013

3/7/2013 - telecon with Holomic LLC

Pre-call:

Denis - the cell phone cytometer is not prssure-tolerant.
Holomic achieves high optical efficiency; can we achieve that?

Ketaki Sood (marketing) , Nevan Karlovac (CEO), Ozcan (scientist)
O'Reilly, Bellingham, Klimov

JGB: Gave MBARI background. Autonomy. Increasing emphasis on microbiology. Physical sampling. Mentioned summer intern program.

Ozcan: what about broad-field microscopy?
JGB: tricky in situ. Contextual sensors for water samples

JGB: What are max flow rates that prototype can sustain?
Holomic: Low so far, Microliters per minute...

Holomic: Current software gives particle counts, not size
Also wide-field fluorescent microscope

DK: Unattended operation is key. Low power, size

Holomic hasn't focused on low power
Holomic: would require significant funding to build instrument for MBARI
JGB: MBARI engineering is capable of significant contribution
Holomic: formulate your requirments, parameters, etc. Holomic sells products. Cell phone cytometer is not yet a product.
JGB: Oceanographic market is quite small.
How important is continuous sampling?
Holomic: Will keep MBARI in mind as they develop the cytometers

Post call:
Need to develop requirements
- particle densities (concentration)
- organism
- flow rates, integration time
- sensitivity, chlorophyll concentration
- excitation/fluorescent wavelengths

Compare to BD, Seaflow
Need to talk with Alex, Scholin, about the above
Example: want to resolve organisms that generate signal detected by EcoPuck; want equivalent sensitivity
Send them a thank-you email, invite them to visit

For next Tuesday:
Target organisms and sizes
How to detect (fluorescence, polarization..)
Characteristics
Natural concentrations

JGB: John Ryan is a good resource for the above
Meet again in 1.5 weeks - March 19
Ask Dorota what organisms BOGS tracks; she's compiling a table

Ask Ryan and Chavez; what organisms would you look for with flow cytometer?

Denis and Tom to interview

2/26/2013

Action items for next meeting

Contact Zhu about cell phone cytometer; latest developments, availability of device - Tom
Look at Zhu et al paper to determine feasibility of reproducing device - Denis
Consider potential embedded imaging intern project - Tom
Review capabilities of Sosik's Imaging Flow Cytobot - Jim

Note: Post-doc candidate Eric Rehm will visit MBARI on March 18-19

Let's explore possible Becton-Dickinson tour when Alex returns (she has several contacts there).

JGB: Cell phones for robotics are very interesting and promising - high capability, low-cost sensors, computing power
Post-doc Eric Rehm will visit on March 18-19

DK: Note that theoretical imaging limit is about 1 micron
DK: Variable bandpass filter via electronic control/piezo-electric control
JGB: Could pulse multiple frequencies on a given particle to measure multiple parameters

Could flow channel be designed to segregate/orient particles based on pressure gradient?

Flow "panel" rather than "stream"?

Investigate flow cytobot - what have they achieved?

Look at citations of Zhu et al:
Microfluidic diagnostics for the developing world:
http://pubs.rsc.org/en/Content/ArticleLanding/2012/LC/C2LC90022J

Millipore Scepter: $3.5K - gives similar data as LISST, which is much more expensive

Approaches:
Coulter
Imaging
Flow cytometer

Ask Zhu et al if device is available

Think about intern project

Denis will look into reproducing cell phone camera

Review flow cytobot work

Becton-Dickinson tour/contact?

2/5/2013

Action items for next meeting:

1. Look at cytometers-on-a-chip literature - Jim, Denis, Tom

2. Fix Confluence access - Tom
Done - please login into Confluence and verify that you can access https://oceana.mbari.org/confluence/display/CytometerTech/Home

3. Update Confluence "Technologies" page with Polychromator reference - Denis

Agenda:
1. Discuss action item status from last meeting (see below)
2. Jim has some information from ONR meeting

Denis: American Society for Cell Biology Trade show
Cytometery development workshop - every year at Asilomar. Shapiro has been co-chair

Denis: Polychromator for flow cytometry (Asahi Spectra) - simplified alignment (about 4x4")
ESE Fluo Sens SD - similar assembly for fluorometer

Jim: attended ONR meeting; saw presentation on Electro-osmosis
Accelerate fluid through a charged tube; potentially eliminate cytometer pump
Could you build cytometer-on-a-chip? Could run at ambient pressure
Try googling cytometer on a chip; multiple hits. Why can't we use one of these?

Denis: Miniature silicone rubber diaphragm pumps on MEMS chip is a common technique

Jim: Why are cytometers still pretty complicated and large?
Take another look at cell phone cytometer...
Check out "google scholar", patent database for "cytometer on a chip"
Several home medical devices in development

Jim: We should cold-call some of these researchers, ask them questions
What are target organisms?
Denis: what are minimum throughput requirements, size ranges?
Jim: talk with Chavez, Scholin, Worden

DAC workshop this week, with Scholin, Delong - we will attend, get an idea of the organism targets

Jim: Eric Reihm has been offered post-doc at MBARI, has not accepted yet.

Action items from last meeting:

1. Set up project library - Tom
Done - confluence page at https://oceana.mbari.org/confluence/display/CytometerTech/Home

2. Refine technology evaluation matrix - Denis
Denis will work on this

3. Check for local relevant trade shows - Denis
American Society for Cell Biology Trade show.
Photonics West - this week
Cytometery development workshop - every year at Asilomar. Shapiro has been co-chair

4. Ask Johnson for Coulter counter briefing - Tom Done
Done - Ken notes that project has been inactive for serveral months, hopes to restart soon (with Ginger Elrod as chief engineer). See https://oceana.mbari.org/confluence/display/CytometerTech/Coulter+Counter

1/18/2013 - Kickoff meeting

Action items:

1. Set up project library - Tom
2. Refine technology evaluation matrix - Denis
3. Check for local relevant trade shows - Denis
4. Ask Johnson for Coulter counter briefing - Tom

Attendees: Jim, Denis, Tom

JGB is recuiting potential post-doc Eric Rehm from University of Washington; he's primarily interested in ocean optics, with extensive software expertise. (Note Rehm's 2007 cytometry publication with Swalwell and Armbrust.)

Would be good to arrange Rehm visit to MBARI, before post-doc decision in mid-February.
Perhaps he could participate in Jim's DAC workshop to be held Feb 6-7.
Jim will pursue travel funding options with George Matsumoto, other sources

How to engage MBARI scientists with cytometry?

CANON PIs: Worden, Scholin, Chavez. JGB notes that we shouldn't expect a lot of engagement from scientists at least during early phases of feasibility project.

Note that Jim is holding a CANON DAC workshop, Feb 6-7; he will see if we can give brief presentation describing our project.

Johnson lab is developing a Coulter counter. We should talk with them about details of their project. Note that Ken Johnson is thinking about how AUV skin surface might be used as Coulter counter detector. JGB notes that at minimum Johnson would be great advisor to our project, although he's not too engaged with CANON

Tom and Denis discussed meeting/lab-tour from Johnson group - Tom will ask Ken

Literature search of existing technologies; we should rank these in evaluation table (refined/codensed version of criteria we presented in proposal)
CytoBuoy
CytoBot
SeaFlow
Cell phone
Other technologies

Denis suggests we check biological, optical trade shows.

We should meet about every two weeks.

Optical detectors for flow cytometry

This page last changed on Mar 26, 2013 by oreilly.

Polychromator for Flow Cytometry, 6 channel, sequential filtering (high efficiency), PMT + optical filter, high throughput

http://www.asahi-spectra.com/

Confocal Fluorescence sensor, 1 channel, a photodiode

FL-SD-xxxx

www.ese\-gmbh.de

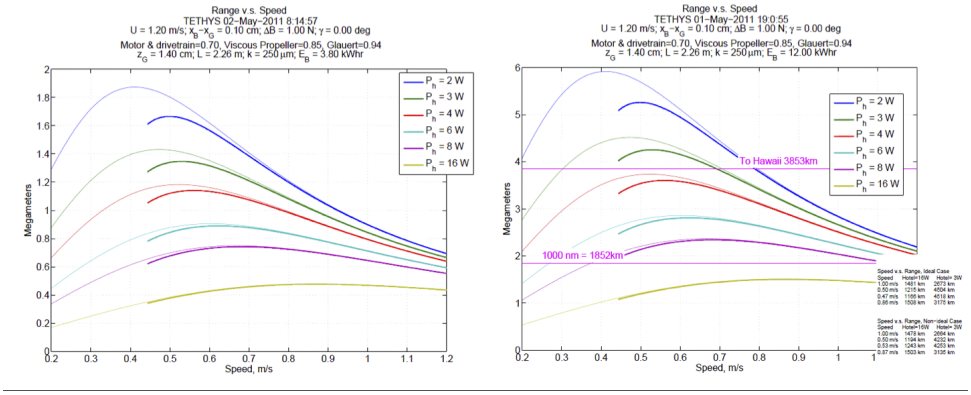
Potential platforms and constraints

This page last changed on Sep 09, 2014 by oreilly.

Vehicle payload constraints

	LRAUV	Wave Glider	Dorado
Power	< 50 W		
Volume	12" x 36" cylinder		
Weight			
Vehicle endurance	30 days	60 days	1 day
Payload endurance			

LRAUV range-vs-speed-vs-payload power:



Questions to science users

This page last changed on Apr 24, 2013 by oreilly.

1. If a cytometer were available on an AUV, how would you use it?
    - what science question would you address? What paper would you write?
    - what observational challenges do you face?
    - coastal zones vs. open ocean, eddies, midwater vs. surface
2. Range of target organisms in mixed populations?
    - size range
    - concentration range
    - methods of discrimination (optical, fluorescent properties, etc)
3. Which features are more or less important?
    - value of imaging (on-demand, occasional, primary)
    - value of physical sorting, pick-and-accumulate
    - real time or post processing
    - fluorescent labeling
    - traditional gating vs. computational population ID (how to find rare and hidden populations)
4. Value of new features of integration on autonomous mobile platform?
    - adaptive behavior, contextual sensors data
    - real-time data for reconnaissance and surveying
    - persistent presence, high-res
5. Any new trends in cytometry, alternative approaches, etc or you aware of?

This page last changed on Apr 22, 2013 by oreilly.

Potential AUV applications

Application	target cells, size, concentration	investigator
Oligotrophic population dynamics	*prochlorococcus*; 0.5-0.8 um, 10^4-10^5 cell/ml *syncechococcus*; 0.8-1.5 um, 10^3-10^4 cell/ml eukaryotic ultraphytoplankton; 2-20 um, 10^3-10^4 cell/ml heterotrophic bacteria; (size?), 10^5-10^6 cells/ml; Hoechst/ DNA stain, blue fluorescence, scatter properties	Worden (Binder et al 1996, CANON ocean eddies group)
Coastal population dynamics	cells/particles > 2 um (Chavez) ~10^5 phytoplankton cell/ml (Li and Dickie (2001) Nova Scotia)	Ryan, Scholin Chavez
HAB detection and survey	*Pseudo-nitzschia*; Width = 2-8um, Length = 40-175um Washington coast 1998 *Pseudo-nitzschia* bloom, 18000 cell/ml (Ref) Washington coast 1997 Pseudo-nitzschia bloom, 13000 cell/ml (Trainer et al 1998) Gulf of Mexico *Karenia* bloom: 20-40 um, ~700 cell/ml (Sosik IFCB presentation) Gulf of Mexico *Dinophysis* bloom: 20-40 um, ~300 cell/ml (Sosik IFCB presentation) Gulf of Maine *Alexandrium* bloom: 40-50 um, ~5 cell/ml (Sosik IFCB presentation)	Ryan, Scholin (CANON HAB working group, Sosik Imaging Flow Cytobot presentation)
Polar survey	From Li et al (2009): Nanophytoplankton: 2-20 um, 450 cell/ml Picophytoplankton: 0.5-2 um, 2500 cell/ml Bacterioplankton: (size?), 2x10^5 cell/ml	Worden, Bellingham (NASA ASTEP proposal) Smith?

SeaFlow and SeaLabel

This page last changed on Apr 24, 2013 by oreilly.

The SeaFlow cytometer was invented by Jared Swalwell of the University of Washington Armbrust Lab. One major SeaFlow innovation is patented "virtual core" technology. Most cytometers form a single-file line or "core" of cells by injecting the cells into a stream of "sheath fluid" when then flows past the light excitation beam. Seaflow eliminates the need for an actual core and sheath fluid by utilizing three positional detectors and software to determine which cells are in proper position relative to the excitation beam, rejecting measurements that don't fall within the measurement region. Swalwell is developing the next generation SeaFlow, called SeaLabel. SeaLabel significantly reduces instrument size and power usage.

2011 NASA ASTEP proposal

2013 MBARI CytoAUV MBARI proposal (not finally submitted; includes SeaLabel description [CONFIDENTIAL])

Performance notes

Measurement time, event rate, power - Swalwell et al describe seawater flow rates on page 472 of their recent paper. Seawater is pumped into the the instrument at a "nominal" rate of 15 ml/min. Only particles in the inner part of the stream are detectable, and only a fraction of those particles are in focus. Only the in-focus "optically-positioned particles" (OPP) can be analyzed. The flow rate of detectable particles is ~1/10 the flow rate of the stream while the flow rate of OPP is ~1/100 the flow rate of the detectable particles. Thus the flow rate of the OPP is ~15 uL/min = 2.5 x 10^-4 ml/sec.

Compute time 'T' to measure N OPP cells at this flow rate 'f', for given values of number density 'p':

T = N / (f * p)

We assume that cells are not necessarily all the same size, but they are all detectable, and assume that each cell is individually detected (i.e. we don't see multiple cells counted as one).

	N=1000 (sigma=3.1%)	**N=500 (sigma=4.4%)**	**N=100 (sigma=10%)**
p = 10^2 cell/ml	40000 s	20000 s	4000 s
p = 10^3 cell/ml	4000 s	2000 s	400 s
p = 10^4 cell/ml	400 s	200 s	40 s
p = 10^5 cell/ml	40 s	20 s	4 s

Note that the virtual core size (and hence OPP flow rate) is adjustable. The example value of ~15 uL/min corresponds to a virtual core diameter of ~6 microns (the physical stream diameter is ~200 microns). For a low number density, the virtual core diameter and OPP flow rate could be increased with only small risk of coincident counting, resulting in shorter counting times. Science likely wants to resolve several constituent populations with different number densities, in which case statistical uncertainties will vary with target population.

Some non-oceanographic instruments

This page last changed on Sep 11, 2014 by oreilly.

Moxi Flow

Submersible Microscope telecon January 31, 2014

This page last changed on Feb 03, 2014 by oreilly.

Questions for Resolution Optics

What is maximum recommended particle concentration? User manual says "In order to achieve accurate images, inline holography requires that a reasonable amount of the reference wave (unscattered light) reaches the camera sensor. Too many objects between the PS and the camera can reduce the amount of reference wave reaching the camera and will therefore result in poor reconstructions." RO looked at 5-10 micron plankton at 5x106 cells/liter with no trouble.

What exactly is output of realtime processing? Predetermined stack of images? Configurable? Can select single image depth for realtime processing. Can save other depths for later offline processing.

Automated image depth determination? This capability will be provided by RO's "Stingray" software, now in development

Realtime particle classification? Realtime particle size distribution, other statistics in one image plane provided by "Swordfish" software

How does spatial resolution vary with image plane depth? Highest resolution (1 micron) when target is against source window. Lowest resolution (4 microns) when target against camera window

How to discrimate between "old" and "new" particles in each image? (not pumped?)
If not a fresh volume every time, how to discriminate shift direction, etc? How to get adequate volumetric statistics?

How to integrate instrument with mobile platform, e.g. flow cell with pump, vs "passive" flow based on vehicle motion? Turbulent vs laminar flow, etc?

Instrument fouling issues? What if particle stuck to window? (e.g. can processing eliminate specific image planes, like against the window?) Smooth sapphire windows may discourage biofouling, but likely to be an issue. Particles stuck to window can be eliminated through software.

What about embedded processing system? RO is working on an embedded battery-powered version; maybe available September 2014?

Julio's questions:
- instrument flow rate
- resolution range (size range of organisms the instrument can image)
- percentage of flow-through volume imaged by the instrument's "eye"
- power consumption 5 Watts
- image storage (memory) capacity and total number of images possible Depends on laptop drive capacity
- options for variable sampling — burst mode versus continuous sampling over a deployment. Possible for the AUV to trigger sampling in an adaptive manner?

ain outcome - MBARI can borrow a Submersible Microscope (older version) for 1.5 months maximum, starting "in April". Sergey or John might be able to visit MBARI to help us get started. Instrument is rated to 2000 meters, but currently has a 10 meter gigabit ethernet cable (R.O. might have a longer cable to loan us). Sample spacer is adjustable on this model.

Notes from the telecon

Here are my actual notes of the meeting - please feel free to comment or correct.

Attendees:
Stephen Jones, Sergey Missan, George McMurtry, John Sampson
John Ryan, Brett Hobson, Francisco Chavez, Steve Haddock, Julio Harvey, Danelle Cline, Hans Thomas, Thom Maughan, Tom O'Reilly

Loaner SM instrument has same optical characteristics as newest version.

Spatial resolution varies with source-to-target distance. Best resolution is about 1 micron when close to source, 4 microns when close to camera.

Imaged volume is about 12 microliters: 2 x 2 mm FOV, 3 mm depth
Sampling rate is 16 fps, 190 microliters/sec
Single plane image transformation at 16 fps, i.e. can keep up in real time. Other planes can be processed later.

Check out the SM gallery

Software packages

Octopus: "manual" processing (hologram-to-image transform, measure size, etc)

Swordfish: High-speed real-time particle characterization (16 fps), historgrams, statistics

Stingray (in development): Automatic target tracking across image planes, morphological features, classifier. Problematic to track when targets overlap.

Particle concentration: if too many particles, there's not enough light for good reconstruction. But RO looked at 5-10 micron plankton at 5x106 cells/liter with no trouble.

Sample spacer - adjustable in older model (the model we'd borrow), fixed at 8 mm in latest model.

Instrument has been deployed for up to 1 day at least.
Sapphire windows are smooth, but biofouling likely an issue for longer deployments.

To process, need reference holograms(s) - to options:
1. Collect clear-water reference at start of deployment, or
2. Alternate between reference and sample images during deployment (not sure how this works operationally - Tom)

Processing requires Windows laptop with Invidia gpu; raw data and images stored to laptop drive.

Instrument requires 12 V power, gigabit ethernet connection to laptop.

R.O. is working on an embedded, battery-powered data logger - maybe available in August or September 2014?

Camera: 2048 x 2048 pixel, 8 bits monochrome, 16 fps, up to 50 fps in binned mode
Holograms and images stored as png, tiff (other formats?)

Data can be analyzed with LabView, other software thanks to standard image formats.

Loaner instrument is rated to 2000 meters, but has 10 meter cable (R.O. might be able to loan us a longer one).

Loaner sample spacer can be adjusted.
Sergey or John Sampson may be able to come to MBARI to help set up and use the instrument.

Sergey will send document that describes method limitations.

Sergey has not seen any processing bottlenecks running at 16 fps for 24 hours.

Technologies overview - 1

This page last changed on Jul 11, 2013 by klimov.

Categories, methods

Method	Notes	Applicability	Adaptability
Flowcytometry in a flow with optical detection - Optical Forward Scatter or (FSC). Seems to be most efficient for optical power utilization. - Optical Side Scatter or (SSC) - Optical Absorption - Fluorescence - Multispectral with single detector: color-space-time coding (UCSD)	FSC seems to be most efficient for optical power utilization and most common "signature" parameter, proxy to the organism biomass SSC is quite dependent on the size and configuration of the scattering organism and if collected at one angle only, can be hard to interpret. Fluorescence is common for taxonomy; signal levels are much lower, and usually require calibration with fluorescent bits. "Traditional" flowcytometry and most common for bench top instruments.	Pros: - established method - seems to be best for taxonomy (with fluorescence) - resolution and ID size range down to sub um Cons: - size range is relatively narrow - method of counting cells is optimized for relatively narrow range of target organism sizes - hard to interpret data for larger cells (> 50um) with complex morphology	Pros: - probably can be adapted in a reasonable size / power configuration (miniaturization of a Seaflow) - CytoSub can propable be integrated to Dorado Cons: - high cost - usually bulky and complex instruments - requiring multiple detectors and alignment - miniaturized Seaflow must compromise on power and sensitivity, and would have even narrower dynamic range
Imaging flow cytometry - Configuration of the channel: round or flat - Primary imaging system, vs. "image on trigger". - Combination with adherent cytometry: Celico (Cyntelect, purchased by Brooks Automation)	Commercial imaging flow cytometers seems to be oriented for oceanographic/ environmental markets. "Image on trigger" helps to identify larger cells with complex morphology as add-on to "traditional" cytometer	Pros: - wider dynamic range - better resolves larger cells with complex morphology Cons: - fundamental limit on low side of dynamic range; optical resolution > 1-2 um, ID limit is > 5-10 um	Pros: - relatively wide detection size range - relatively simple fluid handling/ maintenance Cons: - require processing power; - training/ image database; - harder to implement fluorescence
Holographic Imaging	SHM, Submersible Holographic Microscope (Resolution Optics)	Pros: ???	Pros: - LISST-HOLO adapted to Dorado
Bulk optical properties - Size-dependent diffraction		Pros: - optically, this is fast acquisition method Cons: - can be computationally intensive - bulk optical properties measured as oppose to individual cells - set of assumption on optical properties/ shapes of cells	Pros: - and LISST-100 adapted to Dorado

Mass-spectrometry cytometry			
Microfluidic cytometry	Traditional flow cytometry with cell counting/, with the exception that sample handling is performed in a microfluidic structure usually made of silicone rubber. Emerging technology; attempt to reduce size, cost of traditional flow cytometer; and also to simplify maintenance or confine the fluid handle for toxic/ biohazardous specimens		Pros: - confined fluid path - simplified integration of solid-state detectors and light sources Cons: - how to maintain/ replace microfluidic component
Adherent cell cytometry. - Proxy to membrane properties, mobility, cells size - Resistance in a micro channel. Coulter counter, and proxy to cell volume			

Trends:

Continue to decrease in size and energy consumption.

Use of smaller laser diodes/ solid state lasers.

Increase in detection and precision measurements

Lower cost especially for medical and bio sciences

Challenges

Limited dynamic range vs. unpredictable wide range population composition

Trade-off between size range, concentration range, and throughput is really optimized for *narrow range* of those parameters

If detection volume is set to be able to resolve pulses from small particles, then statistics on larger cells with low concentration is becoming unacceptably slow

Technologies overview - 2

This page last changed on Jul 11, 2013 by klimov.

Options, features

Method	Notes	Applicability	Adaptability
Flow path: - Open space - flow cell (quartz, plastic) - Micro fluidics, microchip. Fishman-R	Most bench top flowcytometers use open space path. Flowcytometers for toxic and hazardous substances, and oceanographic use typically enclosed path.	Design of flow path may affect optical performance, sensitivity	Maintenance: - flow path needs to be easily accessible for cleaning or replacement Catastrophic failure (flooding) - flow path needs to be enclosed
Physical volume vs. detectable volume ("virtual core") - SeaFlow, Sealable	Ability to re-process primary data.		Pros: - Allows processing of undiluted sample - no need for sheath fluid Cons: - virtual core is harder to characterize then physical volume
Sheath fluid, vs. undiluted sample			
fluorescent and calibration bits.	Used for calibration on some (most) benchtop flowcytometers		Cons: - hard to implement in "in situ" instrument
Cell sorting: - Mechanical sorting into "catcher tube". Enclosed path, one population. - Electrostatic deflection	actively separate and isolate particles having specified properties.	seem to be complex to implement and currently does not required	
Focusing: - Hydrodynamic focusing: coaxial laminar flow - Acoustic focusing (Ward)	This is physical sample focusing		
Real time or post processing, or both		Real time processing: required Post processing: beneficial	
Labeling - Fluorescent - Staining (Worden - bacterial communities) - Isotope labeling			
Processing and software			

- Gating and analyzing software - Population identification using computational methods (rare and hidden populations) - Population identification (Worden): trigger on scattering signal is used, and then accumulate data with other parameters. This is "see everything" approach			

Technologies overview - 3 (Specifications and parameters))

This page last changed on Jul 11, 2013 by klimov.

Variables and parameters

Parameter	Notes	Range	
Cell size range, um - detectable - filtration			
Concentration range, cells/mL			
Detection volume, uL, mL			
Flow rate, mL/min, uL/min - per channel cross section - per detection cross-section			
Throughput range, counts/sec. 2E5 (LLNL)			
Signal processing speed (pulses/sec, images/sec)			
Overall energy efficiency; J/count			
Statistics and acceptable errors			
Optical background (Br)			
Detector efficiency (Qr) - determined by laser power, optical design, PMT sensitivity... photoelectrons detected per equivalent fluorochrome molecule			
Linear range (signal : number of photons)			

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This page last changed on Feb 12, 2013 by klimov.

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Visit by Heidi Sosik on 9-11-2013

This page last changed on Sep 18, 2013 by oreilly.

O'Reilly's notes on 10 am meeting.

Attendees: Heidi Sosik, Denis Klimov, Tom O'Reilly

Questions:
Plans for small/low-power instrument?
Can FCB/IFCB be deployed without power/data cable-to-shore?
HS: Working on "low-relief" IFCBT for deployment on Wave Glider, AUV, other platform
Current IFCB takes 30W power

Particle size range overlap of FCB and IFCB?
HS: Difficult; IFCB laser is optimized for microplankton - picoplankton requires higher-power laser. FCB is still original prototype (large) - no funding or plans to refine. CytoBuoy claims pico-micro capability.

Could you replace sheath fluid with virtual core technology, ala SeaFlow (Need to add position-sensitive detector(s))?
HS: Would be concerned about flow-cell cleanliness - core fluid keeps particles moving through flow cell. Very occassionally get dirt in cell - would this be a greater problem with SeaFlow/SeaLabel?

Processing:
HS: Several stages; detect particles (reject particle-free images regions). Edge detection, etc.
Classification
Now using "random forest" approach instead of SVM (2007):
- rf requires less tuning than svm
- svm requires complete characterized dataset for error characterization; rf doesn't
- both methods yield comparable accuracy

How long did the image-processing/classification procedures take per image? On what hardware?
HS: particle detection/empty-image rejection executes in situ on instrument's "Atom" processor. Shore-side workflow in realtime, posted on MVCO website (http://ifcb-data.whoi.edu/)
HS: Image processing is more computationally intensive than classification

Klimov's notes from 10:00, 12:00, 16:00 meetings

Principles of operation:
- FlowCytobot / Imaging FlowCytobot sample path arranged in such a way as sample goes "down hill" due to plankton sinking.
- Newer Imaging FlowCytobot (licensed to Mclane) is developed entirely at WHOI. McLane did some adaptations to simplify production. 8 inch diameter tube, 40 inch long. First unit built in August 2013.
- 5ml sip, then 20 minutes analyzing. This time can be reduced by sipping less, say 1ml then 4 min analyzing, but the period can not be much shorter due to time overhead (pump start/ stop etc) and required statistics.
- this design has emphasis on accurate statistics so flow and volume need to be precise, so syringe pump is used with defined volume.
- Emphasis on "binning" to get particle statistics per "shot" and not as continuous measurement; so "binning" is ok here.
- Also, accurate flow rate is required, due to separation of "trigger volume" and image acquisition volume within a sample cuvette, and fixed time delay between trigger and acquisition of the image. Image is acquired with 1 us light pulse from Xenon lamp.
- Sheath fluid is preventing the contact between sample water and walls of the cuvette, minimizes chance of contamination and particles attachment to cuvette walls, very important feature.
- Sheath fluid is recirculated by filtering sample + sheath by volumetric Fisher filter. Excess water is dumped into environment before filter. Filter lasts 6-8 month deployment easy.

Use of Imaging FlowCytobot on a mobile platform:
- Configuration of the sample path dictates "vertical" position of the instrument so it operation in horizontal orientation could be problematic.
- For glider use, perhaps the Imaging FlowCytobot can be split into set of housings and then arrange them horizontally.
- Suggestion: keep volume of those compartments relatively small (under several hundred mL) to avoid vehicle loss in case of flooding one compartment.

Difference between FlowCytobot and Imaging FlowCytobot:

- Particle ranges: FlowCytobot 1-15 um, Imaging FlowCytobot 5-200um
- Cuvettes have different geometries: FlowCytobot has smaller cylindrical flow cavity for tighter light collimation of laser, and Imaging FlowCytobot has "50 um sheet" of fluid optimized for imaging and larger laser beam for triggering
- Light sources are optimized differently for smaller circular path, or wider flat path

Visit by Jarred Swalwell, Feb 5 2014

This page last changed on Feb 06, 2014 by oreilly.

02/05/2014 /2013 MBARI Jarred Swalwell. Jarred was MBARI seminar speaker. Had discussions with Jarred at 9:00, 12:00, 16:00

9:00 Klimov, Swalwell, O'Reilly

Status: new electronics is done but have a noise on analog board from other (switching) board in a board stack. Due to limited resources, now working on components that can be used for existing systems SeaFlow (synergy). Need to solve:
Flowcell
Modified concept.
Laser (test, select)
Pump
Mechanics.

"Unattended" Seaflow operation of a few days is pretty reliable. Generally shorter deployments (few days, a week) compare to CytoBot

Rosette profiling pump may be a bit fast for cytometry. Need at least 3 minutes sampling same volume to get good population statistics

May come out again in April

Flow cell - hoping to use cheap $10 cell, as opposed to IFCB cell which costs $1000s
But have to frequently change the cell. Jarred uses MicroPump devices, pretty reliable.
Keep clean: consider hydrodynamics near flow cell walls.

Testing new cheap 0.5Watt $500 Chinese-made laser - not too stable but can resolve prochlorococus. (ultrabright.com?) They also make $10k laser which is pretty stable so this is matter of price not technology.

Could do embedded system with Arduino

Camera - would like to take pictures of larger particles.

Round table discussion

16:00-17:00 Klimov, O'Reilly, Maughan, Swalwell, Hamilton, Chavez

Some of Francois' algorithms can be run in real-time

FC: What about machine-learning approaches? E.g. make inferences from clustering and local environment.

JS: Often have to specify centroids to Ribalet's algorithms at the beginning.
A lot of the clustering algorithms originated from medical applications (Fred Hutchinson Inst.). "Flowcast". Written in 'R', which is free (unlike MathLab)

JS: Has done some acoustic applications. Drug-delivery via bubbles, infer bubble material properties from acoustic scatter

JS: Note that size of virtual core can be adjusted. Larger VC results in poorer

JS: Have processed 2012 data - will provide to Francisco/Jason

Fluorescent beads tend to sink; so delivery rate is not constant, but this is not so important as long as some of them appear on cytogram for gating calibration

On "real time": 3 min is sort of enough for cytogram, makes 1 file. Real flow rate is 15 mL/min, virtual core flow rate about 15-30 uL / min

Plans: Fransico is asking what is good for Jarred: 1st, or 3-4 weeks of April?
Jarred: 1st week looks better.

Visit by Peter Lopez 9-10-2014

This page last changed on Sep 11, 2014 by oreilly.

Lopez, O'Reilly, Klimov

Centrifugal elutriation - pre-sort particles

Sampling statistics problem

Partec - flow microscope

Denis - showed SM data
PL: Could you acoustically control concentration in SM measurement volume, to prevent excessive particles?

Round table:

Influx sorting discussion with Camille

Scholin: interested in particle-field distribution, to guide adaptive sampling
CS: Which is more amenable to real-time sampling: scatter/fluor vs morphological?
PL: Need to decide size range. Answer is not always flow cytometry. Look at Shapiro's battery-powered microscope, for remote diagnostics

JB: How small can a flow cytometer be?
PL: lasers are really small now... a lot of cytometers had to be flexible - now more specialized, smaller versions available. HIV/AIDS applications in developing world are driving size/power downward.
CS: What are prospects for in situ cell sorting?
PL: Will show some slides... chip-based, not electrostatic, but mechanical. Working with Livermore guys

FC: Do you sort live material?
PL: Yes - sorting effects on living samples are largely unknown - work to be done . Typically pressurized to 60 psi

PL: Centrifuging technique seems pretty gentle. Acoustic tech hasn't made it into sorting applications yet.

FC: Anyone thought of breaking up cells and analyzing organelles?
PL: Done with e.g. tumor cells. Also lyse cells to extract chromosomes, then run through cytometer

PL: Can get fluorescent beads that bind to specific target - then measure beads in cytometer. Can get that fluoresce in different colors

SS: Can cells be sorted on basis of morphology?

CP: Could we sort environmental DNA?
PL: Need some kind of particle basis for cytometry
TO: Could this be a mass-spec application?
PL: Maybe

PL: No reason why a flow cytometer can't be very small - power is more challenging

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