Efforts to develop a ballast water detecting device

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ABSTRACT

Research of ballast water threating devices cannot follow up the research of repairs of the devices and the research of permanent quality of those machineries. There is a need for follow up system. In this research is being analysed what technology is good enough to support a development of the ballast water bio invasive organisms detection. There are static and dynamic detecting efforts and challenges. Two methods were brought up as good examples for each of them, static and dynamic detection. Methods of detection as DNA method cannot make good results as dynamic detecting method. Charasteristics of DNA method were described in this paper. Another method with some existing devices is already in use for other kind of detections. Spectroscopy has been explained and cytometry as well. Products that use this methodology were listed and the functionality of the system has been explained. The use itself and reasons for it were researched in this paper and the conclusion brought out.

Key words: ballast water treating devices on the ships, professional service and repairs, detection, methods, DNA, cytometry, bio-invasive organisms, scientific research using inducting method.

1. INTRODUCTION

International law brought up Convention of BWM 2004. The Convention requires from certified inspectors to do their best efforts to avoid *not needed stoppage caused by potentional sampling* of all the ships (p.12). That means that the advantage is usally given to respect the contracts of transportation, instead to protect the seas.The inspector can give an order to a designated institution or certified laboratory to take samples and to analyse water ballast that any crew intents to discharge into the sea. That inspection in a laboratory includes analyses to find wether any microorganisms were in water ballast and to find out salinity and kind of salt in the way to determine where from is the ballast water. If the analyses find out no microorganisms or any other reason that would prevent ballast water discharge, all the expenses would be paid by the Ministry that the inspector represents. Those expenses are very big expenses and that could even cause loosing the cargo in next port of call. There comes the need of *cheaper* and faster inspections. New technologies would help to find better ways for that.

Another reason why we need other solutions is on the ships, weather conditions and among the crewmembers. The experience has shown problems of the repairs that happen on the ships. It is very big difference between the correction of the damages in the cargo tanks and in the ballast water tanks on the ships. A good example is correction of the pittings in cargo tanks after the cargo of sulfur acid has been discharged and the tanks cleaned. The repair of the heating coils that exist in cargo tanks (one of the ways how to hold liquid cargo temperature) would be much harder if happened in ballast water tanks. Another example is to derust and correct by welding a constructional problem in ballast or cargo tank when needed. The basic difference is in height of the tanks: cargo tanks are three to ten metres high and ballast tanks are of the height from a half meter up to 1.5 metres . To do any repair or welding job in that height is extremly hard. Same problem could happen with any possible repair of ballast water threating device or welded holders of that device. Any equipement that exists in the salt water is in constant exposure to the damages. The ship's movement in the storms combined with the salt exposure brings up very big risk of the damages or improper functionality of any device, heating coils or other methods of ballast water treatment in the ballast water tanks. Heating coils get loose by the time, the fluid(liquid) in the heating system leaks and could contaminate ballast water. Oil is usually used liquid that brings the temperature into heating coils. It would be impossible to find out where from the heating coils liquid leaks if the ballast inspector found the contamination. Even if the hole was found there are measures and the law that requires how and where is alowed to repair that kind of damage .

One of the solutions to control if the ballast water device/system realy worked in proper way is to give the opportunity to new technologies.

2. DETECTION METHODS

The detection of algae and other bio invasive organisms in the ballast water is important. It is needed to avoid expenses that could happen by wrongfull inspectors' decisions and there is an obligation to protect the nature. The crew and the ship owners take the responsibility of ballast water treatment on board the ships. The experience of using other monitoring devices (even not fixed in the salt water) as, for example, oil discharge monitor, brought many different *overworks* by crew members. The most popular one is to by-pass the oil discharge monitor in monitoring system on board the ships. It is expectable from the crewmembers all over the world to invent other ways to by-pass improper work of the devices in this case of ballast water treatment. To evaluate good or improper work of the ballast water treatment systems on board the ship, requires a good method to detect bio-invasive algae and other microorganisms. Good methods are dynamic methods. A good method that could detect unwanted microorganisms needs to be dynamic and able to scan extremly fast. It is needed also to format data, analyse that and process large data files. Advantages of silico-imaging or scanning is the high acquisition rate of up to 1000 scans per second through a USB interface, and the suitability of the data format for standard flow cytometer data analysis and fast processing of large data files.

2.1 DNA DETECTION METHOD

DNA analyse brought up to the science a new way to answer old questions. DNA analysis method is an initiation to cytometry analyses. DNA analyses are done in laboratory and sometimes days are needed to inspect some objects of detection. DNA tacsonomy is based on the analyse of small segments of genomas. Group of nucleotids of each algae is a different barcode that exists in different algae. Those barcodes filed in a database are used to indentify all algaes of the world. Followed example shows that the time of DNA detection in that case was shorten down to three hours.

A technique used for detecting *Raphidophycean*, a bloom-forming genus of algae, was developed using a specific DNA probe. The design of the probe was based on a sequence polymorphism within the small subunit ribosomal DNA (rDNA) gene of a strain by using fluorescence polarization analysis and the BIAcore 2000 biosensor, which utilized surface plasmon resonance. The specific sequence for *Heterosigma carterae* was determined by sequence data analysis. One pair of polymerase chain reaction probes was designed for use in making the identification. *Heterosigma carterae* rDNA was amplified by. Using a fluoroscein isothiocyanate–labeled or biotin-labeled oligonucleotide probe, the amplified rDNA was selectively detected as an intensive change via analysis or as a resonance-unit change. Although total time for final detection after sampling was within 3 hours, specific rDNA could be detected within 10 minutes after through these detection methods. **[1]**

2.2 CYTOMETRY DETECTION METHOD

The power of the flow cytometric analysis principle is that the cells are passing a laser beam one by one at high speed and their individual light scattering and fluorescence properties are recorded to form an optical *fingerprint* for each cell. This separates the flow cytometric method from bulk methods for fluorescence spectra and/or size spectra for bulk volumes of water where it is much harder to discriminate between the contributions of the various groups in the water since the readings are collected for all particles at once. Flow cytometry allows easy recognition of the different groups in the sample and quantification of their abundance as well as their optical properties (size, pigment) - even the detection of a few rare cells from within a high number of cells from a blooming species.

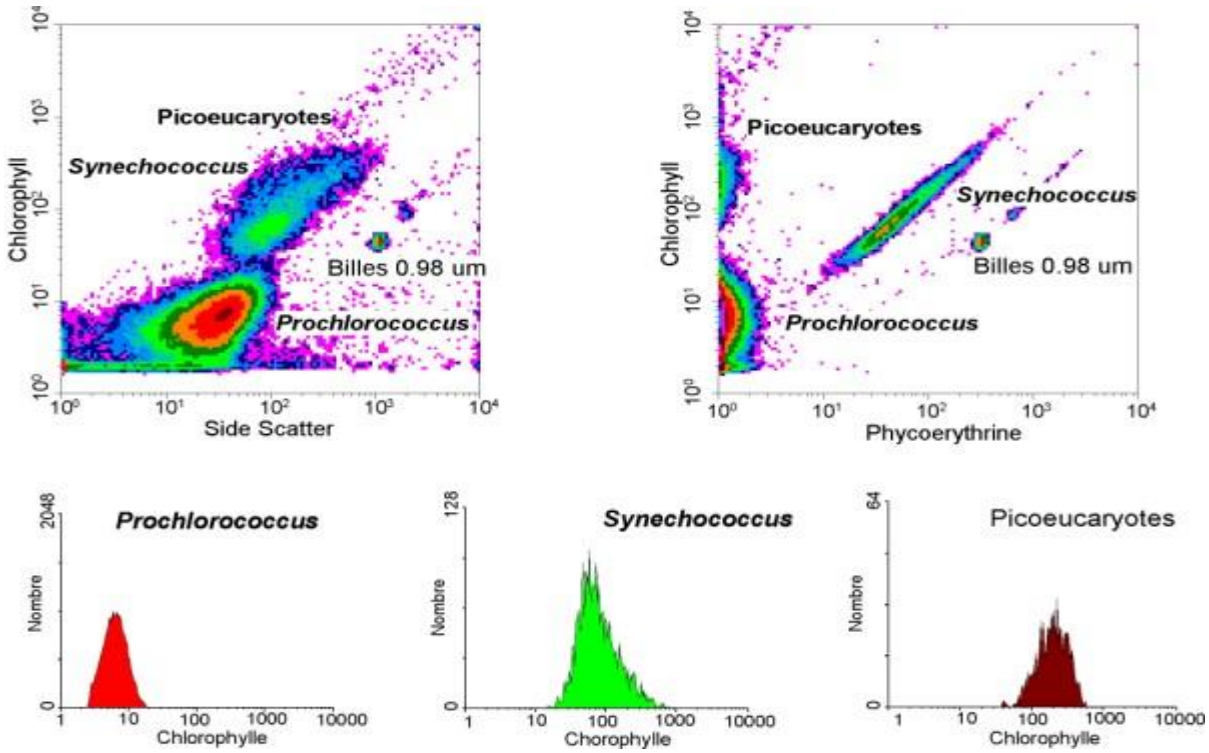

FIGURE 1: Some scans of flow cytometry technology **[2]**

History

The first impedance-based flow cytometry device, using the Coulter principle, was disclosed in U.S. Patent 2,656,508, issued in 1953, to Wallace H. Coulter. The first

fluorescence-based flow cytometry device (ICP 11) was developed in 1968 by Wolfgang Göhde from the University of Münster and first commercialized in 1968/69 by German developer and manufacturer Partec through Phywe AG in Göttingen. At that time, absorption methods were still widely favored by other scientists over fluorescence methods. Soon after, flow cytometry instruments were developed, including the Cytofluorograph (1971) from Bio/Physics Systems Inc. (later: Ortho Diagnostics), the PAS 8000 (1973) from Partec, the first FACS instrument from Becton Dickinson (1974), the ICP 22 (1975) from Partec/Phywe and the Epics from Coulter (1977/78).

The original name of the flow cytometry technology was "pulse cytophotometry". Only 20 years later in 1988, at the Conference of the American Engineering Foundation in Pensacola, Florida, the name was changed to "flow cytometry", a term that quickly became popular.

Principle of flow cytometry

A beam of light (usually laser light) of a single wavelength is directed onto a hydrodynamically-focused stream of fluid. A number of detectors are aimed at the point where the stream passes through the light beam: one in line with the light beam (Forward Scatter or FSC) and several perpendicular to it (Side Scatter or SSC) and one or more fluorescent detectors. Each suspended particle from 0.2 to 150 micrometers passing through the beam scatters the ray, and fluorescent chemicals found in the particle or attached to the particle may be excited into emitting light at a longer wavelength than the light source. This combination of scattered and fluorescent light is picked up by the detectors, and, by analysing fluctuations in brightness at each detector (one for each fluorescent emission peak), it is then possible to derive various types of information about the physical and chemical structure of each individual particle. FSC correlates with the cell volume and SSC depends on the inner complexity of the particle (i.e., shape of the nucleus, the amount and type of cytoplasmic granules or the membrane roughness). **[3]**

Flow cytometers

Modern flow cytometers are able to analyze several thousand particles every second, in *real time*, and can actively separate and isolate particles having specified properties. A flow cytometer is similar to a microscope, except that, instead of producing an image of the cell, flow cytometry offers "high-throughput" (for a large number of cells) automated quantification of set parameters. To analyze solid tissues, a single-cell suspension must first be prepared.

A flow cytometer has five main components:

- a flow cell - liquid stream (sheath fluid), which carries and aligns the cells so that they pass single file through the light beam for sensing
- a measuring system - commonly used are measurement of impedance (or conductivity) and optical systems - lamps (mercury, xenon); high-power water-cooled lasers (argon, krypton, dye laser); low-power air-cooled lasers , diode lasers (blue, green, red, violet) resulting in light signals
- a detector and Analogue-to-Digital Conversion (ADC) system - which generates FSC and SSC as well as fluorescence signals from light into electrical signals that can be processed by a computer
- an amplification system - linear or logarithmic
- a computer for analysis of the signals. **[3]**

The process of collecting data from samples using the flow cytometer is termed *acquisition*. Acquisition is mediated by a computer physically connected to the flow cytometer and the software which handles the digital interface with the cytometer. The software is capable of adjusting parameters (i.e. voltage, compensation, etc.) for the sample being tested, and also assists in displaying initial sample information while acquiring sample data to insure that parameters are set correctly. Early flow cytometers were, in general, experimental devices, but

technological advances have enabled widespread applications for use in a variety of both clinical and research purposes. Due to these developments, a considerable market for instrumentation, analysis software, as well as the reagents used in acquisition such as fluorescently-labeled antibodies has developed.

Modern instruments usually have multiple lasers and fluorescence detectors. The current record for a commercial instrument is four lasers and 18 fluorescence detectors. Increasing the number of lasers and detectors allows for multiple antibody labeling, and can more precisely identify a target population by their phenotypic markers. Certain instruments can even take digital images of individual cells, allowing for the analysis of fluorescent signal location within or on the surface of cell.

Data analysis

The data generated by flow-cytometers can be plotted in a single dimension, to produce a histogram, or in two-dimensional dot plots or even in three dimensions. The regions on these plots can be sequentially separated, based on fluorescence intensity, by creating a series of subset extractions, termed *gates.* Specific gating protocols exist for diagnostic and clinical purposes especially in relation to hematology.The plots are often made on logarithmic scales. Because different fluorescent dyes' emission spectra overlap, signals at the detectors have to be compensated electronically as well as computationally. Data accumulated using the flow cytometer can be analyzed using software. Once the data is collected, there is no need to stay connected to the flow cytometer. For this reason, analysis is most often done on a separate computer. This is especially necessary in core facilities where usage of these machines is in high demand.

Fluorescence-activated cell sorting

Fluorescence-activated cell sorting (FACS) is a specialized type of flow cytometry. It provides a method for sorting a heterogeneous mixture of biological cells into two or more containers, one cell at a time, based upon the specific light scattering and fluorescent characteristics of each cell. It is a useful scientific instrument, as it provides fast, objective and quantitative recording of fluorescent signals from individual cells as well as physical separation of cells of particular interest.

The cell suspension is entrained in the center of a narrow, rapidly flowing stream of liquid. The flow is arranged so that there is a large separation between cells relative to their diameter. A vibrating mechanism causes the stream of cells to break into individual droplets. The system is adjusted so that there is a low probability of more than one cell per droplet. Just before the stream breaks into droplets, the flow passes through a fluorescence measuring station where the fluorescent character of interest of each cell is measured. An electrical charging ring is placed just at the point where the stream breaks into droplets. A charge is placed on the ring based on the immediately prior fluorescence intensity measurement, and the opposite charge is trapped on the droplet as it breaks from the stream. The charged droplets then fall through an electrostatic deflection system that diverts droplets into containers based upon their charge. In some systems, the charge is applied directly to the stream, and the droplet breaking off retains charge of the same sign as the stream. The stream is then returned to neutral after the droplet breaks off. **[4]**

Applications

The technology has applications in a number of fields, including molecular biology, pathology, immunology, plant biology and marine biology. It has broad application in medicine (especially in transplantation, hematology, tumor immunology and chemotherapy, genetics and sperm sorting for sex preselection). In marine biology, the auto-fluorescent properties of photosynthetic plankton can be exploited by flow cytometry in order to characterise abundance and community structure. In protein engineering, flow cytometry is used in conjunction with

yeast display and bacterial display to identify cell surface-displayed protein variants with desired properties. It is also used to determine ploidy of grass carp fry. **[5]**

Particle scanning

Traditional flow cytometers analyze thousands of cells per second acquiring basic total fluorescence and light scattering per cell. This is proportional to particle size and pigment content. What if the separation of the clusters is insufficient to discriminate between species and/or if additional shape information of the particles is required?

In the cytosense instruments the signals from all the detectors are not only digitized continuously, they are also stored for each particle. Since the particles flow through the laser focus in a stretched-out manner these digital profiles are actually length scans of the particle, representing the distribution of its *bodyparts* along its length axis with regard to the their light scattering and fluorescing properties. This fluid driven laser scanning of individual particles is called silico-imaging and uses the normal flow cytometer setup combined with special data grabber boards. The optional *curvature* sensor adds a two-dimensional component to the silico-images using a laser beam polarization setup only available on flow cytometers.

Advantages of silico-imaging or scanning is the high acquisition rate of up to 1000 scans per second through a USB interface, and the suitability of the data format for standard flow cytometer data analysis and fast processing of large data files. Libraries of these fingerprints allow the automatic classification and enumeration of groups and species from many data sets as well as online warning for target (algae) species..

Cytobuoy

CytoBuoy instruments use silico-imaging as basic data format: data-extensive but highly informative optical fingerprints obtained by fluid driven laser scanning of individual particles. Libraries of these fingerprints allow the automatic classification and enumeration of groups and species from large data sets as well as online warning for target of harmfull algae species.

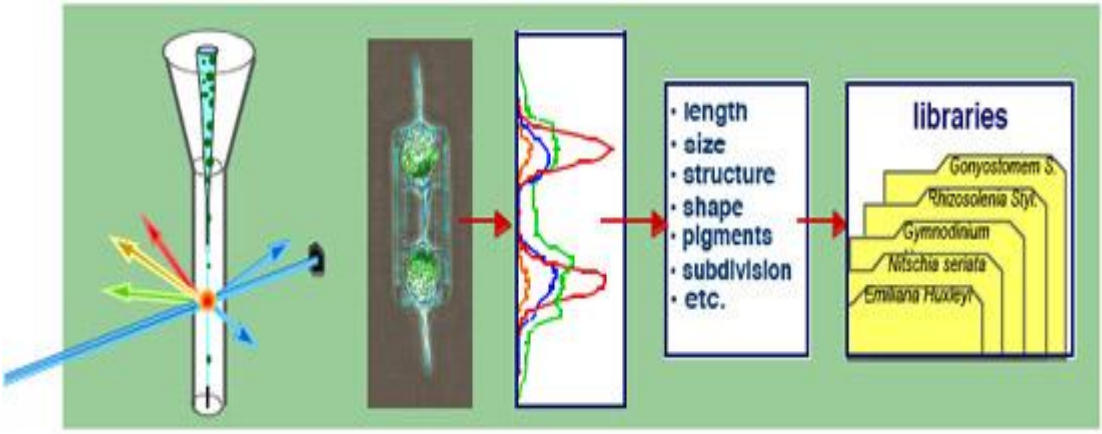

FIGURE 2: Principle of screening and filing data of harmfull algae**[5]**

To be able to take the instrument and put it in/under water it was completely redesigned the flow cytometer to get much smaller and modular. The basic instrument which is a scanning flow cytometer that can hold 2 lasers and up to 10 optical detectors in a 30x45cm cylinder of only 15KGs. The instrument is fully computer programmable with possibility to read out the data files over internet if needed. The same instrument can be placed in a moored buoy or a high pressure submersible housing which makes it possible to do field measurements on real live samples (without filtering - fixatives - transportation - waiting), anywhere, anytime. Applications of the cytobuoy are in screening of phytoplankton cultures, natural samples, detection of rare species, population dynamics research, general phytoplankton monitoring, assesment of biodiversity, bio-indicators, harmful algal blooms, grazing, (micro)zooplankton,

protection of aquaculture, bathing water, resource water, lake restoration, alarm for herbicides, invasive species control and bio-effect monitoring, marine optics and lake or sea truth for remote sensing.

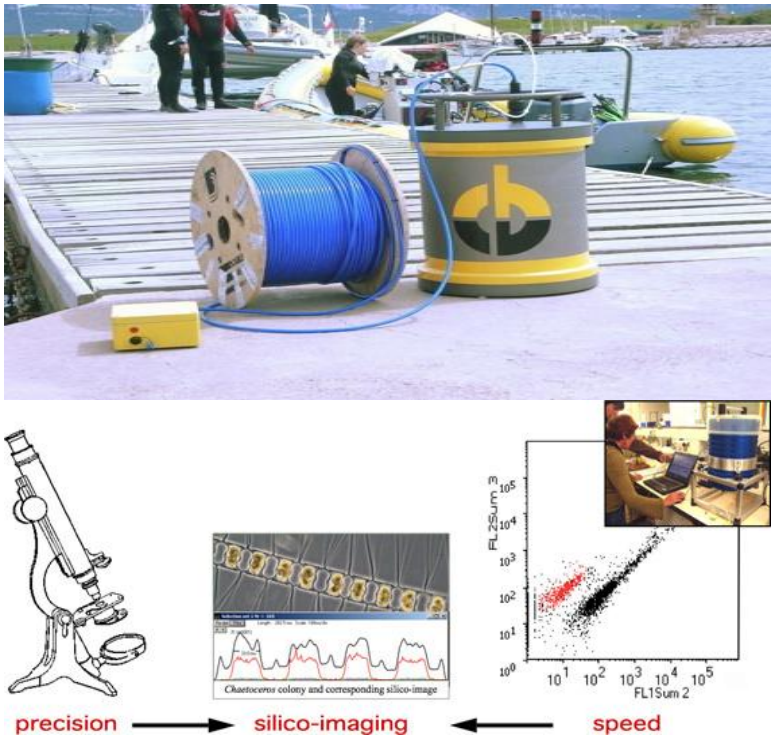

FIGURE 3: Buoy equiped for flow cytometry detection**[5]**

3. CONCLUSION

It is required an instrument that combines a high level of information content and a high level of frequency in sampling times and/or space (coverage). Dynamic ecosystems are requiring sampling at *critical scales*: the temporal and/or spatial scales at which data must be collected in order to resolve patterns and processes (including early warning). In practice this means the flow cytometer could be fixed on ships, under water and on moorings, and should allow a high level of autonomous operation combined with high speed and high throughput. Ballast water inspector can inspect ballast water but he has not to. That is in accordance with Ballast Water Convention. Inspectorate has to pay stoppage expenses if the analyses were negative. Fast analysis method combined with increased fines would diminish any possibility of work against the law.

The fast and quantitative diagnostic capabilities of the flow cytometers may be of great help for the fast screening of ballast water by generating countings and accurate size spectra for sediment particles, phytoplankton and other groups of particles. This can be used to monitor the efficiency of organisms targeted treatments, or even serve as a feed back mechanism to actively control treatment performance. **[6]**

4. RESOURCES

[1] http://www.springerlink.com/content/5wr78nck7m01vheu/
[2] http://en.wikipedia.org/wiki/File:Picoplancton_cytometrie.jpg
[3] http://en.wikipedia.org/wiki/Flow_cytometry
[4] www.cytobuoy.com
[5] http://www.cytobuoy.com/applications/aquaticscience.php
[6] http://www.cytobuoy.com/products/cytobuoy_flow_cytometer.php