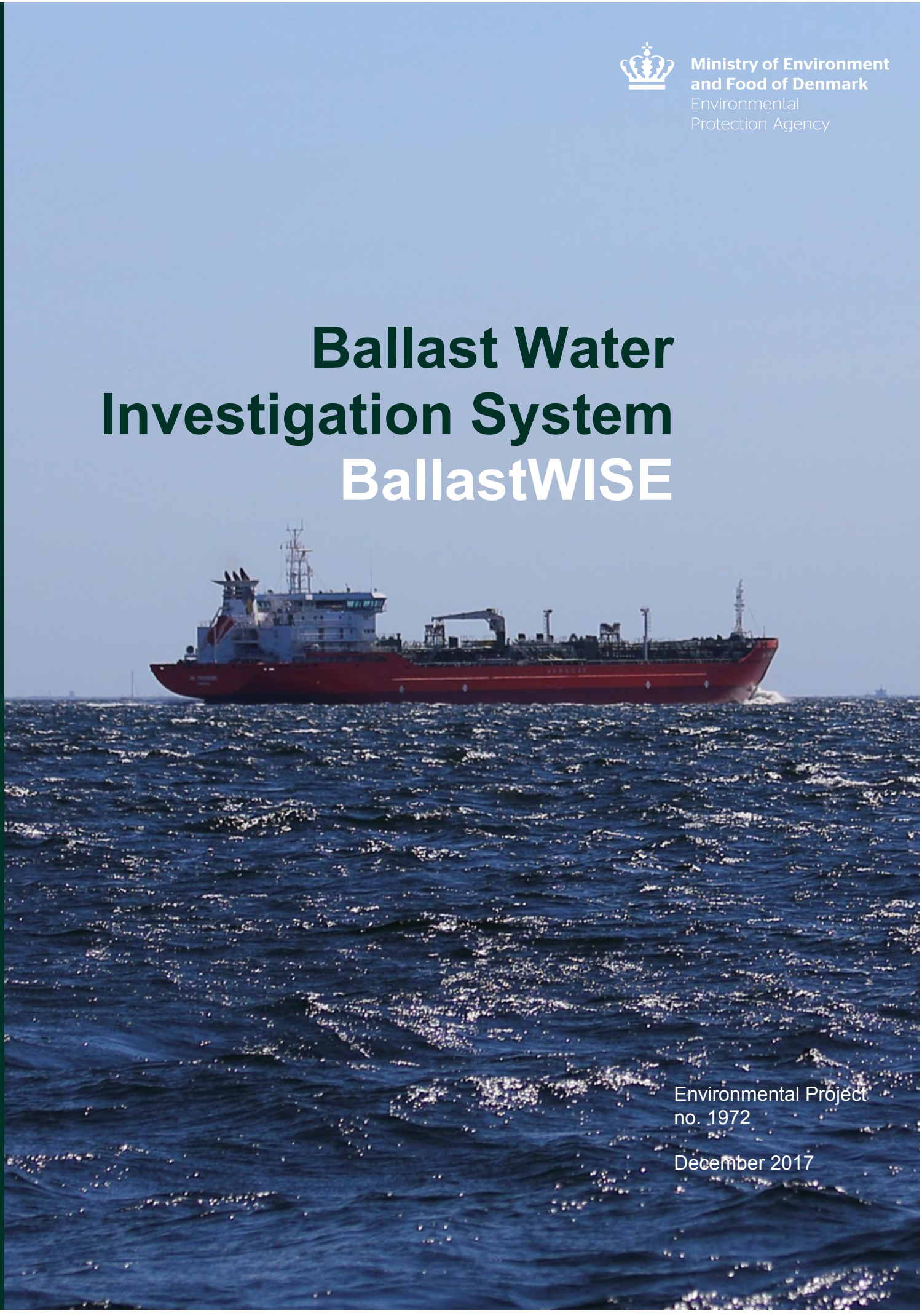
Ministry of Environment
and Food of Denmark
Environmental
Protection Agency

Ballast Water Investigation System BallastWISE



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1. Introduction

Invasive aquatic species present a major threat to the marine ecosystems, and shipping has been identified as a major pathway for introducing species to new environments. The problem increased as trade and traffic volume expanded over the last few decades, and in particular with the introduction of steel hulls, allowing vessels to use water instead of solid materials as ballast. The effects of the introduction of new species have in many areas of the world been devastating.

Quantitative data show the rate of bio-invasions is continuing to increase at an alarming rate. As the volumes of seaborne trade continue overall to increase, the problem may not yet have reached its peak. With the Ballast Water Management Convention, coming into force in September 2017, all ships in international traffic are required comply with the Ballast Water Management Convention by the International Maritime Organization (IMO, 2004).

According to Regulation D2 of the Ballast Water Performance Standard the maximum allowed number of live organisms in ballastwater discharge is less than 10 viable organisms per cubic metre greater than or equal to 50 micrometres in minimum dimension and less than 10 viable organisms per milliliter less than 50 micrometres in minimum dimension and greater than or equal to 10 micrometres.

The IMO Ballastwater Convention (IMO, 20014) has been ratified, and the requirements for the discharge of ballast water will come into force in September 2017. The United States has had requirements for the discharge of ballast water since 2013. Compliance with regulations for ballast water discharge will be assessed by regular inspections on board ships. If the Inspector is not satisfied with the ship's handling of ballast water, he or she can take samples of the discharged ballast water. The problem is that there is no quick and precise measuring methods for quantification of organisms in ballast water. Quantitative analysis of organisms in ballastwater is necessary for the approval of new ballastwater treatment systems, and for periodical monitoring and random checks of installations on ships. Control of organisms between 10-50 µm is a particular challenge, since it is a diverse group consisting of many different algae (phytoplankton), flagellates, ciliates and other microzooplankton. Quantification of organisms between 10-50 µm is performed routinely by DHI for type approval of ballast water wastewater treatment systems. This work is today done manually, using methods that are not part of the port authority control. There is therefore an international need for methods and associated analytical technology that is easy to use and validated across from the conventional methods of quantification of living plankton.

The aim of the BallastWISE project was to develop a system for rapid and automatic evaluation of the concentration of live organisms between 10 – 50 µm by detecting organisms that are motile and/or organisms which contain chlorophyll using image- and motion analysis.

The BallastWISE method is designed for use by port State control officers to evaluate compliance with the D-2 standard, or as part of BWMS to monitor efficiency during ballasting and deballasting operations, and ship operators wishing to carry out self-control.

The BallastWISE technology has been described in an Information paper for IMO (International Maritime Organization (IMO) (MEPC 71/INF.25).

2. Development of the BallastWISE system

2.1 Principles of analysis

BallastWISE detects organisms by their movement and/or by detecting chlorophyll. Some organisms are heterotrophic and they can only be detected by their movement, however most heterotrophic, planktonic organisms in the size above 10µm are actually motile. Some autotrophic organisms swim, while others do not. A BallastWISE analysis is divided into two stages; first an analysis with white light where only moving organisms are detected and where the background is ignored (see image below).

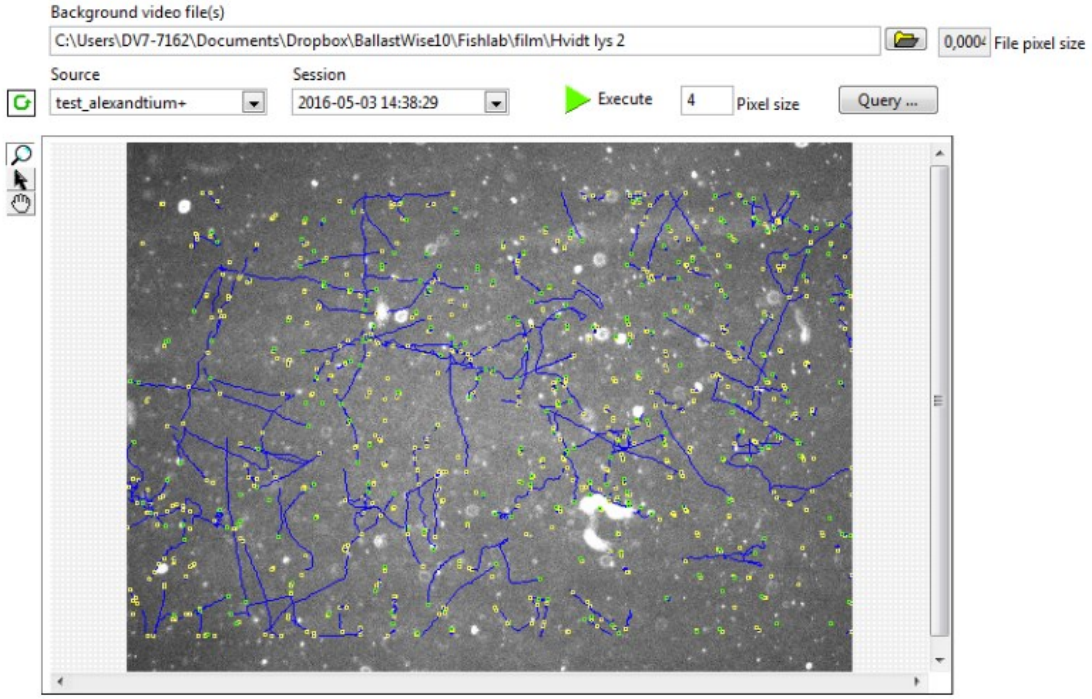


Figure 1. Analysis of a water sample with *Alexandrium* sp. cells (dinoflagellates with a size around 20 µm). White light was used, and only moving organisms were detected while the background noise was ignored. The blue trajectories are tracks from swimming *Alexandrium* sp. cells against a complex background.

Secondly, an analysis is performed which looks at all organisms containing chlorophyll that are detectable in the field of view, both motile and non motile. However, the background is "removed" optically by way of a high pass filter, which makes the light source, and all reflected light from it, invisible (see below).

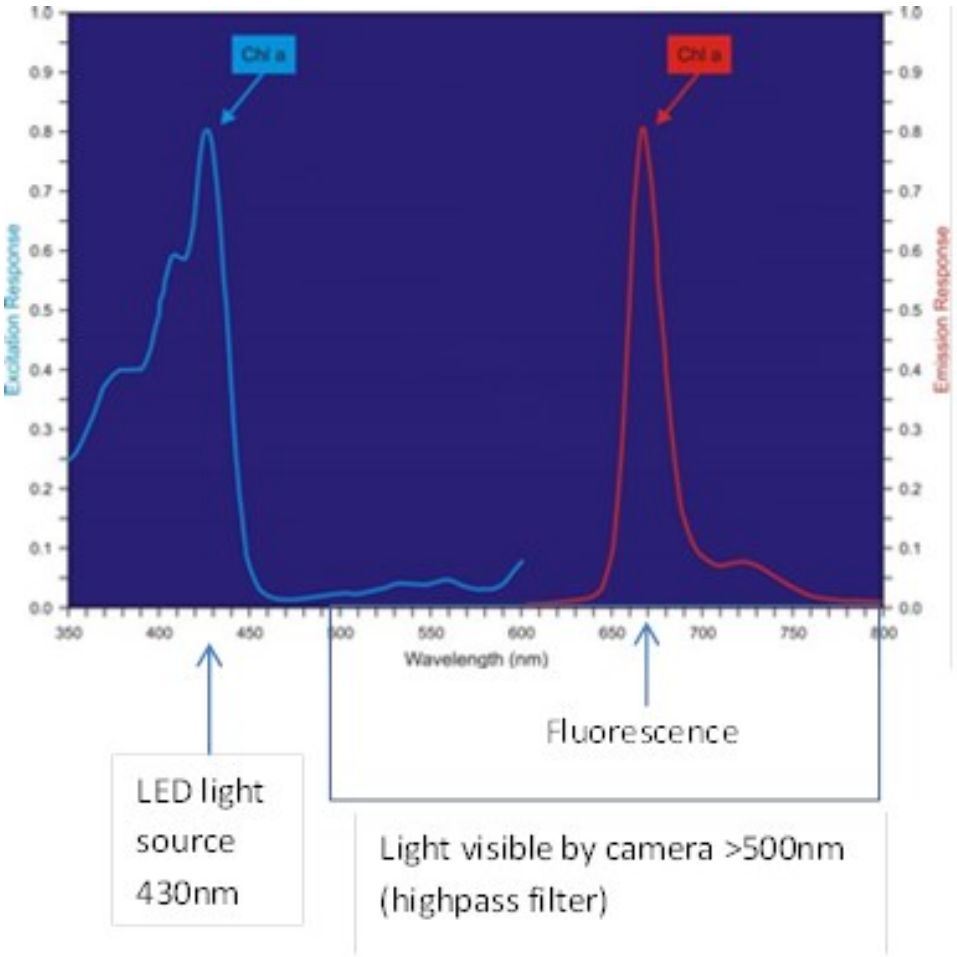


Figure 2. Blue high-power LED light source (430 nm) excites the Chl*a* pigments of phytoplankton cells. The excited cells produce a fluorescent signal at >500 nm and an optical highpass filter will block virtually all other light.

Fluorescence, however, is visible because it occurs at a longer wavelength. The light source is carefully chosen to maximize fluorescence of chlorophyll a (430nm). Light emitting diodes (LEDs) are available that give a narrow output spectrum centered around that wavelength. Below is a first look at the result of looking at a drop of water containing an algal culture with this method. This confirmed that the camera is sensitive enough to see the fluorescence from individual cells.

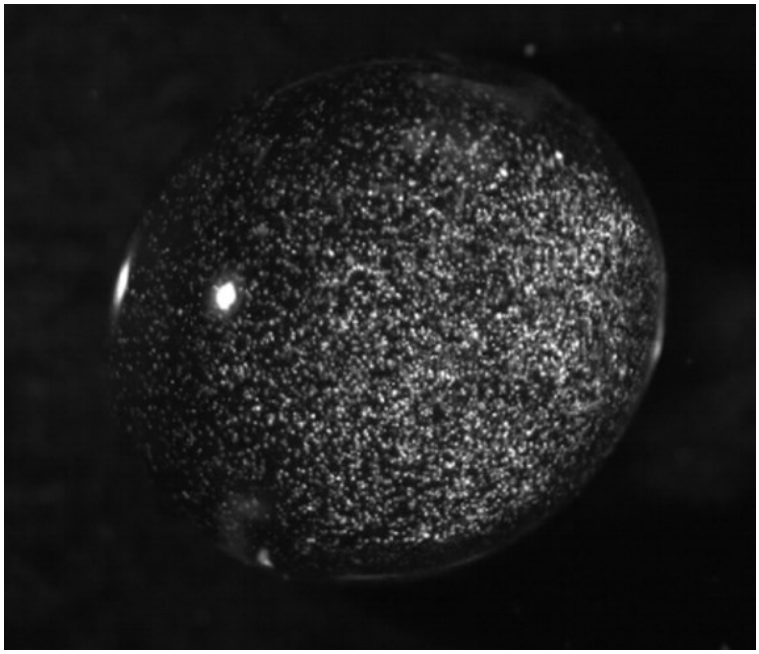


Figure 3. Fluorescent signal from a drop of water containing an algal culture, excited with light at 430 nm.

The window below shows an analysis of *Alexandrium* using the technique. Note the almost completely uniform and dark background where only organisms containing chlorophyll are visible.

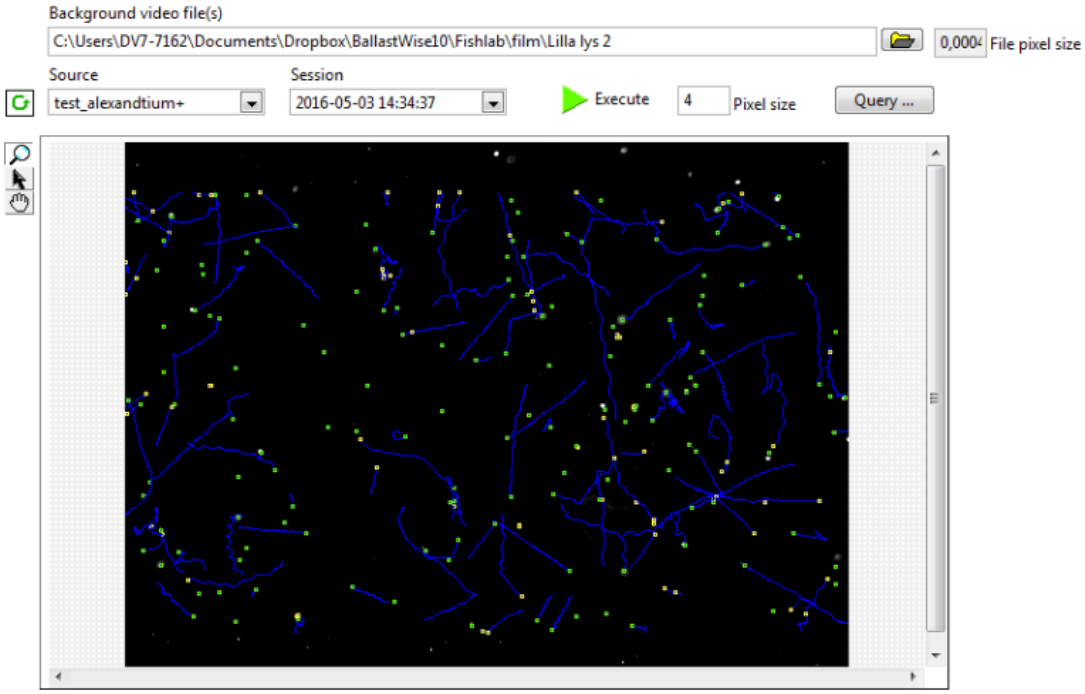


Figure 4. Analysis of a water sample with containing *Alexandrium* cells, as described in Fig. 1. Blue light was used, and all fluorescent organisms, containing Chl*a*, were detected. The blue trajectories are tracks from swimming *Alexandrium* sp. cells.

2.2 Choice of camera

This first prototype consisted of a high quality industrial video camera (1600 x 1200 pixels, 1/1.8” CCD sensor, 4,4um pixel size) and a zoom lens, which is specially designed to give a magnification of up to 1x. The magnification was set to 0.5x, which gives a pixel size of 8,8um.

A frame rate of at least 10 per second was possible to achieve under most circumstances. A glass cuvette of 2 mm thickness was used as a chamber to give a volume within the field of view of 1,4 x 1,1 x 0,2 cm = 0,3 ml. Illumination was by way of custom made miniatures strips, one on each side, and consisting of 3 purple diodes and 2 white diodes on each.

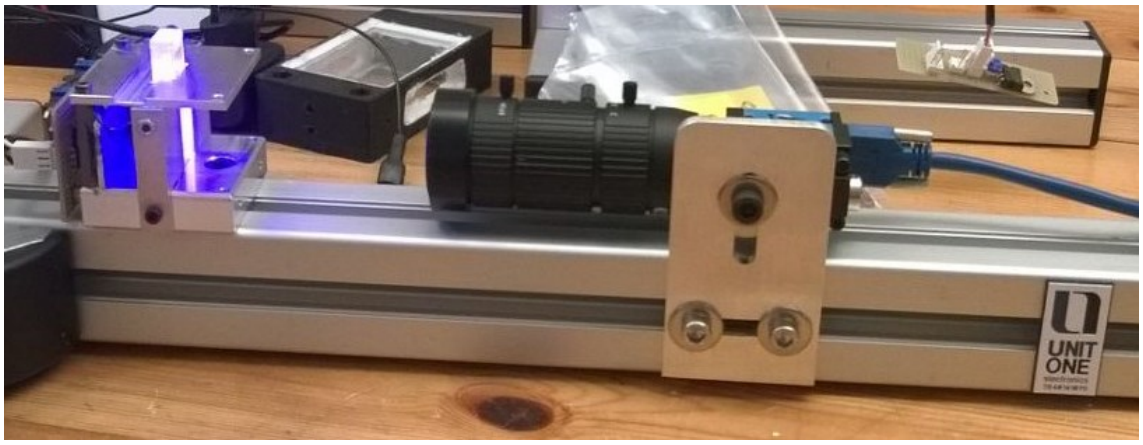


Figure 5. Setup of a simple test system with an industrial video camera (1600 x 1200 pixels, 1/1.8" CCD sensor, 4,4um pixel size) and a zoom lens. On the left side is the light source, and holder for a cuvette with the sample volume.

A first look at a pure culture of the dinoflagellate *Heterocapsa* (25-40um) at different concentrations showed that organisms of that size could be detected (see graph below). Emphasis was on looking at as large a volume as possible while still being able to detect organisms down to 10 um.

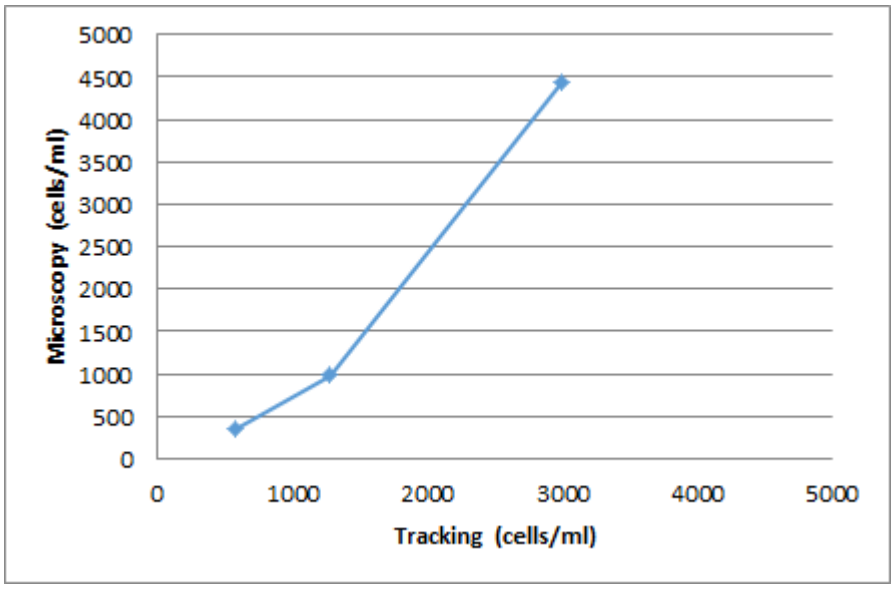


Figure 6. Automatic and manual counts of *Heterocapsa* cells. Video recordings for the automatic counts were made with the system shown in Fig. 1.

DHI started looking at ballast water samples from the ballast water test facility in Hundested and comparing the results from BallastWISE with manual counts using CMFDA staining (the accepted/approved method for detecting the 10-50 µm fraction). The first results are shown below using purple light only i.e. motile and non-motile organisms containing chlorophyll are detected. When concentrations were high, typically for inlet samples (before treatment), the correlations were good.

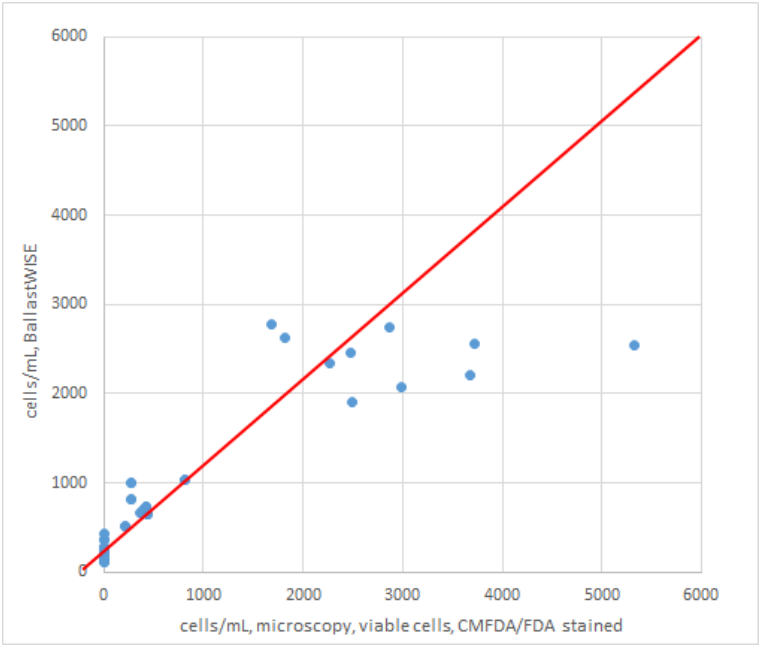


Figure 7. Manual counts and automatic counts of ballast water samples from the ballast water test facility in Hundested. For the BallastWISE analysis purple light was used, for counting autotrophic organisms only. The manual counts were counted using CMFDA staining.

However, at lower concentrations (typically in treated outlet samples), the CMFDA counts were much lower. One of the causes of this was that manual counters ignore organisms in the size range just below 10 µm, whereas BallastWISE, with a pixel size of 8,8 µm was not able to measure size accurately enough. Apart from this, however, it should be noted that chlorophyll content (detected by BallastWISE) and fluorescence from the CMFDA staining (enzymatic activity used by manual counters) is not expected to be closely correlated anyway.

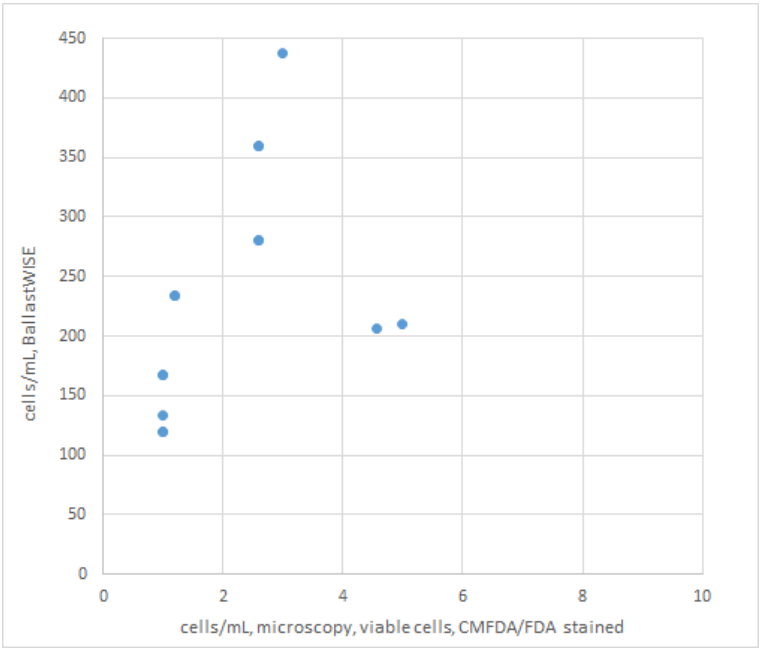


Figure 8. Manual counts and automatic counts of ballast water samples from the ballast water test facility in Hundested using purple light for the BallastWISE (automatic counts), and CMFDA for the manual counts. Automatic counts were significally higher than automated counts because the automatic method was not able to precisely measure the organism size at the given magnification.

2.3 Optical chambers

This prototype is basically the same as prototype 1, but with a higher magnification and smaller chamber. The chamber was replaced with a 1mm thick glass capillary. Magnification was increased to 1x giving a pixel size of 4,4um and the aperture was decreased to f/8 or f/II to give a larger depth of field. This decision was based on the assumption that a higher measurement precision is required. Plotting size distributions as the one shown below (distribution of organism area in um2) of ballast water samples consistently showed something close to a continuous size spectrum. Choosing the correct cutoff point in size to accurately get the size fraction >10 um i.e. >100 um2 resembles a kind of calibration because of the lack of extreme accuracy in size determination.

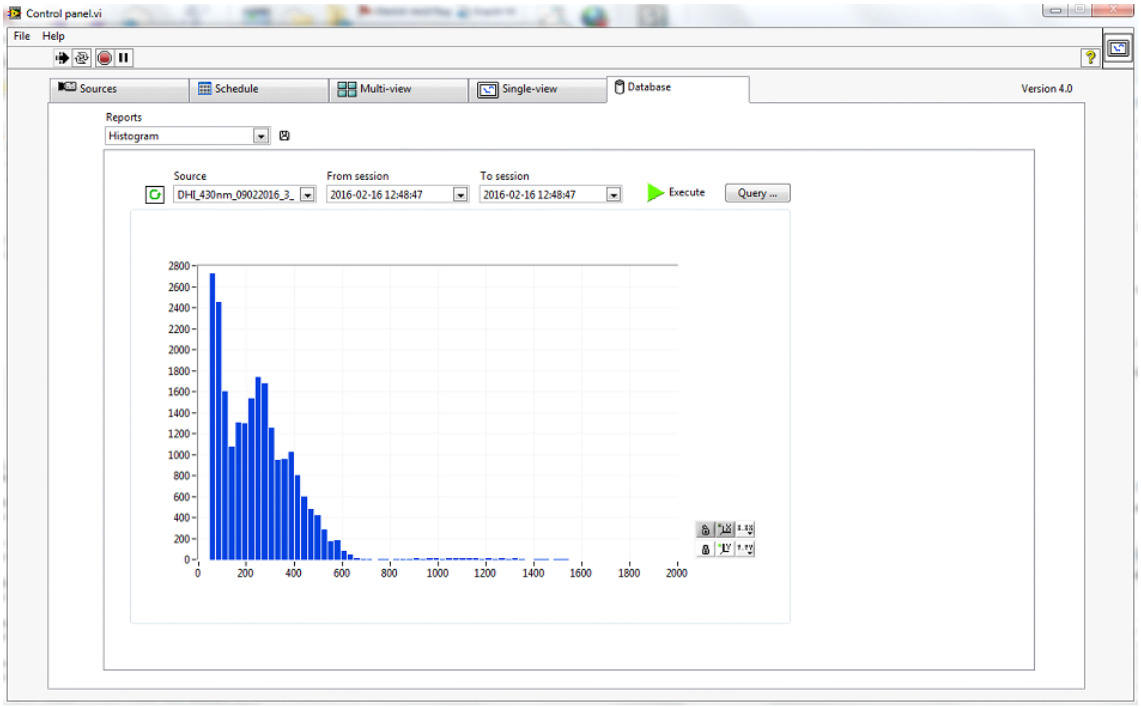


Figure 9. The organisms size distributions measured with BallastWISE prototype system. The distribution of the cell area in µm2 of organism from ballast water samples is shown.

A set of results from DHI of ballast water samples from the test facility in Hundested with a 300 µm2 area threshold are shown below. The 300 µm2 value was chosen as a cutoff point because of a halo effect around objects which makes them look bigger than they are. There are more detailed analysis on the size estimation problem to follow. The correlations have not changed much in relation to the tests with the lower magnification, and in particular, the CMFDA/FDA counts still show up much lower after treatment. It was decided to aim for yet higher measurement precision to resolve the problem.

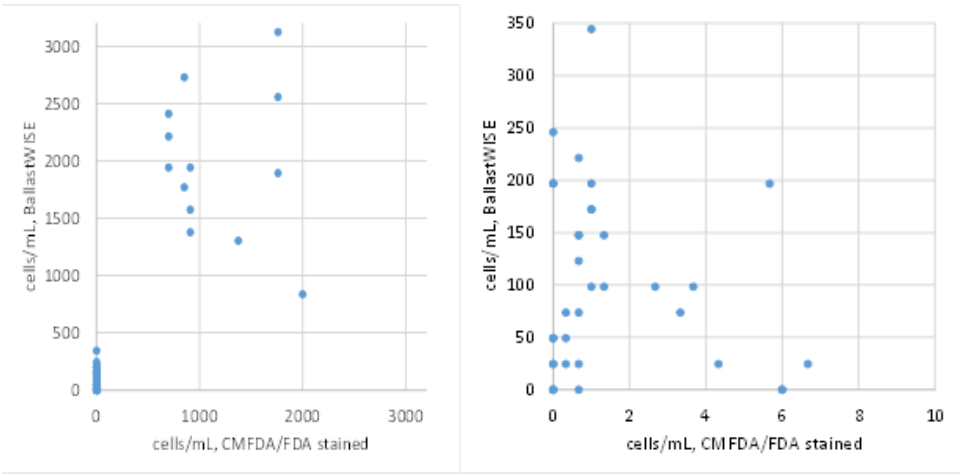


Figure 10. Comparison of manual and automated analysis of ballastwater samples where the automatic system had 1x magnification and pixel size 4.4 µm, and the manual counts were as described above. The BallastWISE counts included cells <10 µm due to inaccurate size measurements. Therefore the CMFDA/FDA counts still showed up much lower that the automated counts.

2.4 Precision optics

In order to increase precision of cell size measurements a new prototype was based on a Navitar 2x Magnistar bi-telecentric lens. This lens has the ability to adjust the aperture. Below is an illustration of the importance of stopping down to (preferably) f/II. Although cells can be seen at larger apertures, they become diffuse halos when out of focus and this creates false size estimations even though they can be tracked. At f/II, this effect almost disappears, as well as making sure that the whole depth of the chamber is in focus.

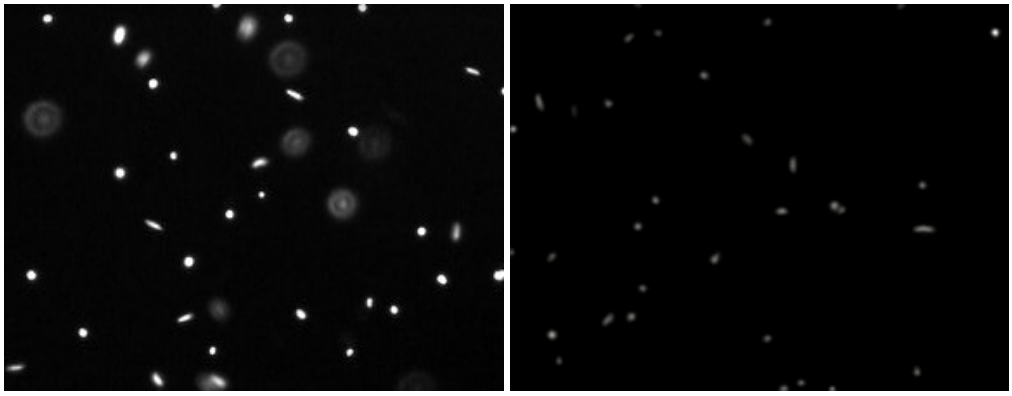


Figure 11. Left: Aperture f/4, showing the cells clearly with a bright halo around each cell, which results in a false size estimate. On the right side the aperture was f/11, which resulted in correct cell size estimates.

DHI analysed ballast water samples from the test facility in Hundested and close analysis of the original videos revealed again the problem of accurately determining the size threshold on the lower limit of 10µm (see below).

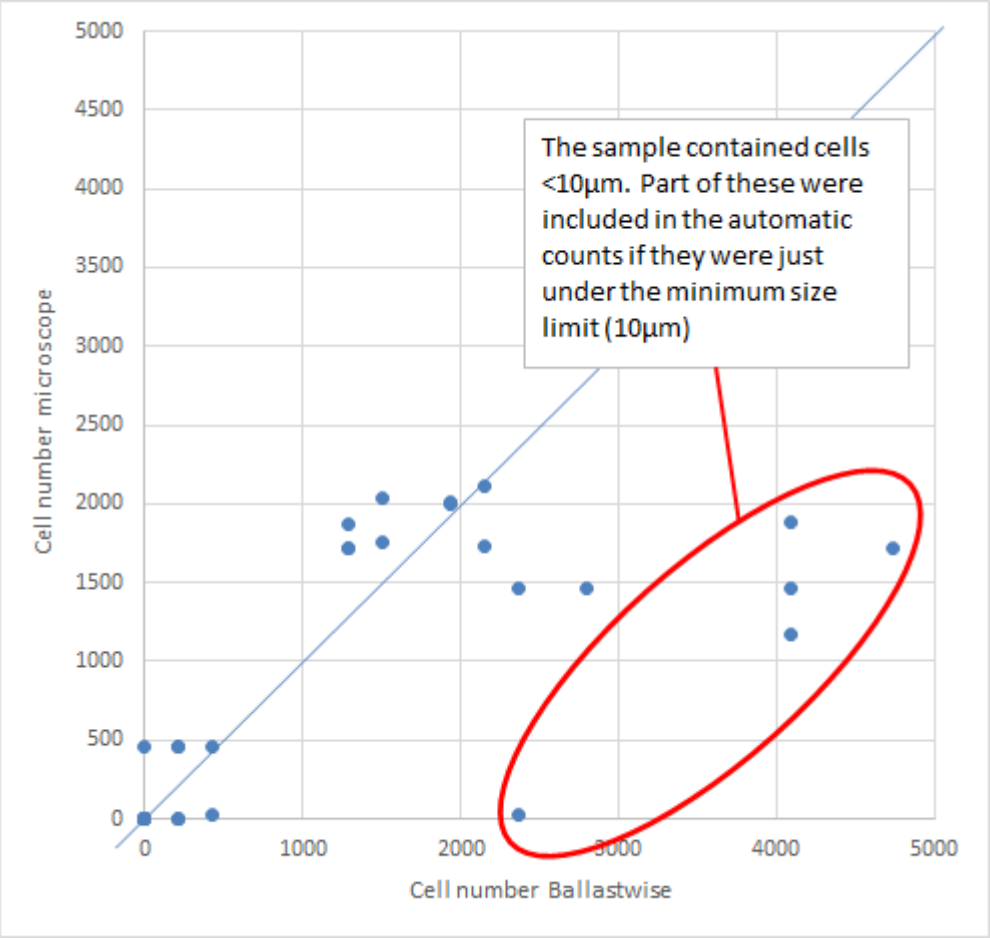


Figure 12. Analysis of ballast water samples from Hundested, where cells close to the minimum size limit of 10 µm were included in the automatic counts.

Fishlab has worked with cultures of a wide variety of organisms within the size range of 10-50 µm. Below are comparisons of sizes measured manually and with BallastWISE in white and violet light.

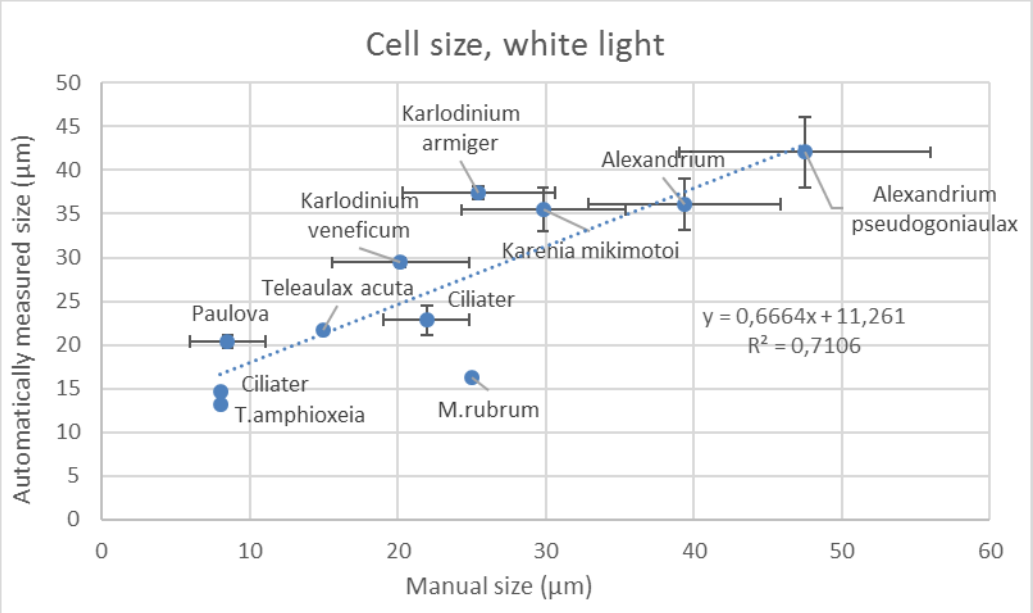


Figure 13. Comparisons of sizes measured manually and with BallastWISE system using white light.

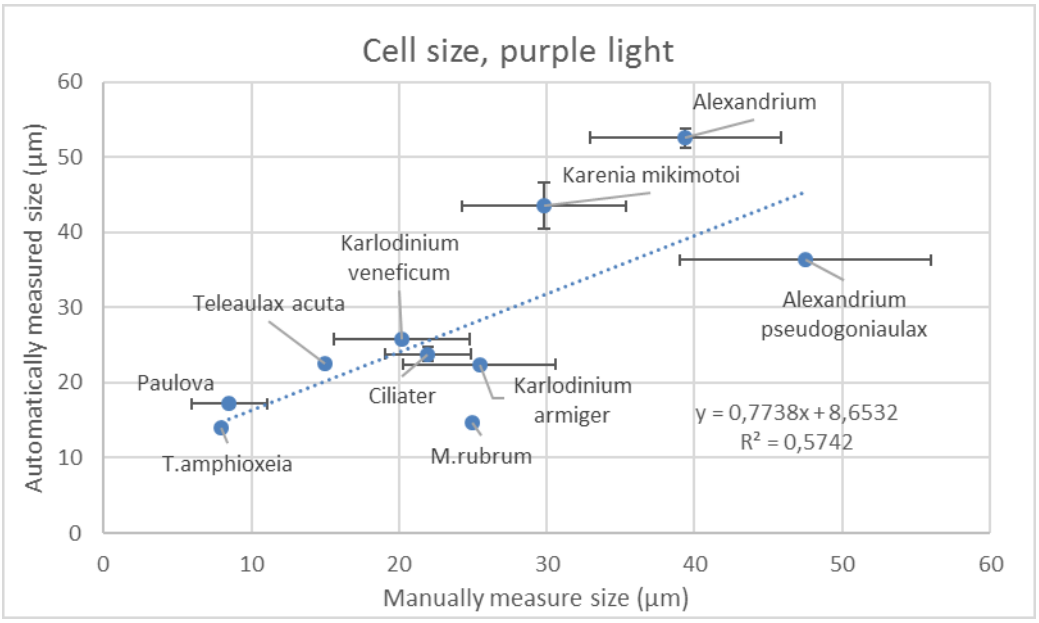
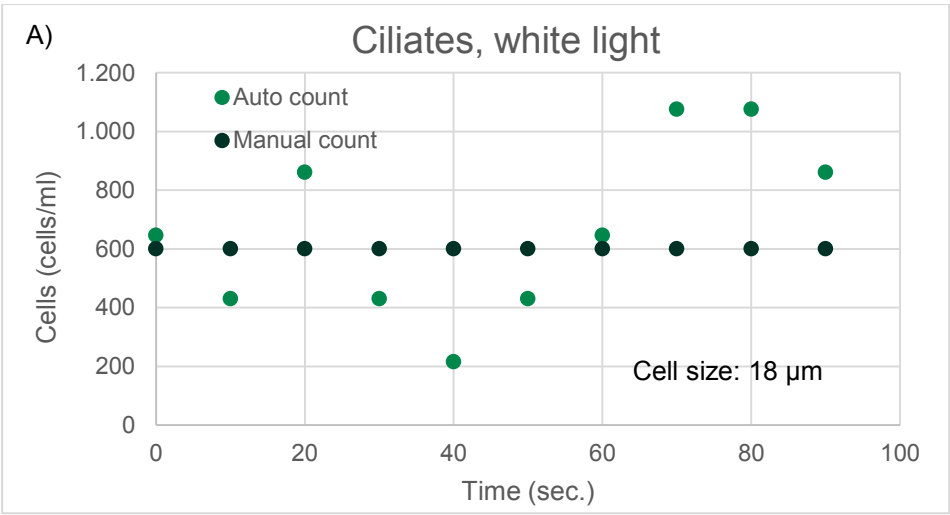


Figure 14. Comparisons of sizes measured manually and with BallastWISE system using violet light.

A number of analysis were performed over time in order to estimate the variation in Ballast-WISE estimates as well as to see what effect chamber residence time has on estimates. The next 4 plots are of larger organisms (15 – 25 µm), and it can be seen that BallastWISE finds them with reasonable accuracy and with relatively good consistency. The drop off with time is usually caused by cells settling on the walls of the chamber. Only swimming organisms were registered during these analysis.



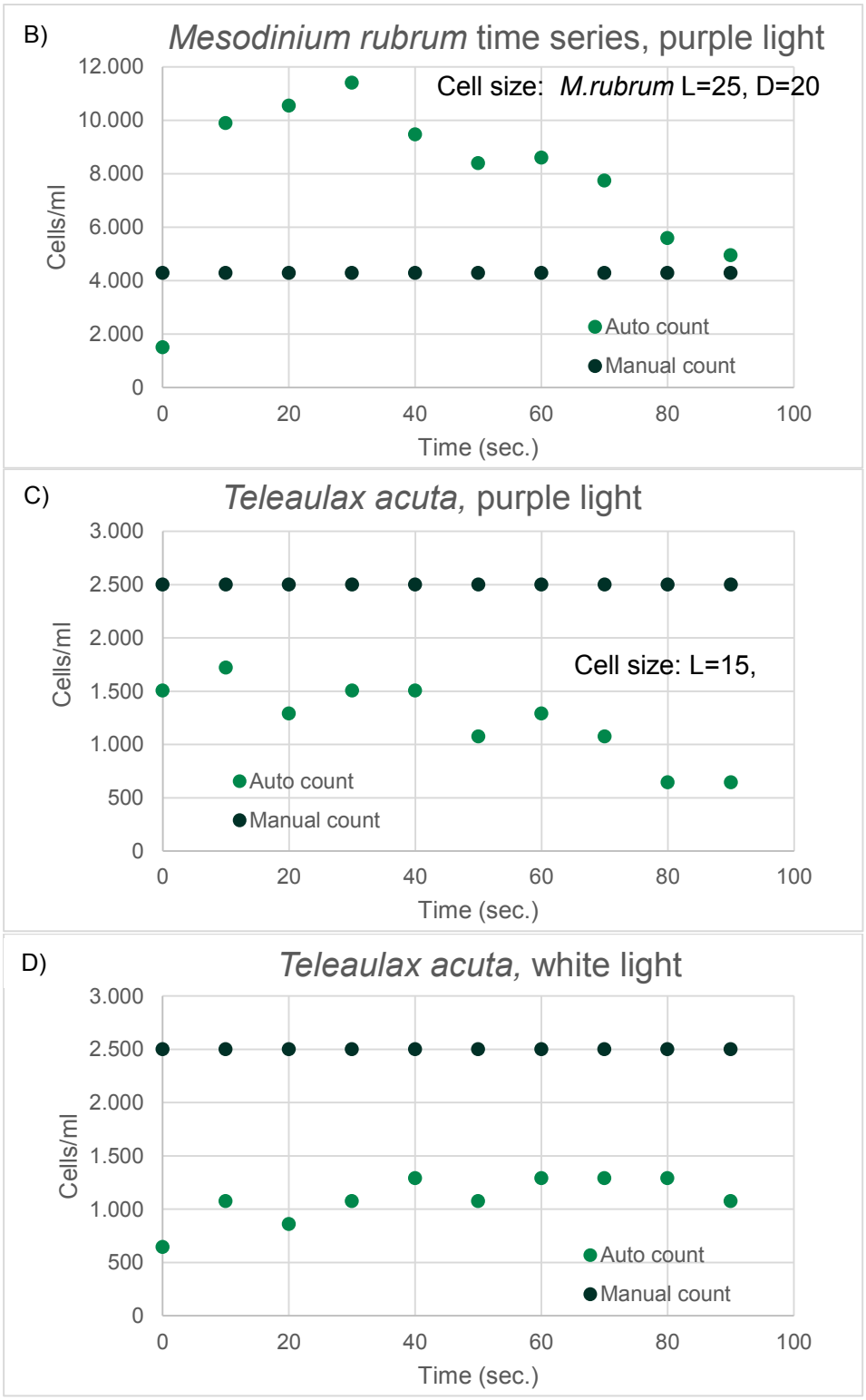


Figure 15. Automatic and manual counts of organisms, over a time span of 90 sec. with measurements every 10 seconds: A) Ciliates, using white light; B) *Mesodinium rubrum* using purple light; C) *Teleaulax acuta* using purple light; D) *Teleaulax acuta* using white light

The next 2 plots, show the ability of BallastWISE to reject cells that are below 10µm. A value of 200 cells/ml corresponds to just 1 cell per video sequence.

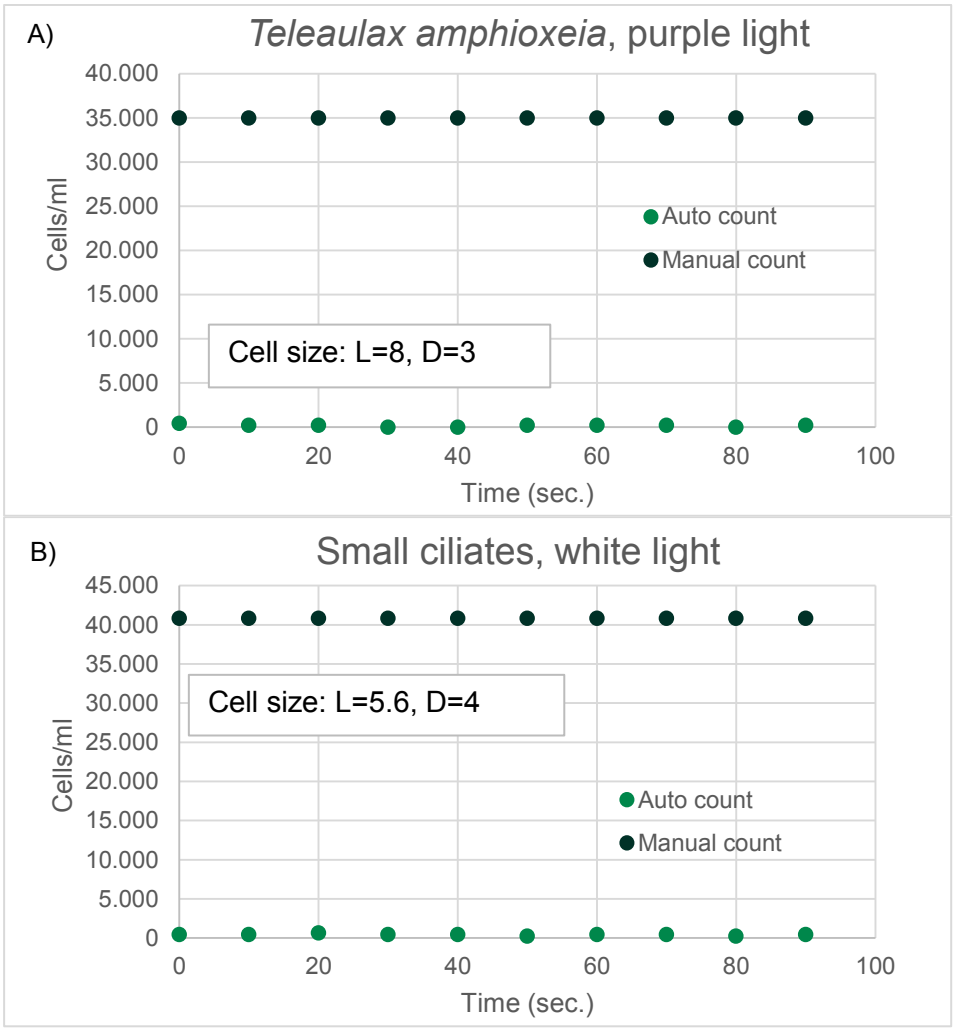


Figure 16. Automatic and manual counts of organisms smaller than 10 µm, over a time span of 90 sec. with measurements every 10 seconds. The cells were not included in the automatic counts due to their small size. A) *Teleaulax amphioxeia* (L= 8µm, D=3µm), using purple light B) Small ciliates (L= 6µm, D=4µm), using white light

2.5 Prototype construction

The final prototype that was constructed within the time frame of the project, consisted of the same camera as was used throughout and the Navitar Magnistar 2x bi-telecentric lens set at f/II. The lens has no focusing option so the camera is mounted on a miniature rack and pinion sleigh. A custom chamber fixture was machines from POM. It accepts a small interchangeable chamber, which is constructed as a sandwich. The top of the fixture connects to the two inflow holes in the chamber with an o-ring seal and the sample can be pumped through the system. However, we decided to replace the small chamber with a 0.7mm flat glass capillary with silicon tubes attached at each end in order to avoid having to clean the channels within the fixture top. This will be the disposable chamber solution for the time being.

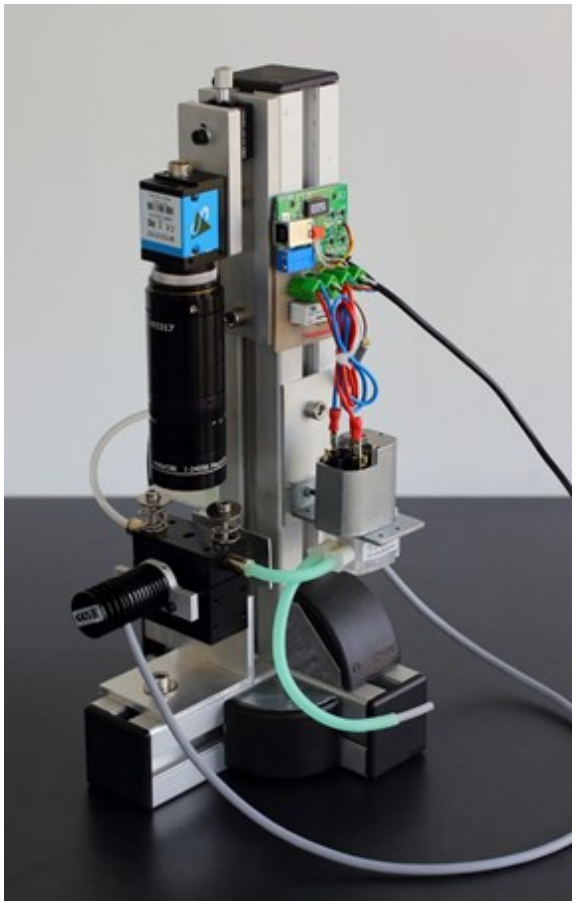


Figure 17. The final prototype, constructed within the time frame of the project, using the same camera as was used throughout and a Navitar Magnistar 2x bi-telecentric lens set at f/II.

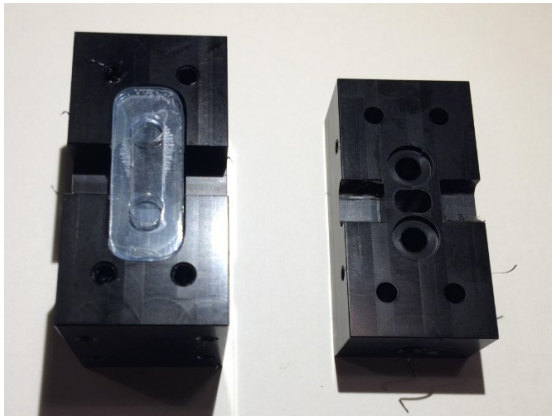


Figure 18. A custom chamber fixture machined from POM. The bottom part is holding a clear measuring chamber, the top part is shown on the right.

A small peristaltic pump creates flow from a sample reservoir through the chamber and out to an outflow reservoir. Custom designed electronics with an on-board microcontroller allow control of lighting and pump automatically from the main BallastWISE application. The small field of view of only 3,5 x 2,5 mm allows for a single and more powerful focussed LED rather than the small strip and this allows for a higher intensity of the purple LED, in particular, without transferring heat to the optical chamber.

The number of analysis with this prototype are limited, but both DHI and Fishlab have reported good usability. Below is a plot of ballast water discharge samples where BallastWISE and

manual counts show similar results, however concentrations are relatively high compared to concentrations in discharge in previous studies where viable counts were much lower.

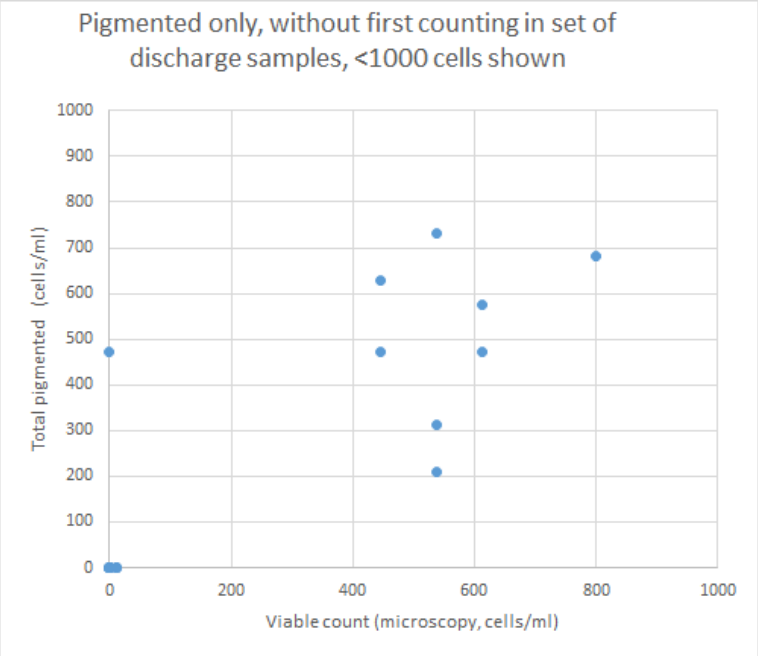


Figure 19. Plot of ballast water discharge samples where BallastWISE and manual counts using the using CMFDA staining show similar results.

2.6 BallastWISE software

The layout of the BallasWISE user interface is shown in Fig. 20. The large area at the left is the video display, which is live during filling, analysis, and emptying the optical chamber. This gives good visual feedback to the operator. The operator types in a sample description in the "Source" field, puts a sample in place, and presses the start button. The analysis will start and the indicators on the panel show how the analysis is progressing and how much time is remaining. It also shows the intermediate results of the concentration of motile organisms and the concentration of pigmented organisms after each field is processed.

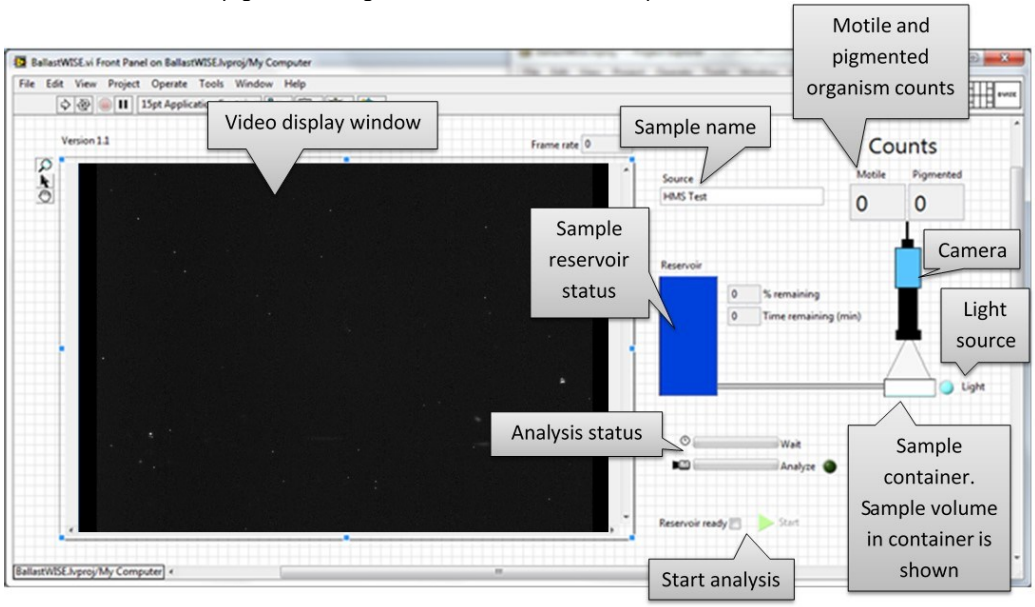


Figure 20. Layout of the BallasWISE user interphase.

2.7 Patenting of the BallastWISE technology

One of the objectives of the project was to investigate whether the BallastWISE technology is patentable, and if it is the case to apply for a patent.

A patent investigation showed that the technology is likely to be patentable. Therefore, the project partners decided to apply for a patent based on the present project (Ballast Water Investigation System (BallastWISE) and a previous project funded by the Danish Maritime Fund, where a system for quantification of organisms >50 µm was developed (Kontrol af ballastvand, project number 2013-064).

After the completion of the BallastWISE project, an international patent application for patent application was filed (MicroWISE, 2017).

3. Conclusions

The BallastWISE project succeeded in developing a system for rapid and automatic evaluation of the concentration of live organisms between 10 – 50 µm by detecting organisms that are motile and/or organisms which contain chlorophyll using image- and motion analysis.

Major findings during the project:

- It is possible for a modern industrial video camera to detect chlorophyll content down to cells sizes of 10um or less using a 430nm LED for excitation and a high pass filter of 500 nm placed in front of the camera
- The smallest moving organisms can be detected and tracked at 2x magnification
- Organisms can be fairly accurately measured by the software in order to determine whether they fit within the 10-50um range, but due to the continuous size distribution of organisms in typical samples, it can be difficult to give an absolutely precise estimate of cell counts with the size range
- The 2x magnification gives a volume with the field of view of 0,35 x 0,25 x 0,07 = 6µl, which means that 160 fields need to be processed in order to process 1ml of sample. However, an ongoing statistical analysis could cut the analysis short if feasible
- The analysis process can be fully automated and will take a maximum of 30 minutes but probably in the order of 10 minutes in practice
- The BallastWISE technology is patent pending

4. References

IMO, 2004, International Convention for the Control and Management of Ships' Ballast Water and Sediments, International Conference on Ballast Water Management for Ships, BWM/CONF/36, International Maritime Organization.

MEPC.173(58) – Guidelines for Ballast Water Sampling (G2) http://www.imo.org/en/KnowledgeCentre/IndexofIMOResolutions/Marine-Environment-Protection-Committee-(MEPC)/Documents/MEPC.173(58).pdf

MEPC 71/INF.25 Harmful Aquatic Organisms In Ballast Water. Development of a versatile methodology using Motility and Fluorescence Assays (MFA) to count viable organisms. Submitted by Denmark

MicroWISE (2017) A method and a device for quantifying living organisms and use of a device. PCT application number: PCT/DK2017/050131

Annex 1. MEPC 71/INF.25 (2017)

INTERNATIONAL MARITIME ORGANIZATION

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MARINE ENVIRONMENT PROTECTION COMMITTEE
71st session
Agenda item 4

MEPC 71/INF.25
28 April 2017
ENGLISH ONLY

HARMFUL AQUATIC ORGANISMS IN BALLAST WATER

Development of a versatile methodology using Motility and Fluorescence Assays (MFA) to count viable organisms

Submitted by Denmark

SUMMARY

Executive summary:	This document describes the principles and preliminary results of a method for evaluating the concentration of live organisms in a ballast water sample by detecting organisms that are motile and/or organisms which contain chlorophyll (MFA; Motility and Fluorescence Assay). The method which is entering its final evaluation is fully automated and can be used as a single assay by port State control officers to evaluate compliance with the D-2 standard. The same approach can also be implemented as part of BWMS to monitor efficiency during ballasting and deballasting operations
Strategic direction:	2
High-level action:	2.0.1
Output:	2.0.1.2
Action to be taken:	Paragraph 6
Related documents:	Resolutions MEPC.175(58), MEPC.252(67) and A.1088(28)

Introduction

1 The International Convention for the Control and Management of Ships' Ballast Water and Sediments, 2004 will enter into force in September 2017, and therefore the number of ships discharging ballast water that have to meet the D-2 standard will increase over the coming years.

2 Article 9 of the Convention stipulates that inspection of a ship may be carried out to verify the presence of an onboard valid Certificate; to inspect the ballast water record book, and/or sample the ship's ballast water in accordance with the Guidelines (G2). The Committee has subsequently developed the PSC Guidelines (MEPC.252(67)) to support the smooth

implementation of CME (compliance, monitoring and enforcement) activities. This document describes a method for estimating live organisms in the size fractions 10 to 50 µm and > 50 µm in accordance with the Ballast Water Management Convention.

3 The monitoring of the performance of a BWMS will become crucial because it raises the confidence that the treatment from type approved systems is adequate. Furthermore, the monitoring of this performance is crucial for the success of the implementation of the Convention.

4 MicroWISE has received support from the Danish Maritime Fund to combine existing technologies to finalize the development of a versatile monitoring system (BallastWISE, patent pending) which may be used for port State control as well as by technology developers/shipowners to evaluate compliance with the D-2 standard and treatment efficacy, respectively. BallastWISE was developed during two separate projects funded by the Danish Maritime Fund (Ballast Water Control System) and the Danish Nature Agency (BallastWISE). The projects were carried out by Bioras (Bioras.com), Unit-One (Unit-One.dk), Fishlab (Fishlab.dk) and DHI Denmark (https://ballastwater.dhigroup.com). The project results are available on the BallastWISE website (www.ballastwise.dk). MicroWISE is a company started by Bioras, Unit-One and Fishlab, which will carry out all activities related to BallastWISE.

Proposed supportive technology

5 The system is based on the expertise of MicroWISE to evaluate motility and fluorescence through assays for counting viable organisms in ballast water. The system described in the annex to this document has been tested through a series of studies carried out at DHI Denmark and is planned to be further evaluated by PML and DHI Singapore, all of which are members of the Global Ballast Water Test Organizations Network (GloBal TestNet). Preliminary results presented in the annex support the adequacy of the approach and confirm that combination of technologies can increase the reliability of testing ballast water for organisms in different size classes.

Action requested of the Committee

6 The Committee is invited to take note of the information contained in this document, in particular in paragraphs 4 and 5 and the annex.

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ANNEX

OVERVIEW OF THE MFA METHOD

Chambers, optics, and resolution

1 The MFA method involves viewing organisms in optical chambers using suitably dimensioned optics. The fraction of 10-50µm requires a resolution of approximately 2µm in order to determine organism size with reasonable accuracy. As an example of a suitable camera and optical configuration, a camera with 1600x1200 pixels and a pixel size of 4.4µm would require a 2.2x lens and a field of view of 3.2mm x 2.4mm. A realistic depth of field is 0.7mm which gives a volume seen by the camera of 5µl. The detection limit is a single cell and the statistical outcome depends on the number of analysis of the chamber volume. The dimensions for an optical chamber for viewing the fraction >50µm are approximately 70mm x 50mm x 20mm (L x W x H) giving a volume of 70ml. Using the same camera resolution as above, the pixel size is 40µm, and the detection limit is also a single cell. In order to detect concentrations as low as 10 per m^3, a filtration through a 50µm mask towards achieving a 1000x concentration would be preferred, but this method still enables testing of unfiltered samples. For both size fractions, a number of chamber volumes would be analysed from the same sample to give a statistically sound result.

Physical framework and analysis time

2 Several chambers volumes need to be analysed to give an accurate result. In order to achieve this, pumps are connected between samples and chambers in order to fill and empty them. Synchronization between sample flow and analysis is performed by the computer. Approximately 60 seconds is suitable for each chamber analysis for the >50µm fraction including a waiting time for the liquid to settle after filling. Only 20 seconds is required for the 10-50µm fraction. 14 analyses for the large- and 50 analyses for the small fractions give a combined analysis time of less than 30 minutes. The filling and measurements are done automatically.

Motion detection

3 Most heterotrophic organisms, and virtually all flagellates and ciliates, are motile. This observation applies to both size fractions. The content of the chambers are analysed on a time base of 10 to 25 frames per second in real time by a computer to which the cameras are connected. Each frame is analysed and individual organisms are detected and measured. The movement of each organism is tracked over time from frame to frame by the computer. In this way, it can be established whether each organism is within the size range and whether it is motile or not. The sequences are also saved as video files for documentation.

Chlorophyll detection

4 Some autotrophic organisms contain chlorophyll and are non-motile while some contain chlorophyll and are also motile. The presence of chlorophyll alone is an indication of a live autotrophic organism, because chlorophyll disappears quite rapidly after cell damage. Chlorophyll within organisms can be detected by recording fluorescence after stimulation at a certain wavelength (violet light at approximately 420ηm). The insertion of a high pass filter of approximately > 500ηm between the chamber and the camera means that only fluorescence is observed by the camera. Chlorophyll is often evenly distributed throughout an organism so that organism size is determined by the overall size of the chlorophyll organelles, which merge into each other to form a single fluorescent object. There are relatively few autotrophic species that

are >50µm so this method is mostly relevant for the 10-50µm fraction. This method allows detection and sizing of organisms that are still against a dark background as well as detection of motile fluorescent organisms.

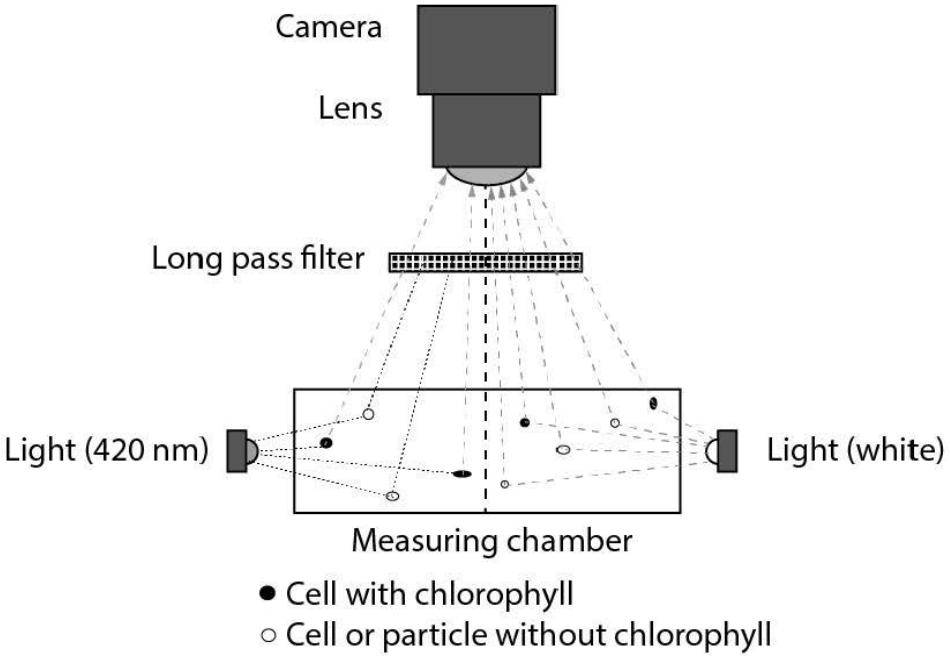

Figure 1: Camera, measuring chamber and lighting. The chamber is divided into two halves to show the two measuring principles. On the left side a 420 nm light shines into the measuring chamber, which excites chl*a*, resulting in emission of light at a higher wavelength from chl*a* containing organisms. This light is able to pass through the long-pass filter, and will be recorded by the camera. On the right side white light shines into the measuring chamber, and all particles and organisms that refract or reflect light are recorded by the camera. The long-pass filter only attenuates the white light marginally. The software processes these images to measure size, motility and presence of chl*a* for each individual organism.

Counting and detection limits

5 The system can detect single cells but the accuracy of counting depends on:

- the volume of the chamber
- the number of chambers that are analysed for a given sample
- the concentration of organisms that shall be detected

Simulations show that this accuracy for the 10-50µm fraction can be as high as 10 cells ± 30% with 95% confidence with a chamber volume of 30µl which is analysed 100x per sample. One litre or more of sample can be analysed for the >50µm fraction. The chamber is completely emptied between each fill and subsequent analysis, so the entire volume of the sample is actually analysed and the accuracy depends more on how the sample is collected and filtered.

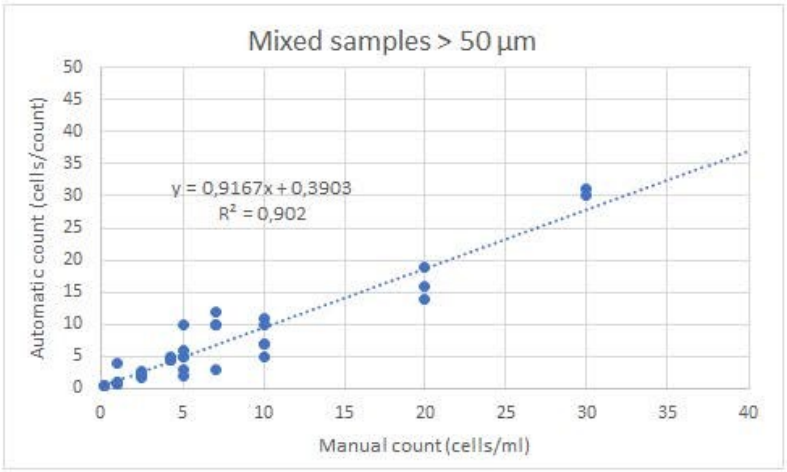

Figure 2: Results from the MFA method and manual counts of organisms > 50µm of specimens from cultures with daphnia, rotifers, ciliates and copepods.

Size determination

6 The resolution for the 10-50µm fraction is approximately 2µm and 40µm for the >50µm fraction, respectively, with current technology. Resolution can be improved with higher resolution cameras and optics so the configuration is a compromise between data bandwidth as well as cost. The relatively high resolution for the smaller fraction enables the system to determine which individual organisms lie within the given size limits with the given accuracy. Size determination becomes more accurate with increasing organism size so the lower resolution poses less of a problem for the larger fraction >50µm. Figure 3 shows size measurements with different species of organisms.

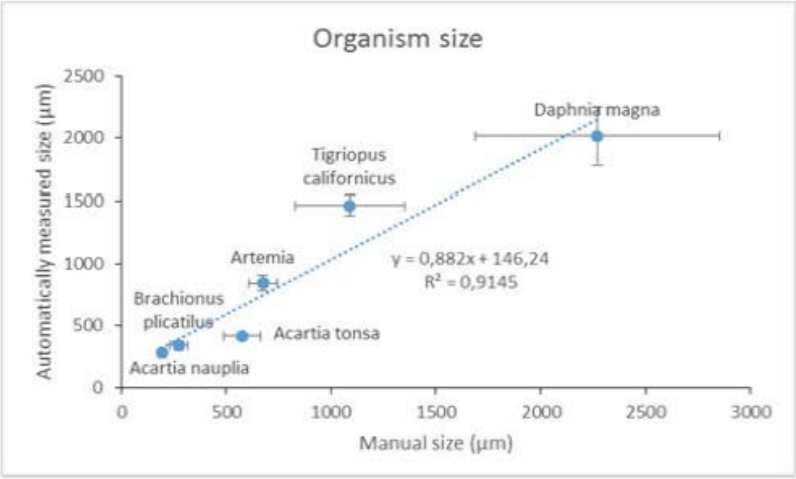

Figure 3: Comparison of manual and automatic measurements of organism sizes for different species and size classes in the range >50µm.

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Ballast Water Investigation System

The BallastWISE project succeeded in developing an automated system for rapid evaluation of the concentration of live organisms between 10 – 50 µm by detecting organisms that are motile and/or organisms which contain chlorophyll in an optical chamber and using image- and motion analysis. It is possible for a modern industrial video camera to detect chlorophyll content down to cells sizes of 10um or less using a 430nm LED for excitation and a high pass filter placed in front of the camera to remove the primary illumination. Movement of heterotrophic organisms is detected using white light. The smallest moving organisms can be detected and tracked at 2x magnification. Organisms can be measured by the software to determine whether they fit within the 10-50um range, but due to the continuous size distribution of organisms in typical samples, it can be difficult to give a very precise estimate of cell counts with the size range. Several measurements need to be performed on for each sample to give a statistically sound result. This is done by controlling flow through a pump and can take between 10 to 30 minutes per sample. The BallastWISE technology is patent pending.

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