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JERICO-S3 DELIVERABLE

Joint European Research Infrastructure for Coastal Observatories
Science, Services, Sustainability

DELIVERABLE #, WP# and full title	JERICO-S3 D5.6 - WP5 - "Best practices document for sampling procedures of biological automatic sensors (imaging-in-flow, automated flow cytometry and multispectral fluorometry)"
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TABLE OF CONTENT

TABLE OF FIGURES

1. EXECUTIVE SUMMARY

The definition of Best Practices for biological sensors stands as an acute need for the JERICO community, due to the multiplicity of procedures for calibration, operation, sampling and flagging. In the case of novel Biological Sensors, they help to understand plankton dynamics and distribution in coastal waters. Our task within WP5 ST7 is to work on the improvement of the readiness of ship-based and autonomous platform observing networks, guaranteeing their robustness, reliability, and long-term sustainability by working on common operational procedures that we could define together and rely on.

This document describes the results of the three polls launched on the JERICO-S3 partners and out of the consortium about three main novel (semi-)automated techniques for observing marine phytoplankton. It reports on the discussions carried out about operational practices summarised from these three polls, during three Workshops on Best Practices for Biological Observations organised by CNRS and CEFAS and held in 2021 and 2022. In addition, we present briefly and discuss the operational practices for sampling procedures related to some targeted innovative biological sensors gathered from various sources. This deliverable therefore seeks to frame these notions in the context of holistic coastal research led by JERICO, for the benefit of the wider scientific community.

The first section details the challenges currently faced by marine microbiologists, and the associated issues and solutions. Three polls on three techniques for monitoring plankton were launched and advertised amongst the JERICO community and beyond. We rely on discussions carried out during the corresponding international online workshops, based on the results from dedicated polls, to define some guidelines for defining best practices for the use of three groups of techniques at high spatial and temporal resolution.

An example of relevant biological sensors (including *in situ* imagers, single-cell characterisation devices, or bulk measurement probes) is then briefly described, with associated Best Practices for calibration, maintenance, data collection, and metadata flagging (even though this last part, as well as the description of the sensor, are already described in previous deliverables and publications).

2. INTRODUCTION

In the rapidly advancing realm of marine environmental monitoring, biological sensors now play a pivotal role in unravelling complex ocean processes, especially in the coastal ocean, from the shore to the continental margin (estuarine, coastal and shelf ecosystems). The Joint European Research Infrastructure Consortium (JERICO) stands as a leading force in fostering collaboration and innovation in the field of marine coastal observatories. In an era of increasing environmental challenges, the need for standardised and efficient monitoring tools becomes increasingly evident, especially for biological data.

Biological sensors are designed to capture and interpret intricate biological signals associated with planktonic cells and organisms within aquatic ecosystems and especially small phytoplankton cells. The interest in best practices for operating and deploying these sensors correspond to the ongoing efforts within JERICO S3 towards measuring synchronously different environmental variables (especially biogeochemistry and biology) at high frequency and spatial resolution and filling observational gaps in under-sampled areas or periods. This will help to understand plankton dynamics and distribution in coastal waters. Our task within WP5 ST7 is to improve the readiness of ship-based and autonomous platform observing networks by guaranteeing their robustness, reliability, and long-term sustainability, by working on common operational procedures for the use of innovative automated sensors, that we could define together and rely on.

These sensors offer a nuanced understanding of marine microbial life. However, to harness their full potential and ensure the reliability and comparability of data across diverse coastal marine environments, the establishment of best operational practices for biological sensors within the JERICO framework is imperative.

This call for standardised operational practices is not merely a technical requirement, but a response to the urgent demand for comprehensive, high-quality, interoperable data (Findable Accessible Interoperable Robust – F.A.I.R.) that can inform robust policies and management strategies. The data management and pipelines to common repositories is explored in the frame of WP6 and corresponding deliverables).

The variability in sensor technologies, deployment methodologies and data processing techniques bring challenges in achieving the desired level of consistency needed for meaningful cross-site comparisons, upscaling of data and the development of a unified understanding of marine ecosystems.

In this context, the formulation of Best Practices for biological sensors under the leadership of JERICO becomes a strategic initiative. These practices aim to harmonise methodologies, calibrations and data processing protocols, fostering a cohesive and collaborative network of observatories across Europe. By establishing a common framework, JERICO can enhance the reliability, reproducibility, and interoperability of biological sensor data, ultimately strengthening the scientific foundation upon which informed decisions about marine ecosystems are made. The establishment of Best Practices for biological sensors within JERICO becomes not just a technical necessity, but a shared commitment to advancing our understanding of marine ecosystems.

JERICO-S3 aims to establish an integrated scientific approach for the monitoring of coastal ecosystems from the angle of regional and local specificities. In this context, WP5 is concerned with the harmonisation of the many types of measurements currently being

carried out in coastal research. While some parameters benefit from a well-defined workflow and a relatively good spatial coverage resulting from past needs and investments (typically physical data, initially of military use), other parameters are much more subject to harmonisation and standardisation issues (Estes et al., 2021).

This is particularly the case for microbiological data, which until recently consisted of only a few parameters of interest, cheap and easy enough to measure in the field (*e.g.,* Chlorophyll-a concentration), and simple enough to interpret in the context of physico-chemical studies or fishing campaigns. Because plankton are at the base of marine food webs (Fenchel, 1988), and because they can influence both higher trophic levels and their environment (albeit to a limited extent, see Volk & Hoffer, 1985), such basic knowledge is often not sufficient to understand the subtleties of their dynamics. Until recently, taxonomists provided the most detailed information on the composition of plankton samples. However, their work was limited to the largest cells and required a significant investment of time by a trained taxonomist with their own biases (Kovalenko & Reave, 2022).

DNA-based methodologies, such as environmental DNA, have been developed since the 2000s and have proved useful in assessing marine microbes' diversity and physiological potentialities (Simon et al., 2009; Dutkiewicz et al., 2020). However, such methods do not provide quantitative information and give limited insight into marine microbial ecology.

A deeper understanding of plankton dynamics calls for much more complexity in harvested data, as well as tighter spatio-temporal coverage. To cope with these emerging requirements, marine biologists have sought innovative sensors, often developed on a case-by-case basis according to the vision of a particular individual or research group. As the technology that emerged in the field did so in a peppered way, to cover different scientific challenges and activities depending on local contexts, there has been no pan-European, federated standardisation effort to date.

This has become a significant issue: the upscaling of microbiological data to wider areas clearly requires common procedures for sensor calibration, operational aspects, measurement settings, data analysis and data management. The present deliverable deals with the first three notions as data analysis, especially data management, is considered in WP6) and tries to cover relevant microbiological sensors for coastal research. The aim is to discuss and go towards a better definition of operational Best Practices for these sensors to properly monitor the planktonic compartment in the context of a holistic appraisal of coastal ecosystems.

The various sensors hereby explored cover an extended range of biological data, from picophytoplankton to zooplankton deployed within the frame of the JERICO consortium (see Deliverable 5.1) and abroad. These sensors can be classified into 3 groups according to the type of information they provide:

- *In-flow* devices (benchtop or *in situ*, including single-cell/particle optical analysers and/or imaging-in-flow devices)

An inner or external pump brings the sample from a flask or a sub-sampling device, by the use of a second and more powerful pump, from under the vessel, up to a flow cell where the analysis takes place. These sensors use lasers and detectors (automated cytometers) to measure and/or trigger on optical properties of individual cells or colonies (*e.g.*, size, fluorescence) followed (or not) by a camera that takes microphotographs of each cell/colony

selected by the triggering protocol. They can also directly take all images that flow through the system (they are then only considered as in-flow imagers.

They are commonly used for relatively fast, high-throughput analysis of microbial populations, providing both quantitative (estimation of population abundance) and qualitative (estimation of cell properties) data (automated flow cytometers). Such sensors allow the characterisation and sorting of cell types based on arbitrary or predetermined mathematical criteria according to the distribution of populations of cells and/or correspondence with sorting systems and taxonomical/molecular identification of microorganisms. In-flow imagers are mostly based on morphological recognition of species and on calculating features derived from images.

- *In situ* imagers

These devices record real-time or even time-lapse images directly *in situ*, without the need for a sampling device. They are used to study the spatial distribution, behaviour, and interactions of microorganisms in their natural habitat. They can also be used to monitor changes in microbial communities, spatial patterns and of responses to environmental changes without disturbing natural conditions.

- *Bulk measurement optical analysers*

This category includes single- or multispectral fluorometers and spectrophotometers that can be measured both in the field (*in situ* casts) or on underway measurements from subsurface pumped waters (FerryBox), see JERICO-S3 D5.1 "Catalogue and checklists for existing biological sensors that will be implemented in JERICO-S3" for more details.

Each of these devices has a specific bias that the operator and data analyst must be aware of. They can also be sorted. Moreover, the information collected by each approach is often complementary and can be combined in integrated observatories for addressing the whole plankton size range (including picoplankton and low-concentrated colonial forms), as stated in previous reports (Karlson et al., 2017; Artigas et al., 2019) and publications (Lombard et al., 2019).

The present work aims to discuss the various operational and calibration procedures that need to be standardised across all sites part of JERICO concerning 3 main techniques providing data on functional and/or taxonomic diversity of phytoplankton (and also zooplankton): i) single-cell/particle optical analysis (automated Flow Cytometers), Imaging systems including both Imaging in-flow, including also automated Flow Cytometers even though most of them do not release any data on optical features (except the CytoSense/CytoSub); ii) *in situ* imaging-systems, and iii) bulk optical devices, mainly multispectral fluorometers.

To address that, three polls were built and discussed among the WP5 ST7 participants, launched in late 2020 and late 2021 (see Annexes 1, 2 & 3) and advertised on the JERICO website (www.jerico-ri.eu), social media, and also sent to the whole JERICO community and beyond, including international working groups as the ICES Phytoplankton and Microbiology Working Group (WGPME).

After receiving the results from scientists' operational procedures established in those polls, we organised 5 online international workshops: two of them (March 9 and 12, 2021) dealt with operational procedures and data treatment/management/pipelines (see D6.5 for this second workshop concerning WP6 activities). The second series of Workshops took place

by June 28 and July 7 and focused on Imaging devices for phytoplankton and zooplankton, and were followed by a hands-on GlobalHAB workshop in Kristineberg hosted by University of Götenborg and SMHI, where three types of automated imaging flow cytometers were tested and compared in both cultures and fjord samples. Finally, by September 9, 2022, we organised the last workshop on in vivo fluorometry.

From the discussions carried out during the workshops, as well as considering further work as the one proposed in the frame of workshops that followed in the frame of WP11 as the one in Villefranche-sur-Mer on imaging data (at CNRS LOV, June 2023) and recently the one on Flow Cytometry operational practices for data fairness (TT-Cyto Workshop, at CNRS LOG, June 2024), recommendations are made, considering operational practices and flags to be implemented in the metadatabase (handled by JERICO-S3 WP6, see corresponding deliverables).

The content is slightly different from set expectations, as no specific recommendations are made to cover the specificities of the different Integrated Regional Sites (IRS) and Pilot Super Sites (PSS), and the different platforms distributed and operated within the current network.

Nonetheless, this document is intended to set a frame to consolidate the establishment of operational best practices for the numerous innovative biological sensors that are used by the JERICO consortium (see D5.1) and beyond. It will be further amended in the future, as more collaborative activities and processes are initiated, in the frame of new projects and, if the 2026 ESFRI Roadmap application is successful, within the Research Infrastructure.

3. Operational Practices for the application of automated Flow Cytometry

3.1.Results from the dedicated questionnaire

3.1.1. General information

A questionnaire on best practices in flow cytometry was launched by late 2020 and announced by email to dedicated international working groups, such as the Working Group on Phytoplankton and Microbial Ecology (WGPME) of the International Council for the Exploration of the Sea (ICES), on the JERICO website (www.jerico-ri.eu) and on social media.

Two workshops were organised by CEFAS and CNRS on March 9 and 12, 2021, in which both operational and data-related issues were discussed, from the results of the surveys received, taking into account previous workshops, projects and experience gathered.

Nine scientists from seven countries responded to the survey on Flow Cytometry (FCM). Protocols from 16 machines were described, 5 from France (CNRS-LOG, MIO and LOV), 3 from the Netherlands (RWS), and one for each of the other countries and laboratories (VLIZ, CEFAS, SYKE, SZN, HCMR, Bigelow, considered (Figure 1).

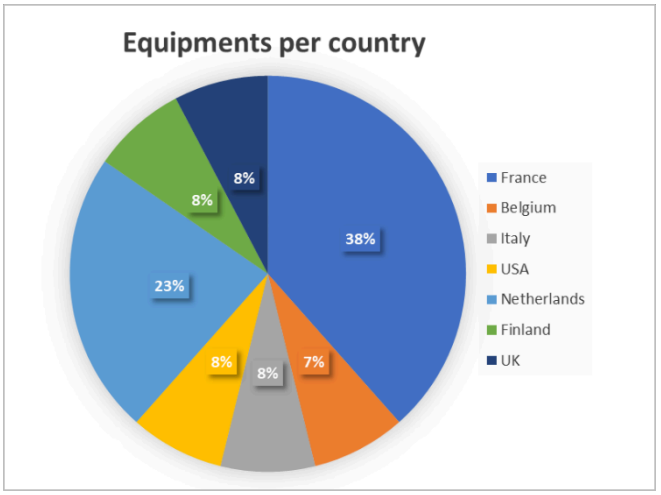

Figure 1 : Equipments (Flow Cytometers) per country (results from the FCM Best Practices poll)

3.1.2. Description of the operational equipment

The respondents work mainly in the marine environment, even though two laboratories (SYKE and RWS) deal with brackish or freshwater phytoplankton monitoring in the Baltic Sea and in Dutch freshwater systems. The machines deployed are either CytoSense/Sub instruments from Cytobuoy (NL) equipped with a camera recently (< 5 years) acquired (since 2016 in 2021) and two still deployed machines acquired more than ten years ago (with or without an imaging-acquisition inner device, optional for this type of machines).

All machines are equipped with at least a blue laser targeting mainly chlorophyll *a* and the standard two filters of 0.1-1.4 µm or 0.1-0.1 µm of porosity allowing the recycling of the sheath fluid, even though some also integrate red lasers or green lasers targeting specifically phycoerythrin and/or phycocyanin for an improved detection and discrimination of filamentous cyanobacteria in brackish or freshwaters. The power of the laser is 50 mW but on one machine laser power goes up to 100 mW for detection of the smallest picocyanobacteria (*Prochlorococcus*) in oligotrophic marine systems.

3.1.3. Deployment

The majority of the instruments are deployed on a research vessel (55%) or ship of opportunity (17%) measuring samples from a circulating continuous underway water supply. No specifications were given about the type of pump used and differences exist according to the vessels considered. The rest of the machines are deployed either on a buoy/mooring fixed station or on the laboratory (Figure 2).

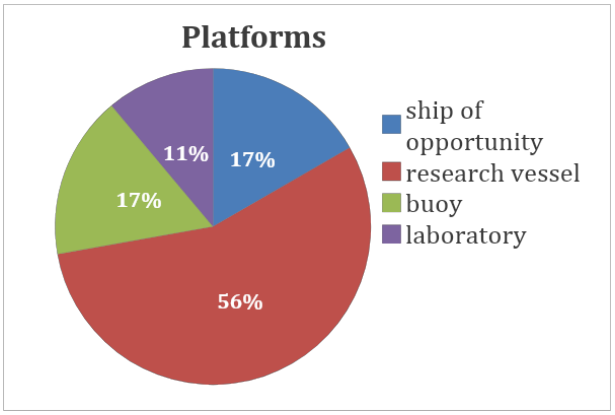

Figure 2: Platforms where Flow Cytometers are deployed (results from the FCM Best Practices poll)

Regarding the frequency of deployment, 22% of respondents use their instrument daily, 21% weekly, 43% monthly, 14% yearly on dedicated cruises (Figure 3).

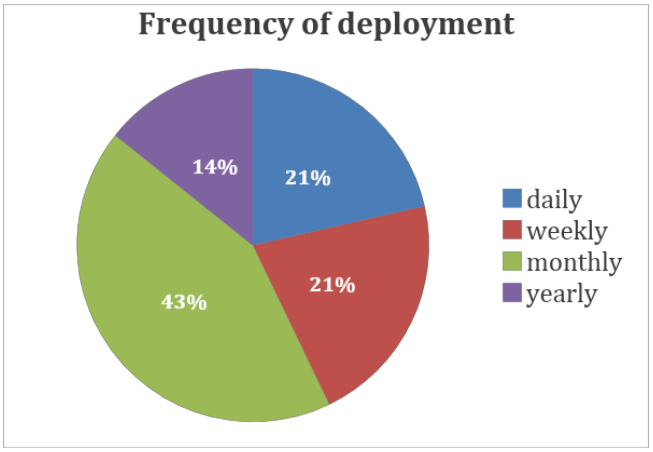

Figure 3: Frequency of deployment of flow cytometers (results from the FCM Best Practices poll).

3.1.4. Maintenance

Most respondents perform an annual revision with the manufacturer's company, where specific parts are replaced during maintenance. In parallel, most of the respondents carry out regular maintenance themselves when it is necessary, in addition to the company's maintenance, according to their use (Figure 4). All respondents keep a log book.

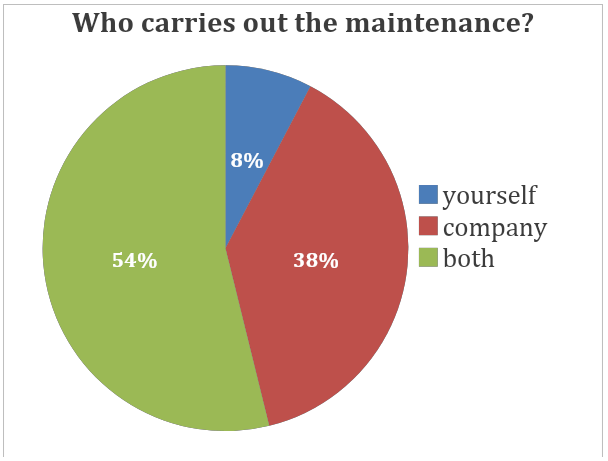

Figure 4: Maintenance of the devices (results from the FCM Best Practices poll).

The frequency of maintenance varies according to the deployment frequency and goes from bi-yearly to once every two years.

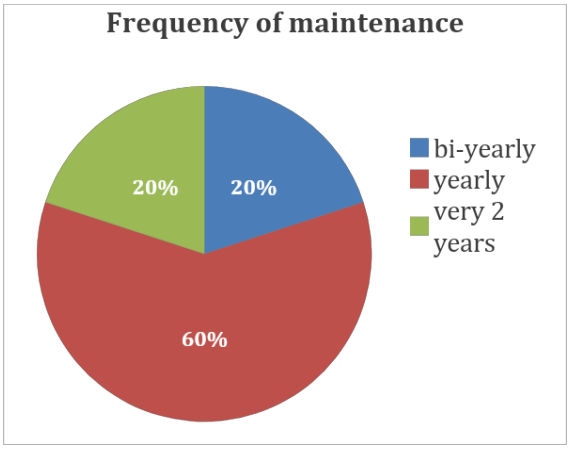

Figure 5: Frequency of maintenance of FCM (results from the FCM Best Practices poll).

The most regular maintenance comprises the replacing of both sheath fluid and sample tubing (Figures 6 and 7) on a yearly basis (57% and 63%, respectively), whereas 29 and 25% of the respondents change them when needed and some do never change them and leave that decision to the company when sending the machine to the maintenance.

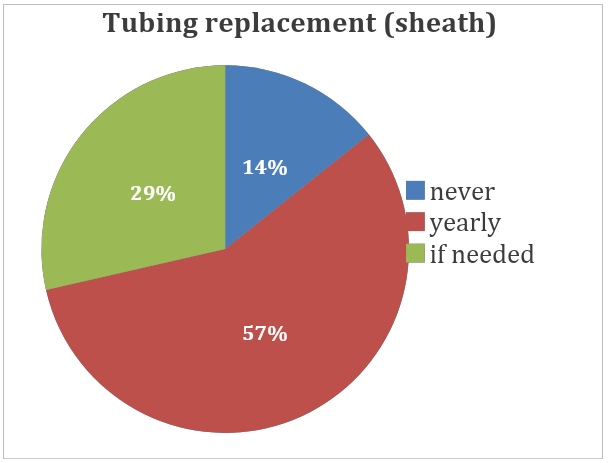

Figure 6: Sheath fluid tubing replacement for FCM deployments (results from the FCM Best Practices poll).

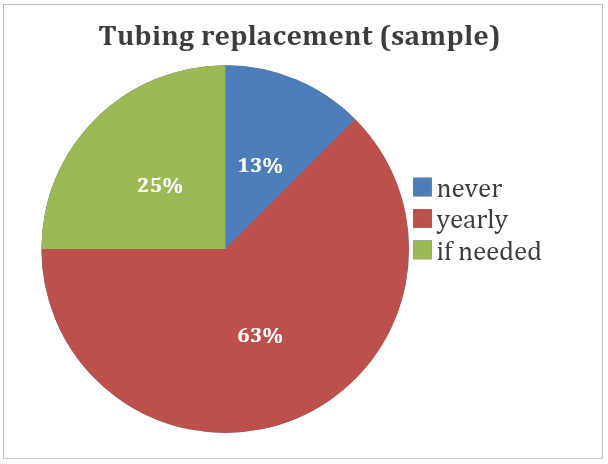

Figure 7: Sample-tubing replacement for FCM deployment (results from the FCM Best Practices poll).

The filters are changed yearly for 63% of respondents (Figure 8), bi-yearly for 12%, whereas ¼ of them change it only when needed (when the sheath fluid gets too dirty)

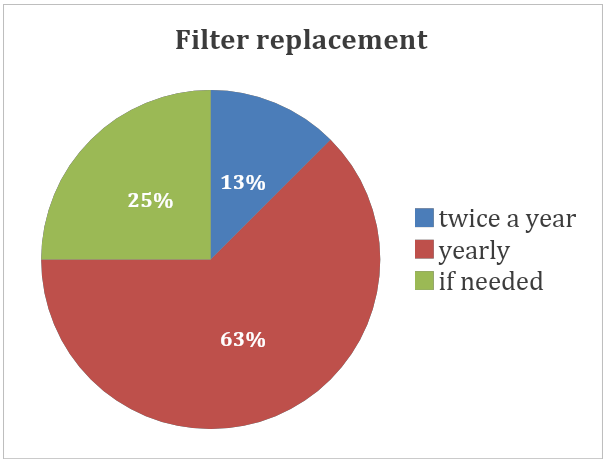

Figure 8: Filter replacement for FCM deployment (results from the FCM Best Practices poll).

3.1.5. Operational Procedures

Measurements are performed following different approaches. First of all, most scientists perform 3 protocols mainly dedicated to the three size-classes of phytoplankton, pico-, nano- and microplankton or including two size classes (pico- and nano-micro) and a dedicated protocol for imaging (adapting the speed of the inner pump and the length of the measurement).

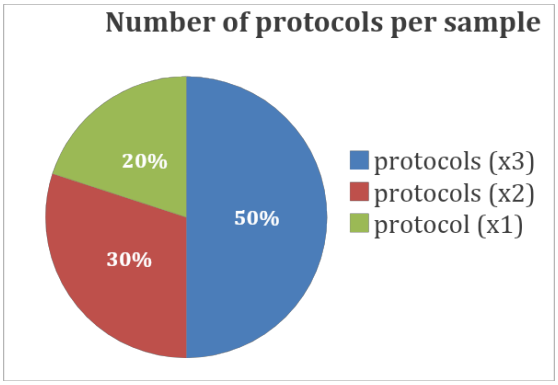

Figure 9: Numbers of protocols applied for each sample (results from the FCM Best Practices poll).

Measurements are carried out on an hourly basis for 59% of deployments, whereas 1/3 of them perform measurements down to 6 times per hour and 8% on a weekly basis.

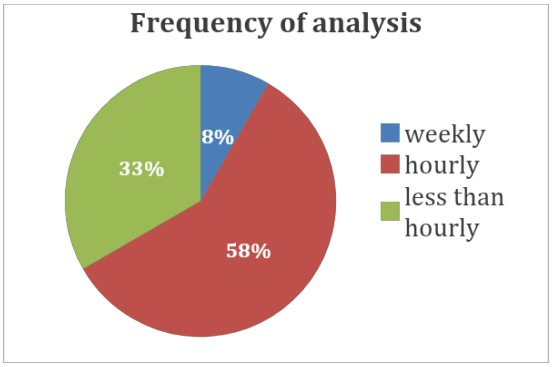

Figure 10: Frequency of FCM measurements (results from the FCM Best Practices poll).

Another step to be clarified concerns the way the machine takes the sample for measurements: pumping the sample either on a discrete basis on isolated samples (pinch-valve if connected to a continuous system) or if the sample is taken directly on a running flow through the chamber and therefore the sample integrates over the duration of the sampling.

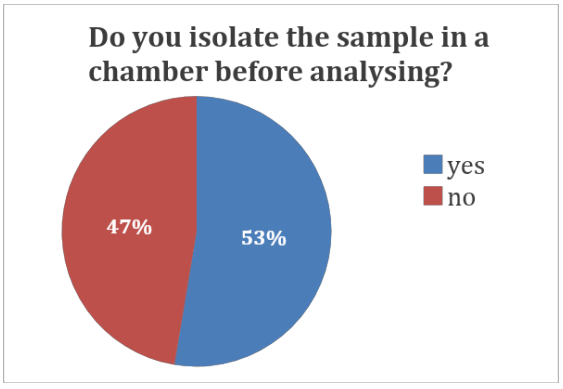

Figure 11: Strategy for sample analysis from sample collection (results from the FCM Best Practices poll).

Calibration (or check) of the sample pump flow is carried out either yearly (37%), every 1 to 3 months (27%) or on a yearly basis, and only 9% recall on the maintenance at the manufacturer's headquarters.

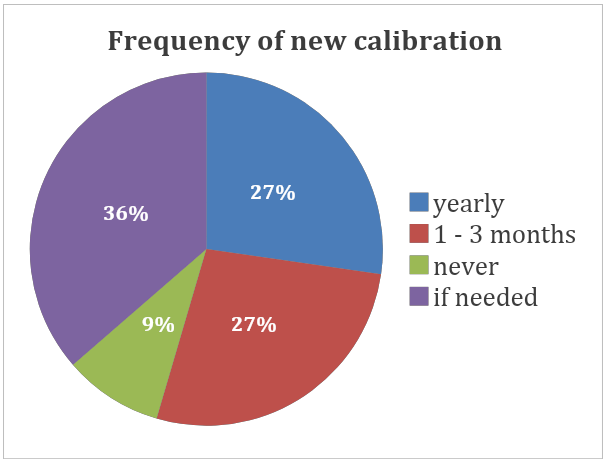

Figure 12: Frequency of machine calibration (results from the FCM Best Practices poll).

Finally, most respondents use beads for size calibration and check of fluorescence drift. However, they do not keep them in the same solution. Most of them use deionised water, whereas 16% use them on a solution of sheath fluid equivalent (same diffraction properties) and only 15% use glycerol for automated long-term deployment submerged in moorings, to avoid beads being stuck together. The frequency of use varies but ¾ of them use them once a day, 12% weekly, and 13% never use beads. The type of beads used for size/fluorescence calibration goes for single type (mainly Polystyrene - PS) one size or multi-size (majority) to multi-type/multi-size combining silicon beads for sizes under 10 µm with larger PS beads.

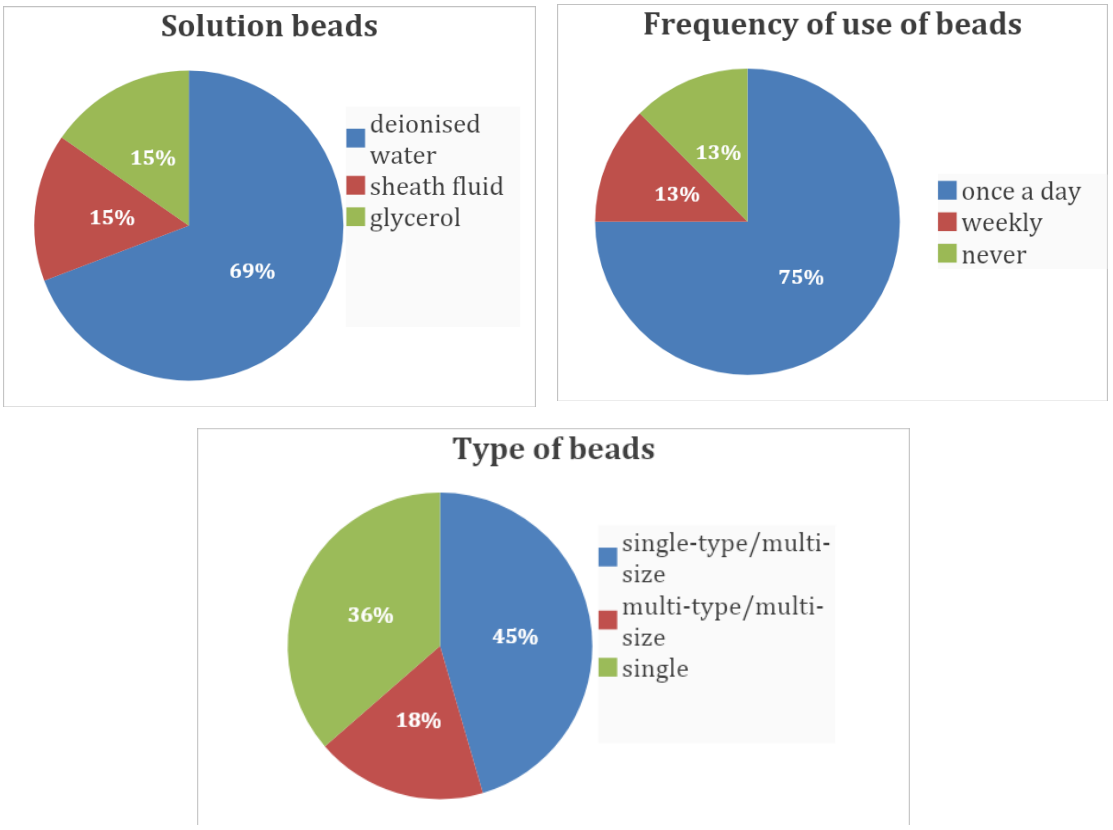

Figure 13: Solution, type and frequency of use of beads on FCM measurements (results from the FCM Best Practices poll).

Finally, a quality control of online measurements is done by all respondents. However, they do not follow the same procedures and do not use the same standards.

3.1.6. Post-measurement data process

Finally, the treatment of raw data, which can be very time-consuming, is carried out mainly manually, by using the dedicated software on two versions (CytoClus 3 & 4), which means that not all scientists upgrade this tool. Only 18% use a combination with semi- or automated (EasyClus) classification and discrimination of optical cell populations (gating).

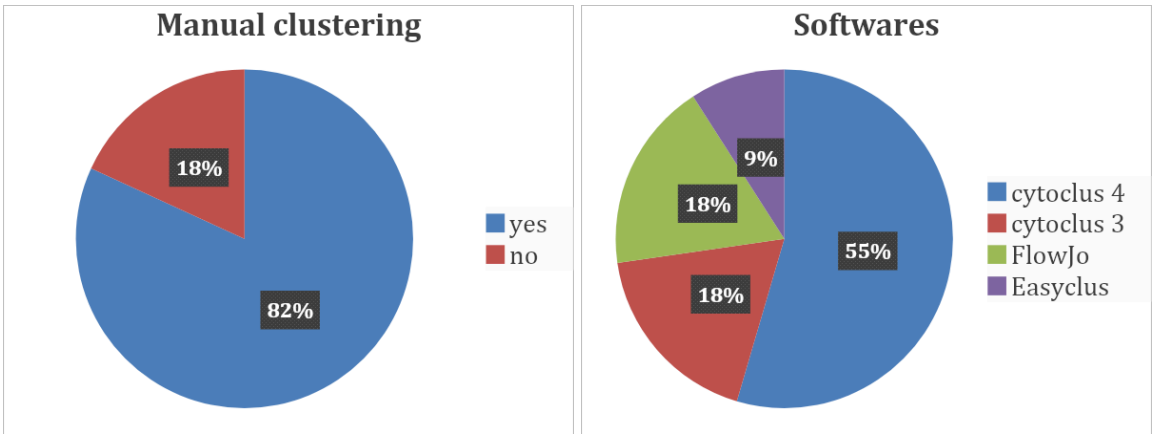

Figure 14: Numbers of protocols applied for FCM deployment (results from the FCM Best Practices poll).

3.2. Discussion carried out during the dedicated Workshop on Best Practices for Flow Cytometry (March 9, 2021)

The Workshop on Best operational Practices for Flow Cytometry was attended remotely by 25 participants online on March 9, 2021.

3.2.1. Lessons learned

Some general trends of operational procedures for analysing and measuring *in vivo* samples with automated flow cytometry were identified and would need to be considered as general operational procedures. However, the complexity of the sites monitored, from oligotrophic clear marine waters to highly turbid and productive coastal systems, under the influence of coastal and estuarine impact, brackish, and/or freshwater systems. The different objectives of the studies make a complete harmonisation of the procedures really difficult to obtain.

Moreover, despite the communication carried out, only nine scientists responded to the survey. The questions were probably sometimes not clear enough to get appropriate answers. Nevertheless, scientists are engaged and their questions and comments need to be taken into consideration (25 scientists attended both workshops)

3.2.2. Round table about next steps to take

All participants agreed on the need to ask sensor providers to better specify sensor performances (*e.g.*, catalogue of the specificities for each sensor, diagnosis after maintenance, details about the changes operated on old machines, troubleshooting guide, requirements for *in situ* automated platforms).

Participants also underlined the need of planning common deployment exercises and sharing of operational practices to apply for shared sensors. In addition to that, a discussion was also carried out on the interest of proposing automated FCM to be put into the coastal demonstration module (c-EGIM) in relation to WP1, WP3, WP4 and WP7, which finally was not possible for safety reasons due to the stability of this machine coupled to the c-EGIM deployed in 2023 at the bottom of the SMILE buoy in the Bay of Seine facing very strong currents.

Communication on the ST7 JS3 approach for developing and agreeing on best practices was made in connection with WP6. A thematic school on flow cytometry applied to marine sciences was proposed by CNRS and hosted by CNRS MIO in October 2022, co-funded by the French Research Infrastructure ILICO.

Decisions to advance together for defining operational best practices for getting the most interoperable F.A.I.R. data were taken and even if it took time to define the best way of proceeding, concrete planning was finally made to organise an international workshop for discussing operational best practices for automated flow cytometry deployments and FAIRNESS of data. A EUROMARINE funding was earned in 2023 (application led by CNRS MIO) to organise the TT-CYTO "Tips and Tricks towards flow CYTOmetry data Fairness" international workshop hosted on June 4-6, 2024 by CNRS LOG in Wimereux (FR), co-organised by CNRS, CEFAS, SYKE, SZN and HCMR. A publication on Best operational Practices for automated FCM is in preparation to be submitted by fall 2024 and further developments will be carried out in the frame of the OBAMA NEXT European project.

3.3. Case study: the CytoSense automated pulse shape-recording flow cytometer

3.3.1. General information

3.1.1.1. Design overview

The CytoSense (benchtop version, Cytobuoy b. V., Netherlands) and CytoSub (submersible version) are automated flow cytometers specially optimised for the study of phytoplankton, that record the whole optical profiles (pulse shape-recording) of individual particles. Similarly, to other flow cytometers, the sample circulates thanks to a peristaltic pump, surrounded by a 0.2 µm-filtered sheath liquid with optical properties close to those of the sample (Dubelaar & Gerritzen, 2000). Sheath fluid circulates at a much higher speed than that of the sample, and is continuously recycled from the sample intake thanks to a gear pump, contrary to most conventional flow cytometers.

The size-range of analysed particles goes from ~1 to 800 µm (and several mm in length), making it possible to analyse the biggest phytoplankton cells as well as chains. For such sizable cells, the volume sampled can be adjusted to relatively high values in comparison to conventional flow cytometers, so as to more reliably count bigger, rarer events. A camera

can be mounted on the design, providing an imaging tool for particles above ~20 µm. Both versions can be automated for semi-continuous measurements, and remotely operated. The CytoSub can be submerged (up to 200 m depth depending on the version) for relatively long periods of time (weeks to months), allowing it to collect time-series in-situ.

An additional module has been developed for the CytoSense as a joint project between CNRS-MIO and Cytobuoy, to target heterotrophic organisms smaller than those typically targeted by the Cytosense: The CytoPro (Silovic et al., 2017). This automated device ensures the staining of a sample prior to its analysis, allowing it to discriminate between auto- and heterotrophic organisms.

3.1.1.2. Functioning

The sample is hydrodynamically focused into a laminar flow, thanks to the significant speed difference with the sheath fluid. Each particle individually passes a 5µm-wide laser beam, and scatters light while doing so, allowing the collection of an optical profile (so-called "pulse-shape") made up of different optical channels.

The most common optical channels are: forward scattering (termed FWS, and FSC in classical nomenclature, corresponding to light collected between 2°-15° of angle), side scattering (SWS or SSC, light collected between 45°-135°), red fluorescence (FLR, light collected on the 668-734 nm wavelength, depending on filter specifications), orange fluorescence (FLO, light collected on the 601-668 nm wavelength, depending on filter specifications) and yellow fluorescence (FLY, light collected on the 536 - 601 nm wavelength, depending on filter specifications). For each optical channel of each pulse-shape, the instrument records numerical values from 15 parameters (Total, Maximum, Length, Average, Inertia, Centre of Gravity, Fill Factor, Asymmetry, Number of Cells, etc) for each particle, generating up to 75 optical characteristics. These values allow for a much finer discrimination of phytoplankton clusters detected by the CytoSense. Classification efficiency is further improved with an image-in-flow device, as each picture taken is coupled to a pulse-shape, leading to a more accurate description of clusters including a taxonomic information type (Lombard et al., 2019).

Although it can vary, laser power is usually set at 50 mW. Higher power is sought to detect the smallest cyanobacterial cells in oligotrophic waters. The detection limit is set by the user appropriately in order to reduce the amount of non-target events recorded, while still retaining relevant information: for example, phytoplankton cells are separated from non-photosynthetic particles on the basis of their red fluorescence (see further).

3.1.1.3. Available training materials and contacts

Documentation allowing to safely set up, operate and maintain the CytoSense/CytoSub is provided by the company. This documentation describes the main components, its optical and fluidic systems, its electronics specifications, and its installation and operation procedures. It also provides a basic understanding of the interpretation of results, details light maintenance and storage recommended practices, and describes various, heavier maintenance procedures. Of particular importance are the sample pump calibration procedure and the resulting error margin. Basic training related to the CytoSense's operation can be found in the dedicated manual provided by the company Cytobuoy b.v.

3.1.2. Maintenance procedures

3.1.2.1. Regular maintenance

The CytoSense recycles its sheath fluid from the sample intake, after a two-stage filtering on 2 µm and 0.2 µm (with new sets of filters of 0.4 µm and 0.1µm) which can be completed by a C filter for detecting the smallest cells in very clear oligotrophic waters. It has to be taken care of on a regular basis, to avoid bacterial growth inside the system.

When the sheath is replaced for sampling purposes, it is also good practice to add ~ 5 mL of biocide to the sheath if the planned operations last for more than a few days. For even longer uses, biocide doses should be added regularly to the sheath anyhow possible. Before putting the sensor out of use for more than a day, the sheath pump should be activated for at least one hour. If it is used only a few times per month or less, it is recommended to run it at least every week or at least once per month.

For processing of marine samples, afterwards it is recommended to flush the sample intake (as well as the PEEK sample loop for CytoSubs) with fresh water if the instrument will not be used for more than a day. If not for use for more than a month, it is recommended to have fresh water as sheath fluid, complemented with ~ 5 mL of biocide running for some time (at least 30 minutes).

If the instrument is not in use for more than a month, it is recommended to activate the sheath pump for at least one hour per month. In such cases it is also recommended to charge the battery for roughly two hours per month.

The recommended biocide solution, initially supplied with the hardware, is Proclin 950. It is supplied with syringes that make it easier to add to the sample inlet.

The sample pump is a gear pump that has to be calibrated regularly, monthly assuming a daily use for less than 10 samples. Before an extended sampling operation calibration should be performed and checked before, and after, data collection in order to estimate the drift.

The flow cell should be cleaned regularly, monthly assuming a daily use for less than 10 samples. To do that, a toothbrush soaked in acetone can be used to gently scrape the inside of the flow cell, from the top-opening.

Laser alignment can be checked from the metadata of any file created. It measures how well-aligned the laser bundle is in relation to the core, where events of interest are recorded. In case the laser is not aligned anymore with the core, it has to be manually realigned. Any misalignment can be checked regularly, and should be corrected as soon as it goes over a certain threshold.

It is recommended to keep the dedicated software (CytoUSB) up-to-date at all times, in addition to the Operating System.

Depending on the type of operation, the internal computer of the CytoSense should be checked and emptied of its acquired data as often as possible, to maximise the system's and the software's' performances.

It is especially recommended to empty the storage before an extended *in-situ* deployment, as a lack of connection to the instrument and a full internal storage will lead to a loss of data.

3.1.2.2. Heavy maintenance

Depending on usage, the tubing allowing the sheath and the sample to circulate gets increasingly fouled by bacterial growth and other biological material. This is especially true for a sampling occurring during a bloom, when the water sampled is loaded.

Moreover, the tubing gets increasingly worn out inside the peristaltic pump, leading to the inclusion of air bubbles in the sample which is to be avoided.

To counter these issues, it is advised to replace the entire tubing of the cytometer after such a sampling, or on a yearly basis for lesser usage.

Likewise, in-line sheath filters should be changed on a yearly basis.

The core's position is fairly stable, but can drift over time, especially if handled or moved frequently: if laser alignment is too difficult or impossible, then the core may need to be realigned manually. Afterwards, aligning the laser with the core is also needed.

The camera's focal plan should be aligned with the core's position. Because this focal plan is quite thin, any deviation of the cell from the core's centre will make it appear blurry. If that happens too often despite an optimised measurement protocol, then the camera's focal plan and the core position will need to be realigned. The camera's focal plan is fixed and should not be dealt with, unless specifically advised by specialised staff. Instead, the core has to be manually moved up to the focal plan, when deemed appropriate.

3.1.2.3. Known issues

Issues may arise both for the software and the hardware part.

The softwares (CytoUSB for data acquisition, CytoClus for data analysis) may have issues that could lead to unexpected behaviour during the acquisition, or errors in the analysis. Issues related to data management, storage, and retrieval may occur, particularly when generating large datasets during long, intense samplings. Rarely, compatibility issues may arise with different operating systems or versions of software dependencies.

For the hardware part, drifts in laser output over time may affect data consistency. Also, degradation of detector performance over time may affect the sensitivity and accuracy of measurements. Finally, components such as motors, valves, and pumps may experience wear and tear over time, leading to mechanical issues and requiring replacement.

3.1.2.4. Standards and calibration

Standards and calibration procedures are paramount to ensure the accuracy, reliability, and comparability of data obtained from the CytoSense, just like for any other flow cytometer. By adhering to established standards and regularly calibrating the instrument, one can generate high-quality data that contributes to robust scientific research and informed decision-making.

Regular calibration is a quality control that allows researchers to detect and correct any drift or deviations in instrument performance over time. By periodically calibrating the CytoSense against known bead samples, it is possible to ensure the consistency and reproducibility of acquired results. Furthermore, standards provide a common reference point for comparing data obtained from different instruments: thus it ensures that measurements are comparable

across different studies and locations. This comparability is essential for synthesising data from multiple sources and drawing upscaled, meaningful conclusions about environmental processes or biological phenomena. Calibration procedures can also help identify and diagnose issues. When measurements deviate from expected values during calibration, it may indicate the need for maintenance or repair of the instrument components.

Calibration may concern either the optical system, or the fluidic system. The latter is managed solely via the operating software, which can start a calibration procedure for the sample pump in order to control sampled volume.

The procedure for calibrating the CytoSense does not fundamentally differ from that for calibrating other kinds of flow cytometer, as it makes use of beads of known size, composition and fluorescence if any. It is advised to use a subsampled beads solution, so as to reduce the risks of contaminating the stock solution over time. Beads can be added in a live sample, or the diluted solution can be sampled with a dedicated protocol. During long, semi-continuous samplings in the field, it might not be possible to add beads to the sample or to make a separate, dedicated measurement. A calibration measurement should be done at least at the beginning and at the end of a sampling. For submerged operations, a module can be added to the CytoSub so as to inject beads regularly for calibration measures.

It is advised to use fluorescent beads, especially for small sizes, as otherwise the corresponding cluster may be hidden by noise. Such beads should be kept in the dark as much as possible. It is advised to sonicate the beads solution before calibrating, so as to reduce the amount of bead aggregates that may form.

3.1.3. Data Collection

3.1.3.1. Sensor deployment

The CytoSense can be used on a benchtop or any other flat surface, as the metal frame ensures stability (figure 15). For discrete samples in the lab, the intake can be placed directly into the container. For semi-continuous samplings (for example during a research cruise), the intake can be placed into a dedicated continuous sampling device, connected to a water supply.

Figure 15: Benchtop CytoSense flow cytometer.

The submersible version, the CytoSub, can be fitted into a pressurised hull that allows for sampling at depth (2 to 200 m depth, depending on the chosen configuration). Before mounting the pressurised hull, silicone grease has to be applied to rubber bands at the top and bottom of the instrument, to ensure proper enclosure. The intake tube should be equipped with a copper filter so as to prevent biofouling.

3.1.3.2. Data acquisition and quality control

Data acquisition should be carefully planned before the sampling occurs, in order to be able to quickly fine-tune acquisition parameters.

First of all, blank (sheath fluid) measurement is required in order to define the electronic noise level. Moreover, attention should be taken to the non-pigmented particles that can be also detected, in order to be able to reduce the size of the measurement file, triggering either on SWS (size for smallest particles) and/or FLR (chlorophyll a content) being aware that smallest pigmented cells can be lost if the FLR trigger is too high. The trigger levels depend on each machine and should be also tested for the different water types monitored on a frequent basis.

A main area of concern is the ratio between pumped volume, and analysed volume. Pumped volume corresponds to the volume of sample that was sucked in the instrument. Analysed volume refers to the volume of sample in which events were recorded. Ideally, both volumes are almost identical but in practice, significant differences may arise and lead to biases in counting cells (need to check that).

Different protocols can be necessary to target the smallest cells (picophytoplankton), very concentrated and nano- micro-phytoplankton, less concentrated. The principles are to consider low-speed flow, the lowest FLR trigger level as possible, short duration and high photomultiplier (PMT) amplification for picophytoplankton optimal detection, whereas the opposite strategy (fast flow, high FLR trigger level, long lasting measurement and lower PMT amplification of the signal and an intermediate protocol could be defined for nanophytoplankton cells. The duration of the measurement is adjusted according to the strategy, either on a discrete sample or continuous flow from a pumping device.

Moreover, for image acquisition, the operator can choose to randomly sample the whole size spectra or to rather focus on > 5µm or > 10µm cells and colonies, and to fix the number of images to be taken accordingly.

3.1.3.3. Known issues

When considering continuous recording, different issues will be possible such as corrupted metadata files, files to be discarded or simply issues with the measurement itself.

When autonomous recording is programmed for a long time, attention should be taken on filling the hard drive, especially when no data can be sent to an inland repository.

Other issues concern core movement as sheath temperature varies (to be solved with motorised injector), see D6.5 as well as a publication on FCM Best Practices to be submitted, leaded by CNRS MIO, after performing new tests on different machines (in collaboration with CNRS LOG, CEFAS and SYKE) and following discussions during the TT-Cyto EUROMARINE meeting by early June 2024. Moreover, clogged tubing can occur

during intense blooms and indications and warnings on the acquisition-dedicated software should be considered.

3.1.4. Data management

3.1.4.1. Quality assessment

Discussed during Workshops of Marseille (October 2022) and Wimereux (June 2024), and D6.5, some issues concerning data management includes metadata check (time, volume, temperature, voltage…) and data check, especially considering reference files with beads in order to follow if different values are recorded over time.

3.1.4.2. Storage

It is recommended to check the memory taken up on average for a fixed number of files on each of the protocols applied.

Moreover, folder organisation needs to be clearly defined for making the easiest as possible manual or automated analysis.

3.1.4.3. Analysis, uncertainties

Issues about clustering - manual vs automated - are still under debate (see results of the FCM poll in the previous section) and were discussed during the last automated FCM workshops. As stated in previous deliverables of JERICO NEXT (Karlson et al., 2017; Artigas et al., 2019), different tools exist as the commercial one EasyClus (Thomas Rutten Projects), the RClusTool (WP 11 VA) and other published ones (Malkassian et al., 2011; Fuchs et al., 2022). A new robust automated classification tool is being built and tested in the frame of an international France, U.K. and Finland collaboration (see details in D6.5).

Uncertainties still remain when addressing the abundance of big, rare cells or colonies as measured volume might never be enough, unless pooling together a series of measurements or making measurements as long-lasting as possible, but then not taking benefit of addressing high frequency and high spatial resolution, which is one of the biggest strengths of the technique. This issue is not restricted to FCM and is common to any phytoplankton sampling and measuring technique, especially important when crossing different systems with autonomous automated recording on continuous underway recording on research vessels and ships of opportunity (Ferry Boxes). Moreover, uncertainties on protocols not being set appropriately, leading to saturation of signals in pulse shapes, or considering colonies as single cells (if trigger level is set too high), should be furtherly taken into consideration.

Finally, one should remind that one of the more interesting values of using this technique to monitor and study phytoplankton refers to the possibility to address not only abundance but also chlorophyll *a* content (Haraguchi et al., 2017) and biomass per cell and per cytometric group (see (Thyssen et al., 2022) and D6.5 for more details) as well as defining functional traits from optical features of signals (Fragoso et al., 2017).

4. Operational Practices for Plankton Imaging Analysis

4.1. Results from the dedicated questionnaire

4.1.1. General information

A questionnaire on best practices for imaging plankton analysis was launched by late 2021 and announced by email to dedicated international working groups, such as the Working Group on Phytoplankton and Microbial Ecology (WGPME) of the International Council for the Exploration of the Sea (ICES), on the JERICO website (www.jerico-ri.eu).

Fifteen scientists from eight countries and eleven laboratories (CNRS LOG and LOV, IFREMER, VLIZ, Havstovan, BIOS, HEREON, SHMO, HCMR, PML and Bigelow) responded to the survey on Imaging Plankton Analysis, Protocols from 6 types of sensors were described, 4 from France (three laboratories), and one for each of the other 7 countries (Figure 16).

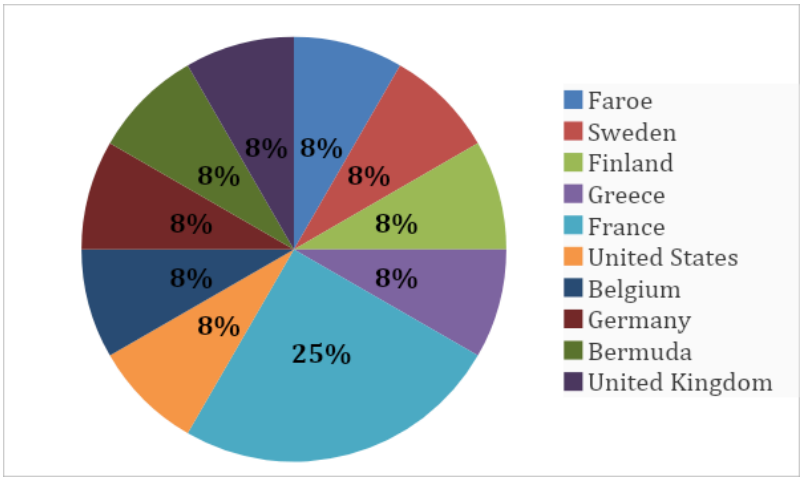

Figure 16: Equipment and related countries resulting from the poll on Imaging plankton operational practices

4.1.2. Description of the operational equipment

Seven different devices were described as currently being used for imaging plankton (see D5.1 for most of their description). The most commonly used imaging device (Figure 17) is the benchtop automated imaging system FlowCAM of different series (3 black and white and 3 providing colour images) and configuration (most with flow cytometry for triggering only pigmented cells, one without it and then imaging all particles, i.e.phytoplankton, microzooplankton and detritus). Other benchtop devices (scanners) are deployed for

zooplankton by PML and CNRS-LOV. The Imaging FlowCytoBot (IFCB) is deployed by two laboratories (SYKE and SMHI), the 3 CPICS are deployed by Hereon and the 3 ZooScan by CNRS LOV and VLIZ. Two types of *in situ* imaging systems are deployed by CNRS LOV (UVPs) and VLIZ (Real Time Video Plankton Recorder).

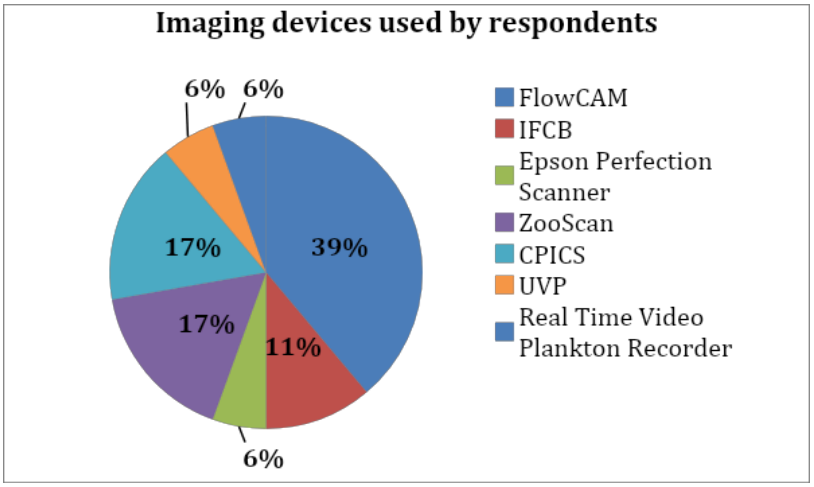

Figure 17: Imaging devices used for Imaging plankton observations (results from the poll on Best Practices).

Institutions work mainly on marine environments, some on brackish waters, few on freshwater. Size range depends on the machine; nanoplankton & microplankton (*i.e.*: FlowCAM, IFCB, CytoSense*), and also on meso/macroplankton (i.e., CPICS, ZooScan).

The majority of respondents (Figure 18) declared working with benchtop imaging systems (either scanners for zooplankton or inflow systems for phytoplankton analysis), ¼ work with *in situ* imaging systems and only two laboratories work with *in situ* inflow devices (IFCB).

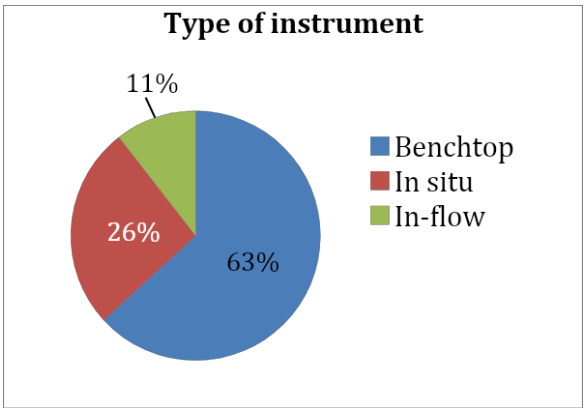

Figure 18: Imaging devices used for imaging plankton observations (results from the poll on Best Practices)

The supply of samples for in-flow imaging sensors either require pre-filtering (53%) or are direct non-filtered (47%) seawater samples (Figure 19). Pre-filtration mesh size (if any) depends on configuration of the machine (i.e., flow cell size for the FlowCAM) or is compulsory (150 µm for IFCB). Concentration on plankton nets is compulsory for zooplankton scanning systems and in some cases for phytoplankton in oligotrophic systems (pre-concentration).

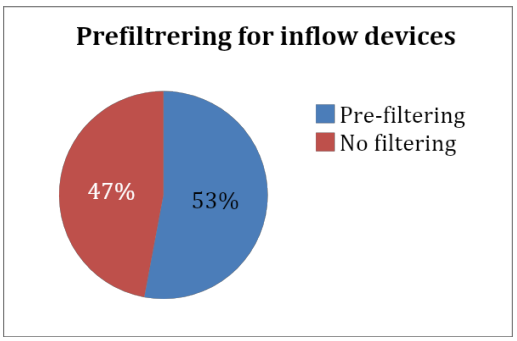

Figure 19: Type of supply of samples for imaging plankton observations (results from the poll on Best Practices)

The imaging method is for half of the devices described on bright fields, whereas almost one third refers to Dark field and the rest function either on auto-image mode or scanning a fixed sample (Figure 20).

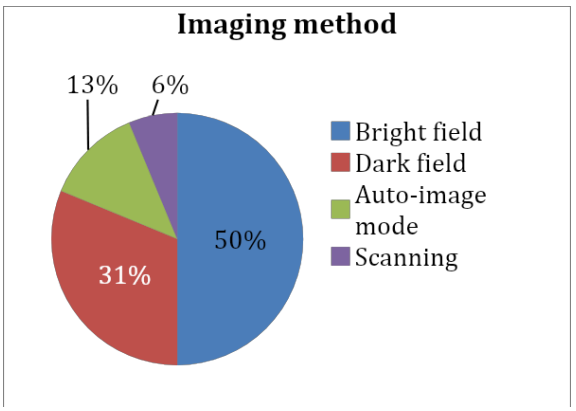

Figure 20: Imaging method applied to for imaging plankton observations (results from the poll on Best Practices)

Size calibration is performed, on the majority of machines, mostly with beads (other: microscope ruler and pollen grains of known size)

4.1.3. Deployment and measurement strategy

The variety of imaging systems are deployed equally on research vessels (28%) or in laboratories (27%). *In situ* stationary autonomous stations cover 12% of the answers, the same proportion for on-deck manual sample analysis. Finally, 6% of the deployments are made on moorings (buoys) or connected (in-flow imagers) to underway continuous pumping and measurements (FerryBoxes) on ships of opportunity (Figure 21).

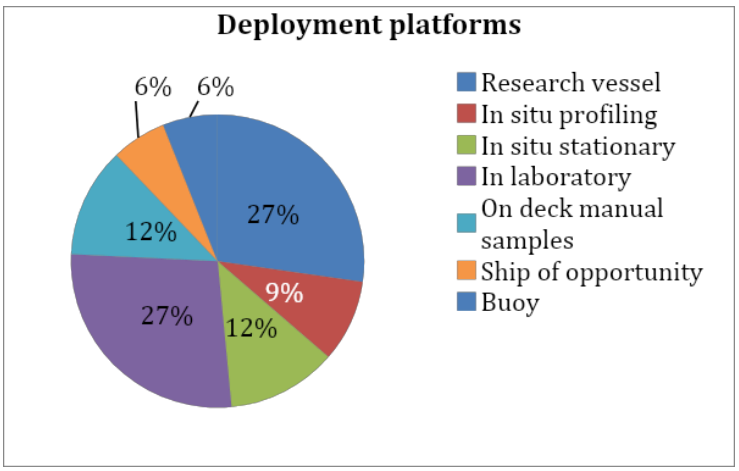

Figure 21: Type of platforms for the deployment of imaging plankton devices (results from the Best Practices poll)

Finally, the measurement strategy concerns mostly (52%) discrete sampling, followed by continuous recording (29%) and profiling devices (Figure 22).

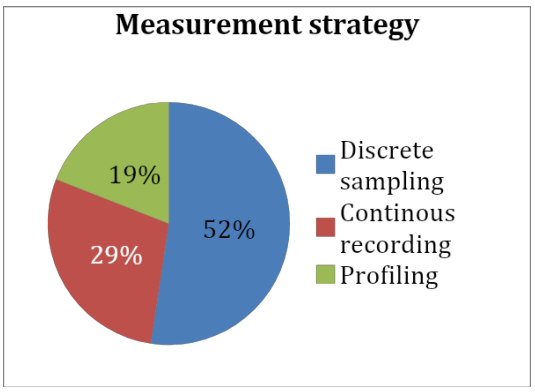

Figure 22: Measurement strategy for deployments of imaging plankton devices (dedicated Best Practices poll)

4.1.4. Samples for imaging, camera settings, spatiality

Cameras are mostly set to auto-imaging, but some are triggered with fluorescence (*i.e.*, FlowCAM if possible)

For in-flow devices as FlowCAM, different flowcells are available flow cells sizes ranges from 50 µm to 600 µm, allowing imaging from nano (5-10 µm) towards big colonies of more than 600 µm, corresponding to magnification going from x 40 to x 100 to x 200.

When considering the type of sample analysed, 40% of respondents analyse non-living samples, whereas 30% use fixative samples mostly for laboratory analysis (Figure 23).

Fixatives are used for zooplankton on the ZooScan as well as for phytoplankton, microzooplankton and zooplankton on the FlowCAM. Phytoplankton is mostly studied with live samples.

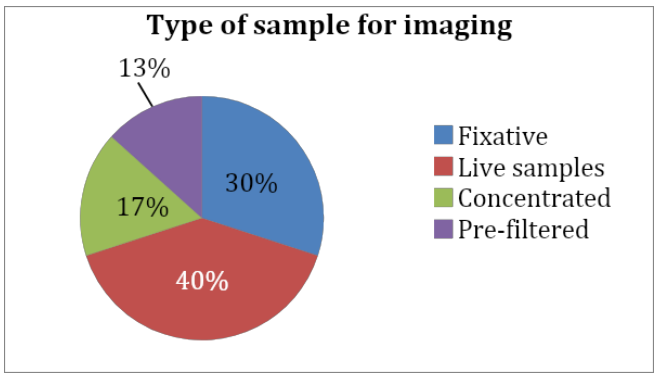

Figure 23: Characteristics of the samples measured by imaging plankton devices (dedicated Best Practices poll)

The strategy of sample/image acquisition for ¼ of respondents refers to discrete sampling of different depths (for phytoplankton analysis), whereas concentration on vertical (15%), oblique (12%) and surface (9%) plankton nets are mainly used for zooplankton imaging (but are also used for phytoplankton concentration in oligotrophic systems). Profiling and continuous recording account together for ¼ of deployments (Figure 24). A few participants specified the maximum depth of their sampling: 42 m (ZooScan), 100 m (FlowCAM), 1000 m (CPICS), 6000 m (CPICS)

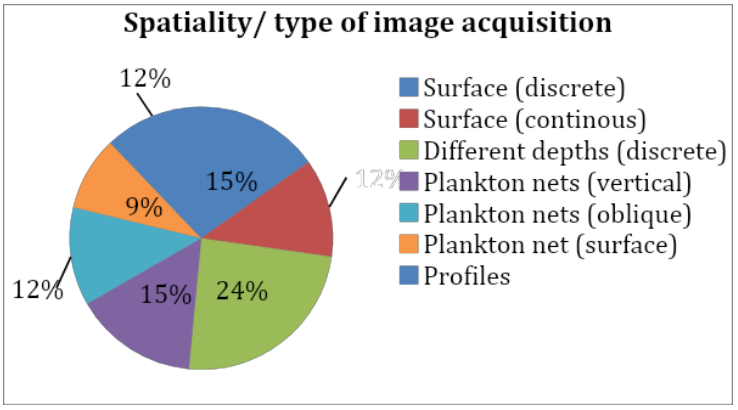

Figure 24: Characteristics of the samples measured by imaging plankton devices (dedicated Best Practices poll)

4.1.5. Particle determination

One important feature in plankton imaging concerns the discrimination of particles on raw images taken (what is called the Regions of Interest or ROIs). The discrimination of particles (cells, colonies) is carried out by the software used during the sample acquisition for image analysis (29%), the one used on raw images (that need a great storage capacity compared to treated images) for determining regions/objects of interest (31%) or the one used for image classifications (27%) (Figure 25). For 13% of respondents, it is possible to set manually the distance between particles for adapting the particle discrimination to different types of phytoplankton (either single-cell or colonial stages).

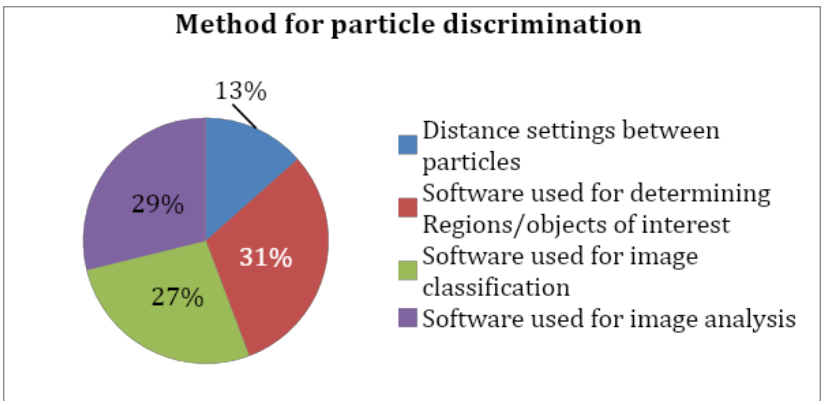

Figure 25: Method applied for particle discrimination by imaging plankton devices (dedicated Best Practices poll)

4.1.6. Maintenance and optics

Maintenance frequency: most machines are only in maintenance when issues occur – they seem generally sturdy and the cleaning is automated

Biofouling solution (*in-situ*): mechanical wipers, UV-light, sodium azide, window cleaner and anti-calc, divers during long underwater submersible deployments

The maintenance is carried out in the laboratory for 53% or respondents, as 47% send it to the manufacturer for maintenance (Figure 26).

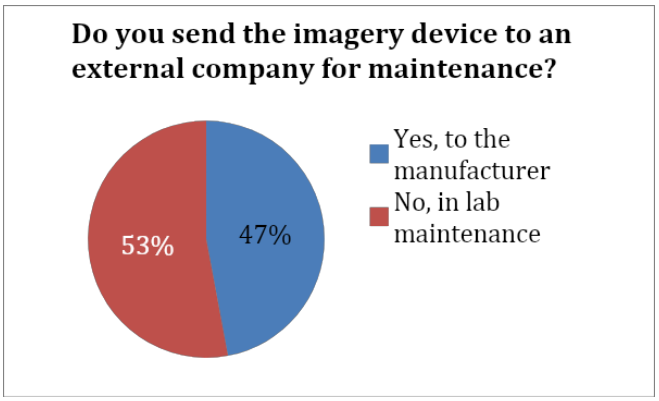

Figure 26: Maintenance of imagery devices (dedicated Best Practices poll)

Example of in lab maintenance for IFCB: Follow automated cleaning protocol when operating the instrument. Replace filter cartridges for sheath fluid approximately every six months

Most colleagues proceed to manual cleaning of device's optics (only 29% wait until sending it to the manufacturer for maintenance) (Figure 27).

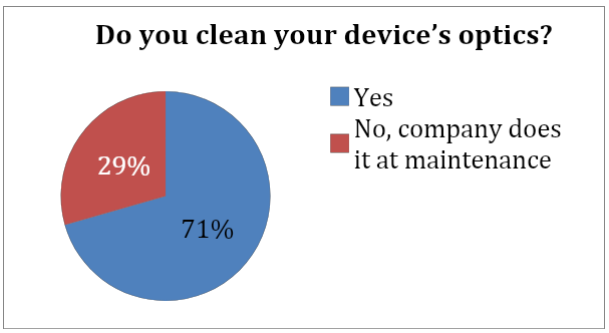

Figure 27: Cleaning of imagery devices (dedicated Best Practices poll)

4.1.7. Analysed volume, questionable sample and magnification

Most users target a minimum of 1000 particles in the analysed volume. Some aim for more: 1500-4000 (ZooScan). Some go with consistent analysed volume (2 – 30 ml for FlowCAM). Magnification ranges from x2 to x20.

Most users (56%) use only one process/magnification for acquiring images (mostly because the device only has one magnification available, allowing anyway different protocols to be programmed) whereas 47% of the respondents change either protocol or magnification for their measurements, either splitting the sample into size fractions (zooplankton), if big cells appear in pre-filtered samples (then moving to lower magnification) or at the opposite, if too many small cells (moving to higher magnification) (Figure 28).

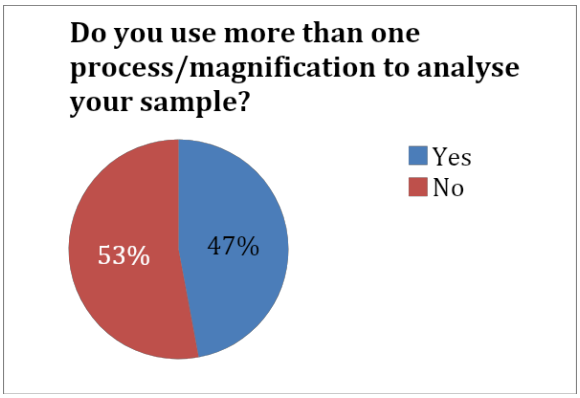

Figure 28: Magnification processes to analyse plankton imaging samples (dedicated Best Practices poll)

Finally, 71% of respondents apply statistical tests for quality control of samples (Figure 29).

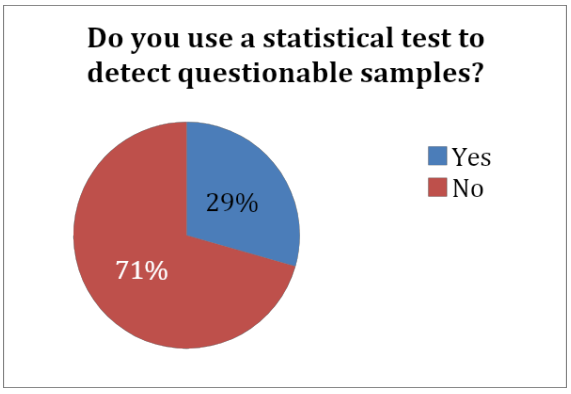

Figure 29: Quality control for plankton imaging (dedicated Best Practices poll)

Other devices are starting to be used for imagery: CytoSense/CytoSub (some characteristics: bright field, autoimage, no pre-filtering, discrete or *in-situ* measurements, etc.).

4.2. Discussions carried out during the dedicated Workshops on Best Practices for Imaging phytoplankton (June 27th & July 7th, 2022) and perspectives

Two online workshops were held online on June 27th and July 7th, 2022 attended by 14 scientists from 12 laboratories.

4.2.1. Lessons learned

This questionnaire highlighted numerous imagers of different characteristics from benchtop to autonomous in situ, targeting a range of planktonic organisms from nano-phytoplankton to big colonies and meso-zooplankton.

For future development, it will be useful to compare them by similar environments and targeted size range/trophic status of organisms (nano/micro or mesoplankton, pigmented or not).

The variety of applications and deployments gathered should help finding complementarity on operational practices by the comparison between machines and institutes

Many of the respondents shared their protocols for their respective machines: with their approval a document could be created and shared with everyone to be discussed and, subsequently, used as a reference, possibly in the frame of a publication in progress leaded by CNRS LOV and/or work carried out in the frame of new projects and networks in which JERICO partners are involved.

We had a good representability with the answers, some questions needed to be clearer (difficulty in the interpretation of the results obtained) and the questionnaire could have been shorter

4.2.2. Round table about next steps to take

The main goal of these workshops was to contribute to the Ocean Best Practice repository and to associate this work to the work and deliverable on best practices in data management (Cabrera et al., 2022) (« D6.4 : "Management Best practices for quantitative imaging systems", recently finished. (https://repository.oceanbestpractices.org/handle/11329/1917).

During the two workshops, we discussed different issues to be considered.

First of all, it is important to remind that some protocols are already available and clearly described, as the one for a low-cost portable device (PlanktoScope) and for the benchtop laboratory zooplankton scanner (ZooScan):

https://www.protocols.io/view/planktoscope-protocol-for-plankton-imaging-bp2l6bq3zgqe/v1

https://www.protocols.io/view/zooscan-protocol-yxmvmk8j9g3p/

These two machines can serve as examples for people who would like to provide a protocol. On this platform protocols are sharable and citable because they come with a DOI. You can

do versioning of the protocols. The aim would be to regroup all the protocols, not just on that platform.

Then discussions included the consequences of the choices made by using one or another device and one or another protocol: *i.e.*, if you decide to trigger on fluorescence or not for taking images with the inflow imaging systems which allow you to choose (FlowCAM, IFCB). If you do so, one will miss an important compartment of the plankton but on the other hand the data will be easier to process by avoiding most of the detritus (except the one including pigmented degraded cells). Moreover, when comparing the performance of different devices, one should focus on the target group (pigments, size) and also compare machines that need pre-concentration/dilution/pre-filtering amongst each other.

It then would be interesting to have more information on which method to use depending on the targeted group or compartment of organisms depending on the ecosystem considered.

Another discussion was carried out on the post-acquisition of images, about storing either raw images or treated images ("vignettes") delimiting the Regions of Interest (R.O.I.s) of the sample. Most users of inflow devices (IFCB, FlowCAM) won't store them as raw images as it takes too much space. In that case, one can adjust the distance between particles (see results of the poll) but only before getting a sample or during the acquisition process.

If formaldehyde is regularly used for preserving zooplankton samples, some laboratories run FlowCAM analyses on lugol samples for addressing phytoplankton diversity and dynamics: attention should be taken in changing the morphology some organisms (as well as the pigment coloration, especially with machines producing colour images).

Some operational practices will differ according to the capabilities in storing images and associated data, on target organisms (size, single-cell, colonial) making some users, as for FlowCAM using different magnification protocols and pre-filtration for the same sample.

When comparing image resolution between devices, microns per pixel value would be more useful information than the magnification settings, because the same magnification might differ between instruments. Concerning benchtop imaging devices (FlowCAM), there is not really a way to avoid different magnification for the same sample to capture the whole breadth of phytoplankton (or of microzooplankton if no fluorescence trigger is applied).

Operational practices need to be investigated further by machine, have a general view of what a machine can do or not and of the choices we can make or not.

It would be useful to have a document to see all the different protocols used for the same type of machines. In that case, best practices will depend on what you're trying to capture. Sometimes you need to re-run a sample several times to capture different organisms mostly possible for manual manipulation on benchtop machines. On automated inflow machines (as the IFCB) – you can program different triggering on the same sample (particle or fluorescence), as possible for CytoSense/Sub machines (see previous section on automated FCM).

Klas Ove Müller (Hereon) refers to an Imaging group in the US – different types of imagers. There is also a Global Ocean Observing Systems (GOOS) initiative, co-working group – heavy imaging instrument users – it would be interesting to have them fill up the questionnaire.

The idea would be to recall networks of experts and users of Imaging Devices as the ITA/ITAPINA which aims to build the All-Atlantic Pelagic imaging community and to develop a framework for operational marine ecosystem monitoring. Recently, colleagues from this network proposed a synthesis of the main requirements for building a network for automated imaging of plankton (Kiko et al., 2023).

We will need to benefit of inter comparison exercises as the GlobalHAB hybrid symposium on automated *in situ* observations of plankton hosted at the Kristineberg Marine Station (University of Göteborg) organised by Bengt Karlson, co-funded by JERICO-S3 and SCOR (https://www.jerico-ri.eu/events/globalhab-symposium-on-automated-in-situ-observations-of-plankton/). During that workshop, attended by some of the participants to the WP5 workshops focused on phytoplankton studies participated in training, demonstrations, and measurements on both a wide range of cultures and in situ samples taken from the fjord. Three groups of devices were deployed on a benchtop mode: FlowCAM, IFCB and CytoSense/Sub (the latter used for both optical characterization and imaging of samples). In parallel, microscopical observations and counts were performed. Results were analysed by young scientists involved. More information and presentations made (tutorials and case studies, operational and analytical tooms) are available at: https://www.globalhab.info/activity/140-globalhab-symposium-on-automated-in-situ-observations-of-plankton

In August 2023, the Operational Phytoplankton Observations (OPO) working group first meeting was held at Bigelow Laboratory for Ocean Science. Nineteen participants from US and international partners (selected through an application process) spanning from early to senior scientist career stages. The working group is focused on developing standard operating and sampling procedures for common particle imaging instruments (PIIs) used to quantify and identify phytoplankton in aquatic environments. Moreover, solutions for data interoperability between different sampling methods and PIIs were discussed and will be addressed in a subsequent best practices document that will be published to both Ocean Best Practices and a peer-reviewed journal. (https://www.us-ocb.org/opo-working-group/). On January 4th, 2024, an online contact was made between some OPO participants and Felipe Artigas. A first presentation of the advance of the work was presented during the ASLO Ocean Sciences Meeting by late February 2024.

Fabien Lombard from CNRS LOV started a document representing a collaborative effort and then supposed to be as inclusive as possible. Beyond a simple comparison between tools, the publication would need to promote their use by defining the real capacities (and limitations) of the considered machines, by proposing recipes to benefit the most of the sensors. Even though we are still far from it, the continuity of the present work would be the way of co-designing a coordinated and cross-calibrated network of observation with interoperable protocols.

4.3. Case study; Imaging FlowCytobot (IFCB)

4.3.1. Design overview

The Imaging FlowCytoBot by McLane is a submersible imaging flow cytometry instrument that integrates imaging and flow cytometry into a single platform. It have been used and deployed since more than a decade in USA and tested in Europe by SMHI in 2916 (JERICO

NEXT workshop) and now regularly used on ferryboxes and/or fixed stations by SYKE, NIVA and recently by CNRS LOG within the JERICO S3 consortium. The design of the CytoBot emphasises a streamlined workflow, combining high-resolution imaging with the rapid throughput of flow cytometry. The system features a flow chamber where cells are suspended in a fluid stream and pass through a series of optics and detectors. This setup allows for both the analysis of physical properties, such as size and granularity, and the capture of detailed images of each cell. The imaging component is equipped with advanced cameras and lighting systems to ensure high-quality visual data, while the flow cytometry component uses laser excitation and photodetectors to measure fluorescent signals. The design prioritises user-friendly operation with intuitive software now under the Linux system (formerly Windows) that integrates imaging and cytometric data, facilitating comprehensive analysis and interpretation.

4.3.2. Functioning

The Imaging Flow CytoBot operates by combining traditional flow cytometry with high-resolution imaging to offer a multi-dimensional analysis of cells. As cells flow through the instrument, they are illuminated by lasers which excite phytoplankton pigments. The emitted light is detected and is used as trigger level for selecting only pigmented cells (see automated Flow Cytometry section). Simultaneously, the imaging system captures detailed photographs of each cell, enabling the examination of morphological features. Both quantitative data and qualitative visual information is computed on a single pass. The software associated with the CytoBot integrates these datasets, offering powerful tools for image analysis and cytometric data interpretation. An important issue to consider is that this software only operates under Matlab license (+ several modules).

1.1.1. Available training materials and contacts

Different repositories provide training and recommendations about IFCB use. First, the manufacturers' website provides manuals, technical updates, papers/media, videos, dashboards, and tools (https://mclanelabs.com/imaging-flowcytobot/ifcb-manuals-0/). Moreover, Heidi Sosik from Woods Hole Oceanographic Institute (WHOI) provides an introduction and links to the repository of data processing and analytical tools (https://github.com/OceanOptics/ifcb-tools). An international forum of IFCB customers is maintained from McLane headquarters.

In Europe, there European IFCB network coordinated by SMHI (Bengt Karlson) allows sharing practices and issues (including operational procedures, processes, and analytical tools):
https://dto-bioflow.eu/sites/default/files/2024-06/SMHI%20Plankton%20imaging%20data%20flow.pptx__0.pdf

An additional IFCB data extraction tool have been built by SYKE (Kraft et al., 2022) on an Open-Source language and is available at: https://github.com/sykefi/syke-pic

2. Best Practices for multispectral fluorometry

2.1.Results from the dedicated questionnaire

2.1.1. General information

A questionnaire on best practices for *in vivo* fluorometry application for phytoplankton analysis was launched by early 2022 and announced by email to dedicated international working groups, such as the Working Group on Phytoplankton and Microbial Ecology (WGPME) of the International Council for the Exploration of the Sea (ICES) and was published on the JERICO website (www.jerico-ri.eu).

Six partners from five countries answered the multispectral fluorometer poll. French and Spanish respondents have described operational practices for three devices, whereas Greece, Finland and Faroe Islands reported only one device (Figure 30).

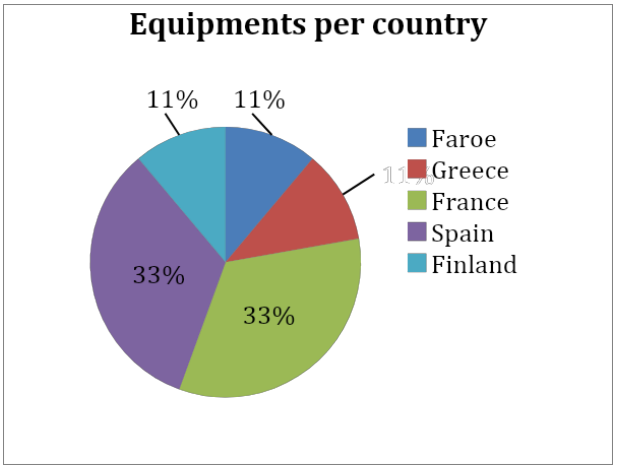

Figure 30: Equipment and related countries resulting from in vivo fluorometry polls

2.1.2. Description of the operational equipment

Seven different devices were described, including 4 multispectral devices: the Fluoroprobe (profiler and benchtop) and AlgaeOnineAnalyser (benchtop) from BBE Moldaenke, the Multiexciter Spectral Fluorometer from JFE Advantec and the Ecopuck Triplet from Wetlabs. The other devices are monospectral fluorometers (Figure 31).

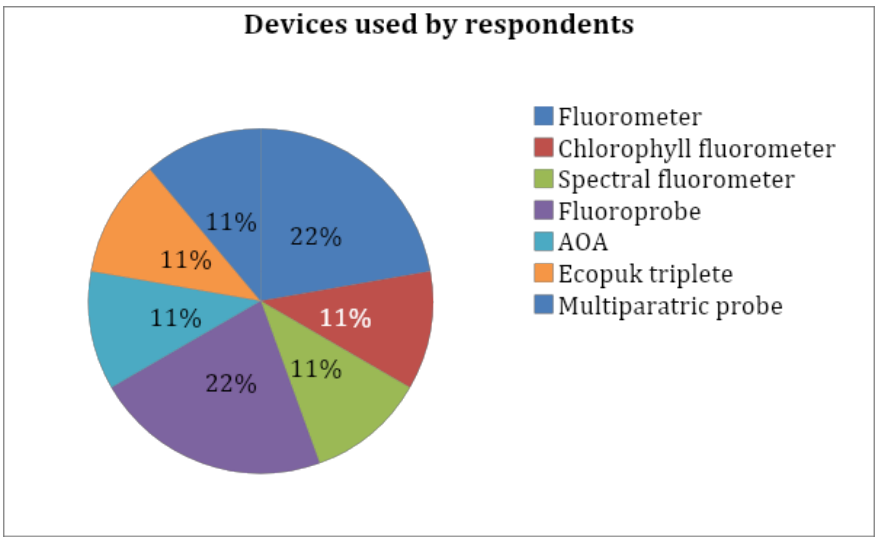

Figure 31: Devices described from in vivo fluorometry polls

Fifty-six percent of the different fluorometers addressing *in-vivo* (total or per pigmentary group) chlorophyll *a* address other variables as temperature, depth, CDOM (as a real variable) or yellow substances (for correcting in vivo chlorophyll a estimates), turbidity (as a real variable) or light transmission to also correct chlorophyll *a* estimation in turbid waters (Figure 32).

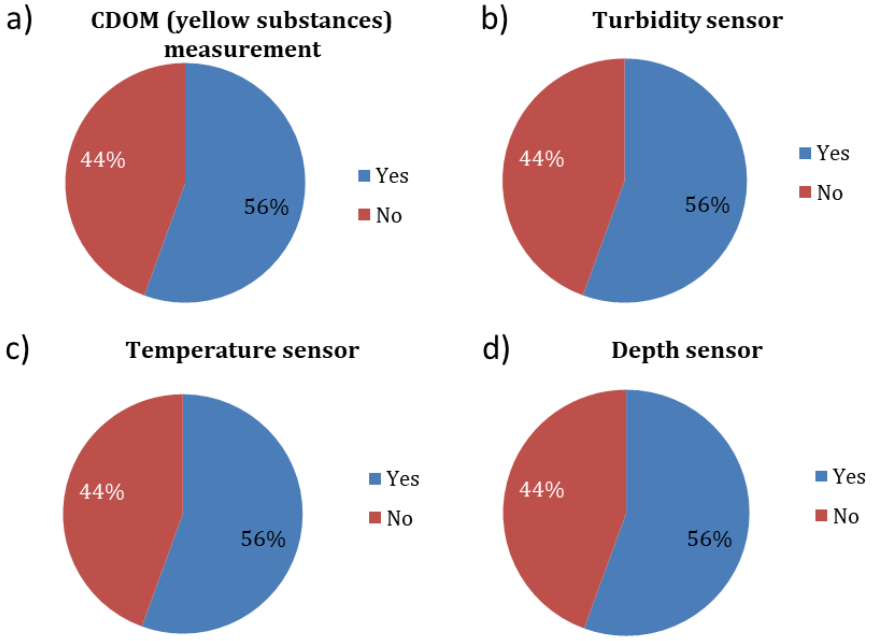

Figure 32: Supplementary sensors associated to fluorometers described from fluorometry polls

2.1.3. Deployment and measurement strategy

The fluorometers described from the poll are mainly (58 %) deployed on research vessels (coupled to a ThermoSalinoGraph or a FerryBox systems), 21% on ships of opportunity (FerryBox) 14% on buoys and 7% on gliders (Figure 33).

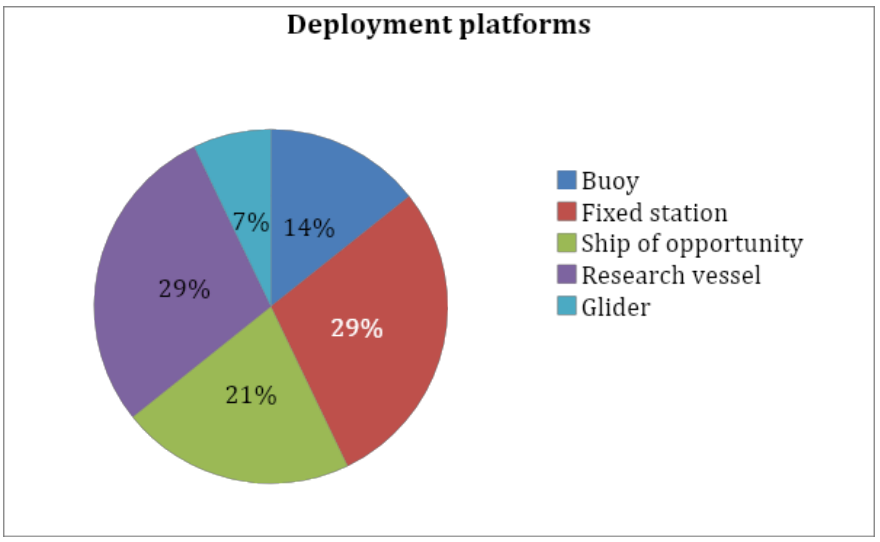

Figure 33: Deployment platforms for in vivo fluorometers described in the in vivo fluorometry polls

The measurement strategy is equally distributed into continuous recording from underway seawater pumping, *in-situ* profiling and discrete sampling (using benchtop configuration, Figure 34).

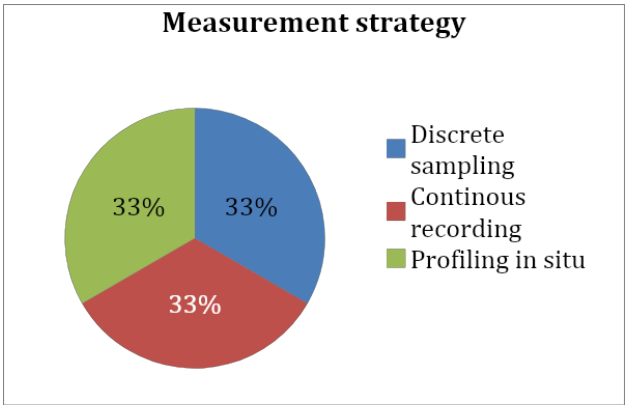

Figure 34: Measurement strategy for in vivo fluorometers described in the in vivo fluorometry polls

2.1.4. Fluorometer stability

In order to ensure the stability of the fluorometer sensors, 56% percent of the respondents tested the stability of machines (Figure 35) in the laboratories, whereas 44% sent the machine to the manufacturer for performing the stability test and re-calibration. The majority of the scientists performing their own calibration proceed to test their machines on filtered seawater (42%) or pure deionised water (33%; Figure 36). Only 17% perform tests on cultures and 8% on a fluorochrome solution. One alternative way is referring to an external fluorometer calibrated by a solid standard to follow the stability of fluorometric measurements.

Finally, 2/3 of respondents do not replace the spectra of pure waters and/or CDOM on their own (Figure 37)

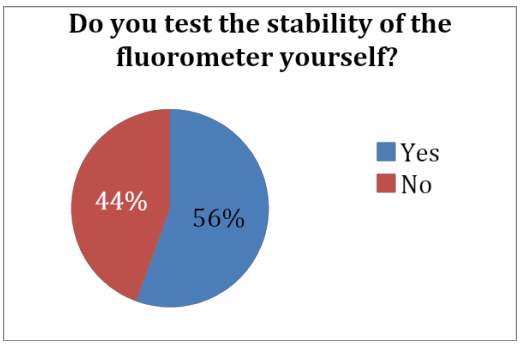

Figure 35: Testing the stability of in vivo fluorometers described in the in vivo fluorometry polls

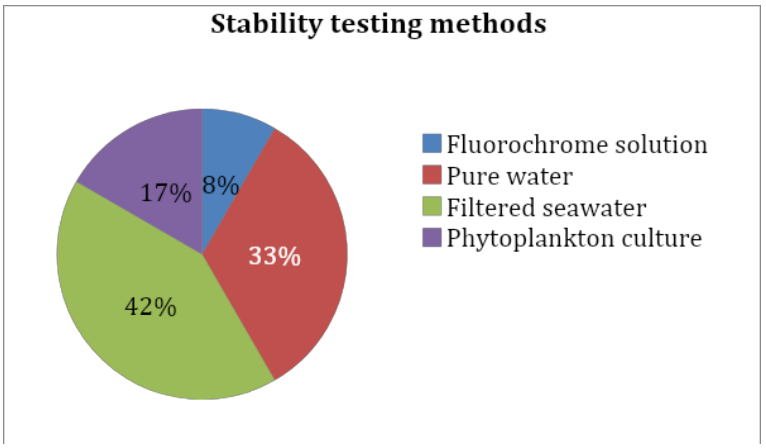

Figure 36: Methods applied for testing the stability of in vivo fluorometers described in the in vivo fluorometry polls

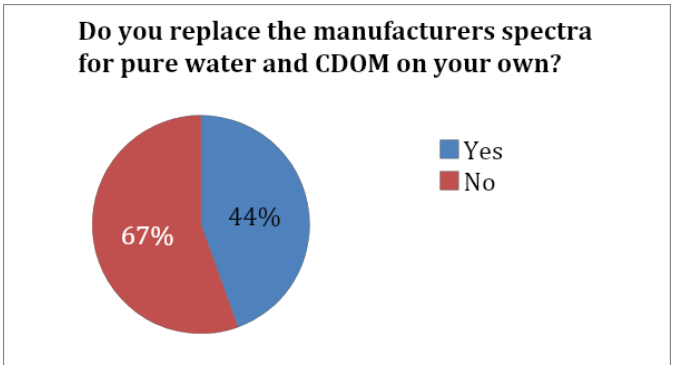

Figure 37: Spectra for pure water and CDOM for in vivo fluorometers described in the in vivo fluorometry polls

2.1.5. Phytoplankton fingerprints and data analysis

Considering multispectral fluorometers, 2/3 of respondents do not produce their own fingerprints (Figure 38), whereas the rest of them have tested and/or implemented their own fingerprints.

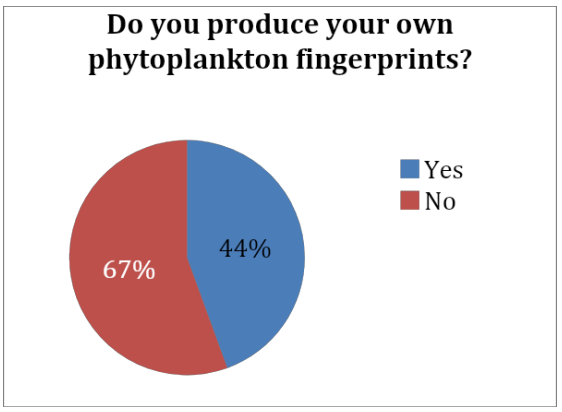

Figure 38: Phytoplankton fingerprints for in vivo fluorometers described in the in vivo fluorometry polls

When referring to the type of data analysed on multispectral fluorometers, 1/3 of respondents analyse LED raw data (either by choice for Fluoroprobe/AOA or because it is the only data available (MultiExciter), 28% rely on manufacturer's defined groups, 22% rely on specific fingerprints built on natural blooms and 17% from monocultures of single species (in addition to those already proposed by the manufacturer's and updated at each maintenance (Figure 39).

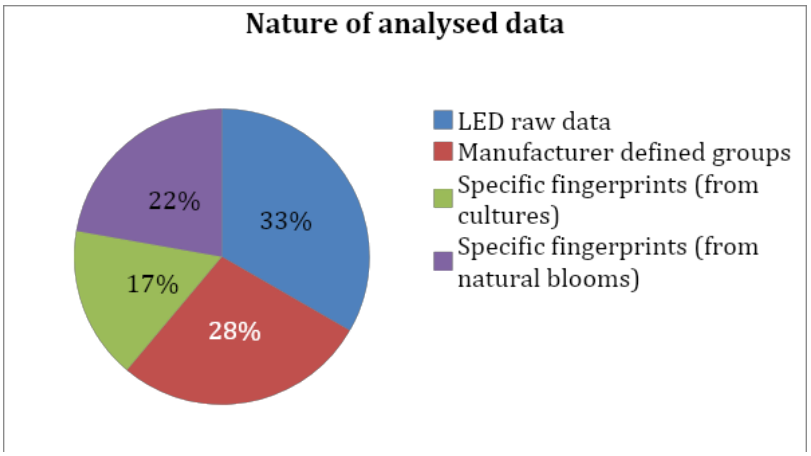

Figure 39: Type of data analysed from in vivo fluorometers described in the in vivo fluorometry polls

2.1.6. Maintenance

The majority (78%) of respondents send the devices to the company for (re-)calibration and maintenance (Figure 40). The rest of them either do not rely on the re-calibration proposed (as it will remove the supplementary fingerprints added by the user) or find it too difficult to do so (company really far from the user's laboratory). Half of experts/users send it only when an issue occurs, whereas 38% of them keep a maintenance/calibration rhythm of two years (as for CTDs) and 12% send it to the manufacturers after each long deployment (Figure 44).

Finally, only 44% proceed to a deep cleaning of the device's optics (especially after long deployments; Figure 42) whereas the rest rely on the manufacturers to do so.

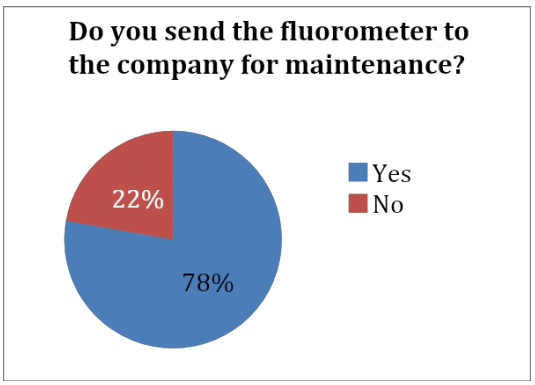

Figure 40: Maintenance on in vivo fluorometers described in the in vivo fluorometry polls

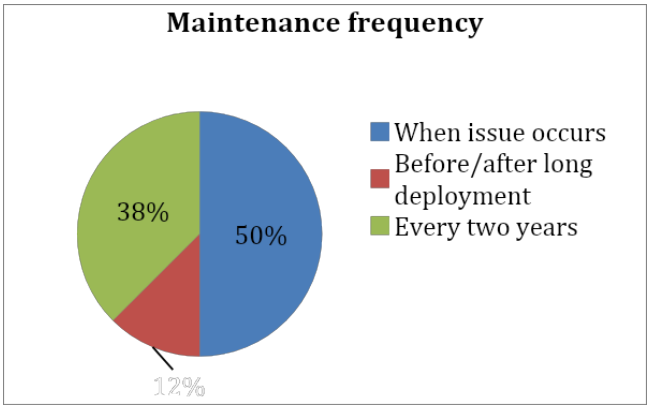

Figure 41: Frequency of maintenance for in vivo fluorometers described in the in vivo fluorometry polls

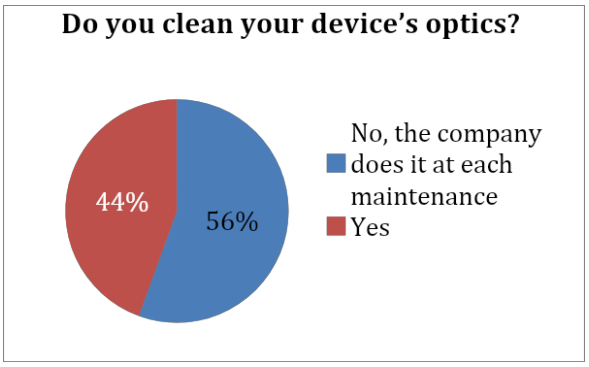

Figure 42: Optic's cleaning on in vivo fluorometers described in the in vivo fluorometry polls

2.1.7. Sample validation

The majority of respondents (56%) does necessarily apply other methods to calibrate/intercompare/validate the results obtained addressing total chlorophyll *a* or chlorophyll *a* per spectral groups (Figure 43).

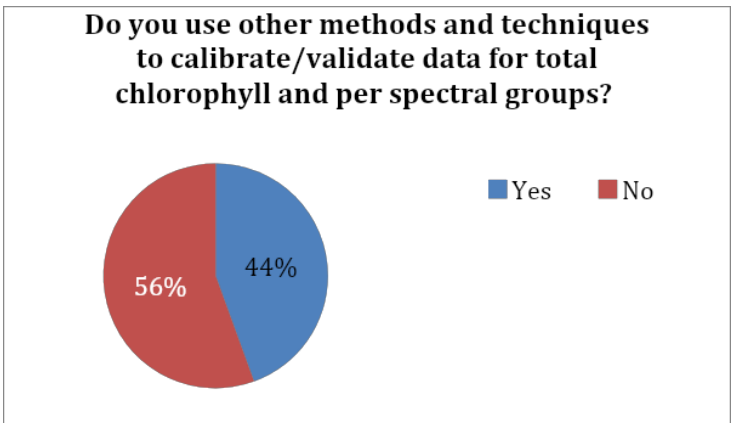

Figure 43: Validation of data from in vivo fluorometers described in the in vivo fluorometry polls

Among the 44% of respondents who does calibrate/intercompare/validate their fluorometric data, 45% of them does it compared to an in vivo fluorometer which is calibrated by a solid standard, whereas the rest compare spectral groups and/or total chlorophyll *a* data with pigments assessed by HPLC and/or in vitro fluorometry (for total chlorophyll *a* estimations) (Figure 44).

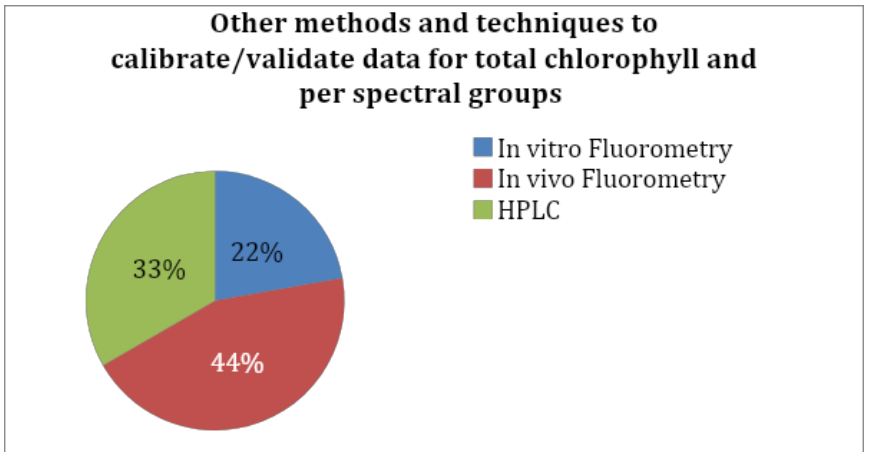

Figure 44: Methods for data validation for in vivo fluorometers described in the in vivo fluorometry polls

Finally, the majority (56%) of questionnaires describes the need of performing dark acclimation of samples (either for discrete or continuous underway sub-surface pumping samples) whereas the rest performs in situ measurements (fixed sub-surface or profiling) without having the possibility of dark acclimation before measurements and can experience non-photochemical quenching (which need to be addressed for the Quality Control of data) (Figure 45).

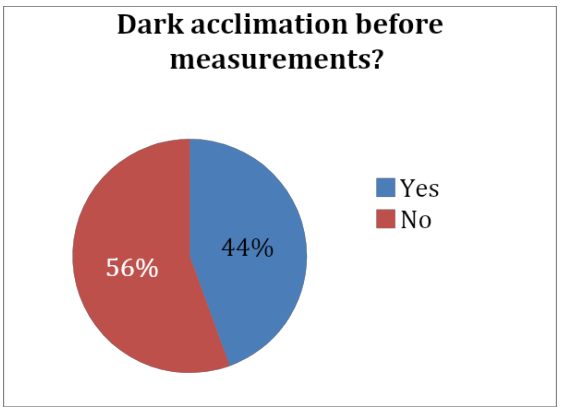

Figure 45: Dark acclimation before measurements on in vivo fluorometers described in the in vivo fluorometry polls

2.2. Discussions carried out during the dedicated Workshops on Best Practices for in vivo fluorometry for phytoplankton detection (September 9, 2022) and perspectives*

One online workshop was held online on September 9, 2022 attended by 8 scientists from 6 laboratories and five countries. .

2.2.1. Lessons learned

This questionnaire highlighted the use of multiple fluorometers of single and multiple wavelengths. The results should help find complementarity in operational practices by the comparison between machines and institutes

Many participants shared their protocols for their respective machines. With their approval, a repository of protocols shared with everyone would help reach a consensus per device and, subsequently, define a reference for best practices. This work will be continued in the frame of new projects in which JERICO partners are involved as the OBAMA NEXT European project on novel approaches for addressing and mapping marine biodiversity.

However, the poll gathered a very low participation. Moreover, some questions might need to be clearer as it was difficult to carry interpretation of the results obtained.

2.2.2. Round table about next steps to take

One first remark concerns the fact that the deployment of several monospectral machines can give multispectral results if combined. Thus, we might need to extend the poll to all types of *in vivo* fluorometers used for monitoring phytoplankton and, especially, phytoplankton blooms.

Most laboratories rely on single- wavelength fluorometers (as the Seapoint from CEFAS) deployed *in situ* on buoys/moorings and/or for continuous underway measurements coupled to a FerryBox operated in both research cruises (on board Research Vessels) and in ships of opportunity (Ferries). SYKE deploys ten different phycocyanin sensors and a Multiexciter device to monitor phytoplankton and cyanobacteria dynamics, both in Ferry and in fixed

autonomous stations as Utö in the Baltic Sea (see JERICO NEXT reports of Petersen & Möller, 2017 and Möller et al., 2019).

One important complementary measurement concerns the yellow substances addressed mainly to correct total chlorophyll *a*, whereas others seek for addressing properly the Chromophoric Dissolved Organic Matter (CDOM), especially in coastal waters.

Concerning multispectral devices, some laboratories (such as CNRS LOG) have built their own fingerprints on the main species responsible for phytoplankton blooms and which can be characterised by their specific pigment composition, as *Phaeocystis globosa* tHouliez et al., 2012). When one makes a fingerprint, care should be taken when sending the device for calibration as the manufacturer would delete it. In the case of haptophytes, after several discussions, the BBE Moldaenke Company now proposes a haptophyte fingerprint when sending the machine for calibration or maintenance.

Getting access to raw fluorescence data (per LED) would allow comparing and following the sensitivity of the device when compared to other fluorometry measurements used as standards and/or to chlorophyll *a* and major pigments' concentrations addressed by HPLC.

It is also recommended to send the machine every two/three years to the manufacturer to to follow any possible drift in sensitivity and analytical performance of the devices (Salter et al., 2020) even tough for some devices the cost is too elevated (Multiexciter from JFE Advantech Co) in Japan.

When processing the data from *in-vivo* multispectral fluorometers, it is possible to process raw fluorescence data from each LED by applying a spectral principal component analysis (Alexander et al., 2022). However, it is not possible to provide LED data in a traceable manner, in comparable units. The manufacturer doesn't give conversion factors.

An important nuance in literature – calibration is really not the same as empirical conversion of fluorometric data into chlorophyll *a* concentration: some reports find a big variability in the data collected over 20 years, see Salter et al., 2020. For multispectral fluorometers who provide such calculation correcting the raw data with fluorescence of yellow substances and turbidity (as Fluoroprobe), even though more accurate than monospectral fluorometers addressing in vivo chlorophyll *a*, the in vivo fluorescence/chlorophyll a will also experience bug variability. As data needs to be uploaded to databases, the units of measurement should be considered, maybe nuanced so it is not taken for a pure concentration.

The relative contribution of each spectral group to the estimated total chlorophyll a is to be taken mostly as indicative for those reasons and because the algorithm proposed to define them (Beutler et al., 2002) does not consider the variability of spectra within and amongst phytoplankton pigmentary groups and taxa.

Therefore, as different methods can be applied to obtain data, it can be useful to specify in the metadata how one obtains it to lessen confusion and misinterpretation

Finally, after discussion, we find out that harmonisation is quite complicated as it is not possible to force people to work a certain way. Nevertheless, operational procedures can be compared and we can move forward to report on the best way of performing good measurements (Möller et al., 2019). It might be even more important to consider

standardising how people process and report on *in-vivo* fluorometric data (see Karlson et al., 2017 and Artigas et al., 2019). A set of guidelines designed and agreed upon will be the main challenge and goal to achieve for moving towards better interoperable phytoplankton *in vivo* fluorometric data.

2.3. Case Study: Fluoroprobe

2.3.1. Design Overview (from BBE Moldaenke)

The BBE FluoroProbe is a highly sensitive multispectral fluorometer dedicated to the analysis of in vivo chlorophyll with algae class determination. Individual profiles are taken during profiles, underway or discrete measurements for four spectral groups at a time: green algae, blue-green algae/cyanobacteria, diatoms/dinoflagellates and cryptophytes, considering the principal manufacturers' groups, with the possibility of adding new more specific groups. This enables the analysis of the occurrence and distribution of these groups at a higher frequency and in real time than laboratory analysis of photosynthetic pigments and/or species. This device has been used for a decade now by some JERICO partners as well as external marine laboratories mainly in Europe.

Possible interferences due to yellow substances are eliminated by an integrated CDOM correction factor.

Chlorophyll *in vivo* measurement can be affected by particulate matter in the water body. Therefore a correction is essential when the turbidity is too high. The BBE FluoroProbe with the optional transmission sensor provides automatic turbidity correction using the latest BBE++ software extension. Turbidity correction with the FluoroProbe makes chlorophyll determination even more reliable.

The BBE FluoroProbe can quickly and reliably address the chlorophyll content of different algae classes. The measurement data can be displayed in real-time on a PC or be stored in the probe for subsequent evaluation.

2.3.2. Functioning

The Fluoroprobe's core functionality revolves around measuring and analysing fluorescence emitted from samples under specific excitation conditions. The process begins with the excitation phase, where the device employs a high-intensity light source to excite the pigments present in the sample. This excitation light, filtered through the chosen excitation filter, isolates the appropriate wavelength needed for the fluorophore.

Once excited, the fluorophores (pigments in the case of phytoplankton) in the sample emit light at a longer wavelength. This emitted light is then collected by the optical system of the Fluoroprobe. The emission light passes through an emission filter, which isolates the specific wavelength of interest to ensure that only the relevant fluorescence signal is measured, thus minimising any background interference.

The photodetector within the Fluoroprobe measures the intensity of the emitted fluorescence. This data is processed by the integrated software, which provides quantitative and qualitative insights into the sample.

Results are displayed on the user interface, where researchers can view real-time data, conduct analyses, and export findings for further examination. The software also supports functionalities such as calibration, data correction, and statistical analysis, ensuring comprehensive data evaluation.

The fluorescence of algae due to excitation by visible light mainly depends on the presence of chlorophyll-a, a common pigment in the plant world. The occurrence of other pigments is typical for the different algae classes. Interactions between these different pigment systems with chlorophyll-a result in a special excitation spectrum for the spectral/pigmentary algae groups (to be nuanced, see previous section).

The special patterns of this algae fluorescence - so-called fingerprints - are used in the bbe fluorometers for the quantification of different algae classes. The excitation light sources are LEDs with different wavelengths. The fingerprints of four algae classes and of yellow substances are already stored in the FluoroProbe. Special user-defined fingerprints can also be added as *Planktothrix rubescens* (for Phycoerythrin cyanobacteria in freshwaters) or *Isochrysis galbana* (Haptophytes in marine waters, after a previous work on Pheaocystis globosa blooms and cultures carried out by CNRS LOG - Houliez et al., 2012).

2.3.3. Available training materials and contacts

Main recommendations are found in the manufacturers' website (https://www.bbe-moldaenke.de/en/products/chlorophyll/details/fluoroprobe.html). A synthetic description is provided in D5.1 (Gallot & Artigas, 2021). Moreover, recommendations and example of applications in coastal marine systems from brackish to marine coastal systems can be found in JERICO-NEXT deliverables (Karlson et al., 2017; Petersen and Möller, 2017; Artigas et al., 2019; Möller et al., 2019).

3. OUTREACH, DISSEMINATION AND COMMUNICATION ACTIVITIES

The three dedicated polls on operational best practices for automated flow cytometry, imaging devices, and *in vivo* fluorometry were advertised on the JERICO-RI website (see Annexes) as well as on different mailing lists and networks, including ICES and IOC experts groups/

Moreover, a dedicated workshop international workshop (TT-CYTO "Tips and Tricks towards flow CYTOmetry data Fairness"), hosted on June 4-6, 2024 by CNRS LOG in Wimereux (FR), co-organised by CNRS, CEFAS, SYKE, SZN and HCMR was promoted, through the JERICO S3, EUROMARINE, and OBAMA-NEXT websites, as well as via direct contact using Cytobuoy's mailing list. The workshop welcomed engineers, technicians, students, and scientists with varying levels of flow cytometry experience. During the event, participants expressed interest in ongoing communication for sharing experiences, ideas, troubleshooting, and improving data handling and management.

Concerning imaging approaches, the summer GlobalHAB hybrid symposium on automated *in situ* observations of plankton was hosted at the Kristineberg Marine Station (University of Göteborg) organised by Bengt Karlson. It was co-funded by JERICO S3 and SCOR, it was

advertised on the JERICO S3, the Global Ocean Observing System, the SCOR and the IOC UNESCO websites, as well as on GlobalHAB. A summary was published on the "Harmful Algae News n° 71" (http://www.e-pages.dk/ku/1549/)

The advances and discussions about the operational procedures and practices, inter comparison and calibration issues, as well as case studies applying either one or the three approaches described in the present deliverable, were disseminated in workshops of different working groups (as ICES WGPME Phytoplankton and Microbial Ecology, WGIMMT on Integrated Morphological and Molecular Taxonomy, IOC Working Group to Investigate Climate Change and Global Trends of Phytoplankton in the Oceans - Trends PO, Imagine/Imaging the Atlantic – A Pelagic Imaging Network Approach, etc.).

Moreover, co-authors and participants,to this deliverable and workshops presented the advances and applications of these approaches in national and international congresses and symposia, coordinating the "ASLO 2021 Aquatic Sciences Virtual Meeting Special Session 28: Aquatic microbial community structure and dynamics: new insights from non-destructive high throughput automated single cell analysis" in June 2021 and presenting oral communications and posters at ASLO Ocean sciences Meeting 2024, the International Congress on Harmful Algae (ICHA 2023) in Hiroshima, EuroGOOS 2021 and 2023, ICES ASM 2022, FerryBox Workshop 2022, amongst others. Finally, presentations were also made by invitation to present the advances on networks (IT/ITAPINA) or other projects (MESAQUA).

4. CONCLUSIONS

During the JERICO S3 project timeline, it was possible to capitalise on the exhaustive documentary work carried out within JERICO-NEXT (see deliverables related) and to move forward through an open discussion based on the results of the polls and the experience gathered within PPS and IRS (WP3 and WP4). Moreover, external inputs from non-JERICO Partners and exchanges were effective during the four dedicated workshops and in different international expert groups and international networks (including experts from many European countries and abroad). Even though we could not define the final Operational Best Practices for the three techniques/approaches considered, we contributed actively to the improvement of awareness about good operational practices, that helped in the choice of sensors that were deployed in WP7 coast-EGIM in the Bay of Seine in 2023 and we were able to also contribute to the definition of Best Practices for data provision to databases (WP6) and proposed to VA tools for data processing (on FCM and Imaging).

The work towards the definition of Operational Best Practices on novel automated flow cytometry, imaging, and in vivo multispectral sensors for monitoring phytoplankton (FCM and in vivo Fluorescence) and phyto- and zooplankton (in situ/in-flow/benchtop imaging) is still a long way to go. Nevertheless, we are confident that with the effort gathered together, through a wider audience, we will be able to move towards the objective. We will benefit from new networks of experts as well as from our involvement in recently awarded EU projects dealing with marine biodiversity as OBAMA NEXT, DTO-Bioflow, Bio-Ocean 5D, ANERIS, DiverSea. Finally, monitoring programs at national levels started to consider including some of these novel and automated techniques for coastal biological monitoring. The success of this implementation will be related to defining and following the best standard operational procedures possible. The continuing of consensual and rigorous discussion would make that possible with the final goal of obtaining F.A.I.R. data to provide to the community of sciences, environmental managers, and society.

5. ANNEXES AND REFERENCES

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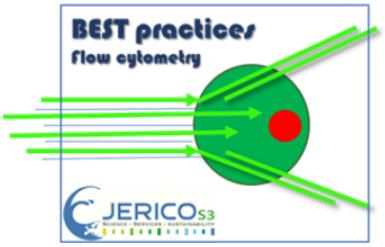


In JERICO-S3, we continue our efforts towards measuring synchronously different variables (especially biogeochemistry and biology) and filling observational gaps in under-sampled areas to understand phytoplankton dynamics and distribution in coastal waters. Our task is to improve the readiness of ship-based and autonomous platform observing networks by guaranteeing their robustness, reliability, and long-term sustainability.

A questionnaire (not longer than 15 minutes to fill) aims to collect the different practices followed by the users and to define the best practices for in vivo automated (including online) flow cytometry. The results will be presented and discussed during a virtual workshop early next year. Participants will be invited to join through existing networks.

The questionnaire is available to complete online.

Deadline 8th of January 2021.

Best practices for plankton automated imagery

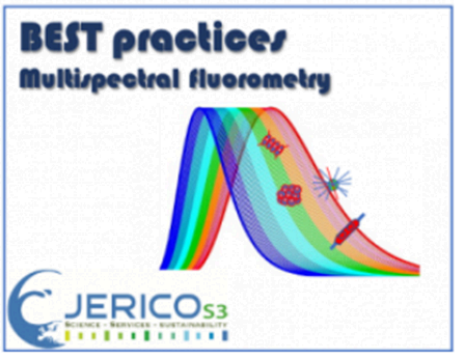
Join our quest! In JERICO-S3, we continue our efforts towards measuring synchronously different environmental variables (especially biogeochemistry and biology) at high frequency and spatial resolution and filling observational gaps in under-sampled areas or periods, to understand plankton dynamics and distribution in coastal waters. Our task is to improve the readiness of ship-based and autonomous platform observing networks by guaranteeing their robustness, reliability, and long-term sustainability. We are pleased to present you our questionnaire on automated imagery (in vivo/in situ, in vivo/in flow, in vitro) for plankton analysis.

This questionnaire (not longer than 15 minutes to fill) aims to collect the different practices followed by users and to help us define common best practice guidelines.

The results will be presented and discussed during a virtual workshop by mid-June. All participants will be invited to join.

Best practices for in vivo fluorometry

Posted on 14th April 2021 |



Join our quest!

In JERICO-S3, we continue our efforts towards measuring synchronously different environmental variables (especially biogeochemistry and biology) at high frequency and spatial resolution and filling observational gaps in under-sampled areas or periods. This helps to understand phytoplankton dynamics and distribution in coastal waters. Our task is to improve the readiness of ship-based and autonomous platform observing networks by guaranteeing their robustness, reliability, and long-term sustainability.

We are pleased to present you our questionnaire on in vivo fluorometry (single wavelength or multispectral) for phytoplankton biomass and pigmentary groups analysis.

This questionnaire (not longer than 15 minutes to fill) aims to collect the different practices followed by users and to help us define common best practice guidelines for in vivo fluorometry.

Complete the questionnaire