DELIVERABLE TITLE:

JERICO-S3 D5.1 Catalogue and checklists for existing biological plankton sensors that will be implemented in JERICO-S3

DELIVERABLE NUMBER: **D5.1, WP5 Task 5.3, Subtask 5.3.3 – ST 7**

WORK PACKAGE N° and NAME: WP5-NA4: Harmonisation of integrated Multiplatform & Multidisciplinary systems

Authors: Gallot, C. & Artigas L.F. (CNRS-LOG)

Contributors: Créach, V., Borst, K., Brunetti, F., Cabrera, P., Cantoni, C., Deneudt, K., Eikrem, W., Frangoulis, C., Grégori, G., Karlson, B., King, A., Lefebvre, A., Lindh, M., Lizon, F., Lombard, F., Möller, K.O., Rühl, S. Lars- Naustvoll, L.J., Pfannkuchen, M., Rombouts, I., Salter, I., Seppälä, J., Steemann, L. & Vitorino J.

Involved Institution: Lead: CNRS-LOG; Partners: SMHI, SOCIB, NIVA, CNRS-MIO, CNRS-LOV, CNRS-BOREA, HCMR, CNR, AZTI, NORCE, IFREMER, CEFAS, IRB, VLIZ, Hereon (previously HZG), SYKE, IH, OGS, FAMRI.

Due date // Submission date: 31/03/2021 **//** 05/08/2021

Nature: **R**

(R = Report, P = Prototype, D = Demonstrator, O = Other)

Dissemination level: PU

PU = Public, PP = Restricted to other programme participants (including the Commission Services), RE = Restricted to a group specified by the consortium (including the Commission Services), CO = Confidential, only for members of the consortium (including the Commission Services)

GRANT N°: 871153
PROJECT ACRONYME : JERICO-S3
PROJECT NAME : Joint European Research Infrastructure for Coastal Observatories - Science, services, sustainability
COORDINATOR : Laurent DELAUNEY - Ifremer, France - jerico@ifremer.fr

DOCUMENT TECHNICAL DESCRIPTION

Document ID	JERICO-S3-WP5-NA4-D5.1 -05.08.2021-V7

JERICO-S3 DELIVERABLE

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

DELIVERABLE n° WP and full title	JERICO-S3 WP5, Task 5.3, Subtask 5.3.3 - D5.1 : Catalogue and checklists for existing biological sensors that will be implemented in JERICO-S3
Description	The task 5.3.3 aims at progressing towards the definition of best practices on the implementation/deployment of biological automated sensors following the JRAPs activities performed in JERICO-NEXT (MS5.1). The focus is made mainly on phytoplankton functional diversity using flow cytometry and multispectral fluorometer. Phytoplankton and zooplankton diversity will be addressed by in-flow and *in situ* imaging. This task will define operational and calibration procedures, determine flags to be implemented in the metadata base (WP6), develop specific recommendations according to the IRS and PSS specificities and platform types for sampling strategy (D5.6).
Lead beneficiary	CNRS - Laboratoire d'Océanologie et de Géosciences
Lead Authors	Clémentine Gallot & Luis Felipe Artigas (CNRS-LOG)
Contributors	Véronique Créach, Kees Borst, Fabio Brunetti, Patricia Cabrera, Carolina Cantoni, Klaas Deneudt, Wenche Eikrem, Costas Frangoulis Gerald Gregori, Bengt Karlson, Andrew King, Alain Lefebvre, Markus Lindh, Fabrice Lizon, Fabien Lombard, Klas Ove Möller, Saskia Rühl, Lars-Johan Naustvoll, Martin Pfannkuchen, Isabelle Rombouts, Ian Salter, Jukka Seppälä, Lars Steemann, João Vitorino
Submitted by	Luis Felipe Artigas

REVISION HISTORY

Revision	Date	Modification	Author
V1.0	05/06/2021	First version	Clémentine Gallot & Luis Felipe Artigas
V2.0	21/06/2021	First set of modifications	Clémentine Gallot & Luis Felipe Artigas
V3.0	01/07/2021	Second set of modifications and message sent to all JS3 Sub-task 5.3.3 participants	Clémentine Gallot & Luis Felipe Artigas
V4.0	28/07/2021	Third set of Modifications according to the suggestions	Clémentine Gallot & Luis Felipe Artigas after comments sent by

		received from JS3 Sub-task 5.3.3 participants	Véronique Créach, Wenche Eikrem, Andrew King, Klas Ove Möller, Jukka Seppälä, Saskia Rühl, Ian Salter
V5.0	29/07/2021	Fourth set of modifications	Clémentine Gallot
V6.0	04/08/2021	Last modifications	Luis Felipe Artigas
V7.0	01/09/2021	Approval	Julien Mader

APPROVALS				
	Name	**Organisation**	**Date**	**Visa**
Coordinator	Delauney Laurent	Ifremer	10-08-2021	X
WP Leaders	Julien Mader	AZTI	01-09-2021	X

Diffusion list			
Consortium beneficiaries	Third parties	Associated Partners	other
X		X	

TABLE OF CONTENT

Sommaire

TABLE OF CONTENT 3

1. EXECUTIVE SUMMARY 4

2. INTRODUCTION 4

3. MAIN REPORT 5

4. OUTREACH, DISSEMINATION AND COMMUNICATION ACTIVITIES 19

5. CONCLUSION 20

6. REFERENCES 21

EXECUTIVE SUMMARY

This report will take on the WP5 - Harmonisation of integrated multiplatform & multidisciplinary observation, Task 5.3 - Procedures and best practices for observing biological and biogeochemical variables from JERICO-RI platforms, Subtask 5.3.3 - Biological automated sensors (Lead: CNRS; ST7).

As technology develops it allows for more precise detection, characterisation, counting, imaging and processing of plankton variables, better estimation of plankton biomass, better definition of either functional or taxonomic groups as well as assessment of photosynthetic parameters and estimation of primary productivity. For years, past and present JERICO members have tested, inter compared and deployed automated instruments and techniques, many of which are used in several different research facilities, and have developed protocols, usage optimization and operational practices. There is a need for gathering current operational practices and to follow it by discussing common practices that will become best practices after common definition and agreement amongst experts and current users. The deliverable D5.1 is devoted to establishing a catalogue and checklist for existing biological sensors that are being implemented in JERICO-S3 and proposes a map of the sensors potentially operating in the different regions corresponding to Pilot Super Sites (PSS) or Integrated Regional Sites (IRS). Together with the many outreach questionnaires, workshops and activities associated to WP5 Task 5.3 - Subtask 5.3.3 (including JS3 MS25), it aims to survey the different uses and practices of biological automated sensors in order to harmonize as much as possible their use between the different actors of JERICO-S3 who are deploying the sensors in the great variety of European coastal systems.

1. INTRODUCTION

Phytoplankton forms the base of most marine food webs. The number of phytoplankton taxa in the sea have been estimated to be over 10 000. All of them are primary producers but the ecological function of the different taxa varies. Many species do not rely on light as the only energy source but also feed on other organisms (mixotrophy). Phytoplankton vary in size and shape; the size range is approximately 0.8 µm to 1 mm. Colonies of cells may be a few mm in size. Zooplankton is their primary consumer, thus being the link between phytoplankton and higher trophic levels. Their size range goes from microzooplankton (20–200 µm), mesozooplankton (0.2–20 mm) and macrozooplankton (2–20 cm). Traditionally plankton is monitored by collecting water samples or deploying plankton net hauls to concentrate them, and analysing them manually using microscopy. During the last two decades, novel methodologies have been developed to be able to process a much larger number of samples compared to classical monitoring and microscopy analysis, and to do it automated and autonomously. This current report will focus on biological sensors technologies currently used by partners of the JERICO-S3 project (some of them already described, tested and compared in former JERICO-Next reports such as 2.4.2 (Petersen et al, 2017), 3.1 (Karlson et al., 2017) and 3.2 (Artigas et al., 2019). Thus, this report ties together previous JERICO-NEXT reports, biological sensors publications and technical sheets compiled for the WP7, task 7.3.

Within the WP5 focused on the harmonisation of integrated multiplatform & multidisciplinary observation, the task 5.3.3 aims at progressing towards the definition of best practices on the implementation/deployment of biological automated sensors following the JRAPs activities performed in JERICO-NEXT (see MS5.1). The focus is made on phytoplankton functional diversity using flow cytometry and multispectral fluorometry as well as both phytoplankton and zooplankton diversity addressed by in-flow and *in situ* imaging. This task will define operational and calibration procedures, determine flags to be implemented in the metadata base (WP6) and develop specific recommendations according to the IRS and PSS specificities and platform types for sampling strategy (D5.6). To move towards physiological measurements as well at the interface with biogeochemistry (primary productivity), the fast repetition rate fluorometry (FRRF) will be discussed as an emerging technology for measuring primary production.

Deliverable D5.1 is devoted to establishing a catalogue and checklist, for existing biological sensors that are being implemented in JERICO-S3 as well as a map of their deployment in European coastal ecosystems.

2. MAIN REPORT

2.1. Plankton automated imaging systems

In the following sections, we describe plankton automated imaging systems through in flow, in situ and benchtop techniques.

2.1.1. Imaging in flow (in situ or ex situ)

- **Imaging FlowCytobot** (McLane Research Laboratories)

Figure 1 – The IFCB out of its case (source: McLane Research Laboratories)

The Imaging FlowCytobot (IFCB, Fig. 1) is an instrument using both flow cytometry and video technology capturing high resolution images and measuring chlorophyll fluorescence (Olson and Sosik, 2007). It has built-in features, such as self-cleaning, allowing for long periods (over six months) of *in situ* deployment (Lombard et al, 2019). It combines cytometry and imagery to capture plankton (Kraft et al, 2021). It generates high resolution (3.4 pixels per µm) images of suspended particles in-flow, in the size range <10 to 150 µm. The instrument continuously samples at a rate of 15ml of sea water per hour, and, depending on the target population, it can generate on the order of 30,000 high resolution images per hour. The IFCB uses a combination of flow cytometry and video te chnology. Sheath fluid (filtered seawater) focuses the flow of the sample and the plankton organisms are analyzed individually, each organism is imaged. Autofluorescence from the organisms triggers a camera and a flash. The optical and image data are then transmitted to the computer in real time, through an Ethernet communication (Karlson et al., 2017). The images are analyzed using advanced image analyses and machine learning algorithms (Olson and Sosik, 2007). Initially a phytoplankton specialist trains the algorithms for identifying the organisms. After training this is an automated process, although quality control is still needed. Figure 1 shows the IFCB internal instrumentation.

- **FlowCAM (**Fluid Imaging Technologies)

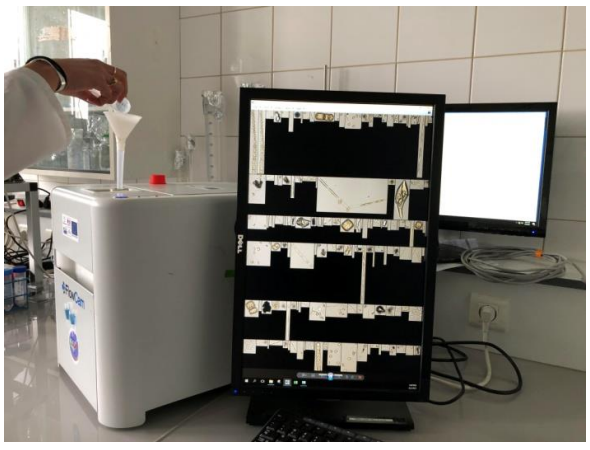

Figure 2 – The FlowCAM 8000 series (source: LOG)

The FlowCAM (Fig. 2) combines selective capabilities of different technologies: flow cytometry, optical microscopy and fluorescence detection. It can generate high resolution images of particles in-flow, in the size range 2µm to 2000µm, depending on the combination "magnification/flow cell" used for the optical system (Karlson et al., 2017). The sample is pulled by a syringe pump into a flow cell with known dimensions, located in front of a microscope objective which is connected to a camera. Each captured particle is imaged and pasted onto a "collage" file grouping multiple images, limiting file size. For particles detection, two operation modes can be used: "Trigger" or "AutoImage". The first one uses a FCM configuration and the scattering of a laser light when a particle passes through and calculates a fluorescence value to compare it with a fluorescence threshold value. If the obtained value is higher, the camera is triggered to take an image (comes with the additional triggering configuration). The "AutoImage" mode is suitable for high particle concentrations samples and allows to calculate the concentration based on the total amount of particles, the

analyzed volume that depend on the flowcell size, image frame dimensions and number of frames (Natunen et al., 2017). Fluid Imaging has a few different models of FlowCams catering to more specific needs such as the FlowCam-nano and FlowCam-macro that target respectively nanoplaknton and zooplankton, one of which is pictured in figure 2. Although primarily used to analyse discrete samples in bench-top mode, the FlowCAM has the capacity to be integrated into underway seawater supplies and analyse samples continuously at pre-defined intervals using the built in scheduling-software. The software also provides the capacity for semi-autonomous classification of plankton using machine-learning algorithms built into the software, although users are required to validate and correct images and quality control is an important task.

- **ZooCAM** (IFREMER)

The ZooCam is a benchtop instrument developed by Ifremer, designed to be deployed onboard research vessels, imaging Zooplankton samples from net tows or a direct source. Seawater is pumped, filtered and sent into a 5L agitator. The sample obtained after plankton net trawls or continuous pumping can then be manually added through a funnel, a pump then passes about 2cm^3 of sample through an optical cell (Ifremer, 2015). The pump speed can be adjusted between 0.28 l per minute to 1.7 l per minute (Colas et al, 2018). A camera then takes pictures at a rate of 15 images per second (Ifremer, 2015). It has a 5.3 µm resolution on which a telecentric 0.5X is mounted (Fig. 3; Colas et al, 2018). Each particle generates an individual vignette that has already foregone some processing. A software then compares the produced image with a reference vignette and suggests a classification. A correction with the human eye leads to software improvement (Ifremer, 2015). This semi-automated image classification allows for real time sample analysis on board (Aubert et al, 2017). Figure 3 schematically represents the picture taking process as well as the complete ZooCAM processing of samples.

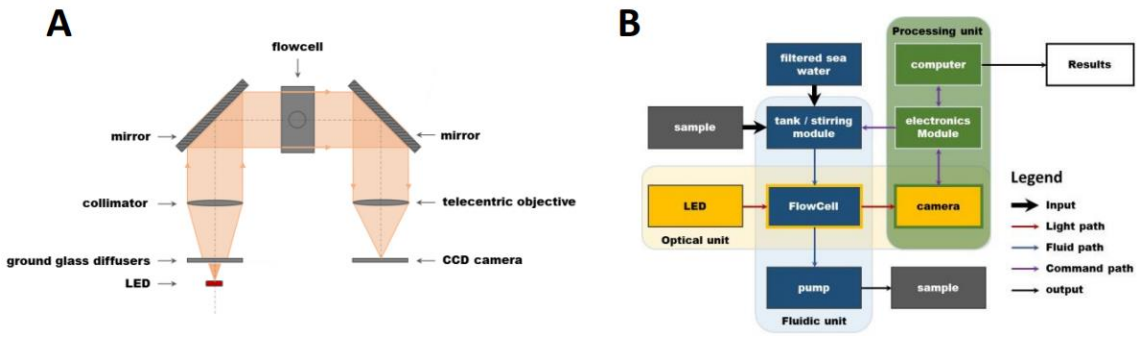

Figure 3: Schematic representation of the ZooCAM (source: Colas et al, 2018)

- **LISST-Holo2** (Sequoia Scientific)

Figure 4 – The LISST-Holo 2 (source: Sequoia Scientific)

The LISST-Holo2 (Fig. 4), takes images at very short shutter times, scanning for a relatively large volume, rendering holographic images of plankton. This digital holographic camera measures the volume, number and shape of large particles ranging from 20-200 µm (Graham and Smith, 2010). The LISST-Holo2 advances the technology that was built into the original LISST-Holo and can capture holograms a hundred times faster. The instrument can be towed and deployed on a buoyant body, as there is an umbilical from which it can draw power. It can be deployed on long periods of time before starting to suffer from biofouling (up to 5 months) (Anderson et al, 2018). Data processing software is included; Holo-Batch processes

a selected group of holograms, whereas Holo-Detail processes detailed manual study of individual holograms (LISST-Holo, 2021).

- **PlanktoScope** (Plankton Planet, Stanford University)

The PlanktoScope (Fig. 5) is a build-your-own instrument allowing for a frugal and cost efficient microscope. It can be mounted in two different modes: fluidic or static. For the fluidic mode, the sample is dragged into a flow-cell by a peristaltic pump at a precision of 1.7 ml per minute with a 1.5 µm resolution (Pollina et al, 2020). It then goes in front of a Pi Camera (v2.1 - 8 megapixels). Each particle taken by the camera is processed into a vignette, see figure 5 (Planktoscope, 2021).

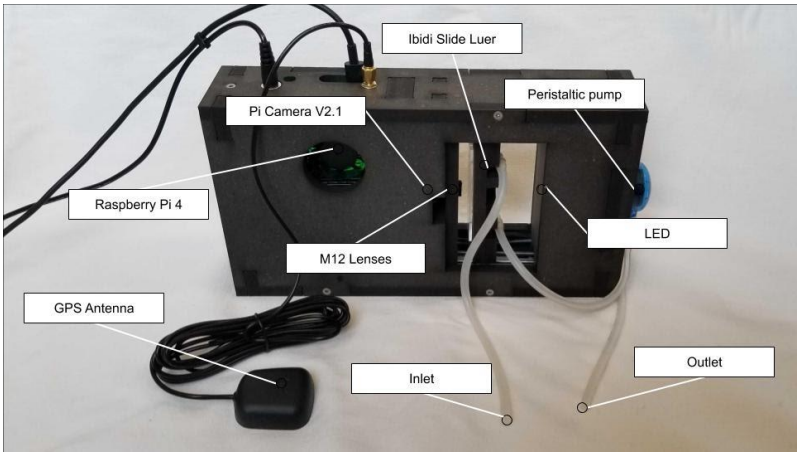

Figure 5 – Planktoscope mechanics (source: Plankctoscope)

2.1.2 In situ imagers

In situ instrumentation is non-invasive by nature and can target more fragile cells that would be damaged by sampling, such as net tows (Lombard et al, 2019). The following instruments can be deployed on more or less extended periods of time and can be mounted on different types of support.

- **Underwater Vision Profiler UVP5** (Hydroptic)

Figure 6 – The UVP5 (source: Hydroptic)

The Underwater Vision Profiler or UVP (CNRS patent, Fig. 6) is an instrument able to record objects illuminated in a slab of water of known volume (Guidi et al, 2008). It is designed to study large (>100 µm) particles and zooplankton simultaneously and to quantify them. It has a 4 MPix camera with a field of view of 180x180 mm^2 (Lombard et al, 2019). The system makes use of computerized optical technology with custom lighting to acquire digital images of zooplankton *in situ* down to depths of 6000m. Mounted on a CTD rosette frame, it acquires only in-focus images in a volume of water delimited by a light beam issued from red light-emitting diodes (LEDs) in 100µs flashes. The typical light beam illuminates an area of 4x20 centimeters which gives a sampling volume of 1 liter per image (Karlson et al., 2017).

- **Underwater Vision Profiler UVP-6LP** (Hydroptic)

The UVP6-LP is a miniaturized and low price version of the UVP5 (Fig.7). It has been designed for low speed, limited space and low power vectors like profiling floats, gliders, floats, moorings and AUVs. It has the same quality standards as the UVP5 and provides a higher resolution of 5 megapixels. It acquires only in-focus images in a volume of water delimited by a single red flashing light illuminating a volume of 0.65 Liter. It can be used for particles greater than 600 µm. Images can be imported on EcoTaxa for particle recognition and classification (Haëntjens et al, 202). The UVP6-LP is inter-calibrated with UVP5std and UVP5hd systems. Just like the UVP 5, it can transmit real time particle abundance to the vector and records vignettes of selected organisms and large aggregates. It is worth noting that the UVP6-LP cannot be utilized on CTD rosette due to its 1.3Hz low acquisition frequency and 500µS flashes. This sensor could be mounted on the GLIDERS used in JERICO3 where it is suitable.

Figure 7 – The UVP6-LP (source: Hydroptic)

- **CPICS Continuous Plankton Imaging and Classification Sensor** (Coastal Ocean Vision)

Figure 8 – The CPICS (source: Coastal Ocean Vision)

The CPICS (Fig. 8) is an underwater microscope using dark field illumination to capture high-resolution colour images, encompassing organisms from 1µm to several cm. This instrument is non-invasive, providing images of fragile phytoplankton, zooplankton and marine aggregates (i.e. "marine snow") (Gallager, 2016). It is mounted with a high magnification, six megapixels colour camera, imaging a volume of seawater adapted to the magnification at the high frequency of > 10 frames per second. The CPICS can be deployed *in situ* on numerous platforms (Lombard et al, 2019). The latest version has an embedded processing feature allowing for real-time region of interest extraction and on-board classification (Coastal Ocean Vision, 2021). Image features including texture, colour pattern, morphology and shape are used to train a classifier based on a CDNN YOLO random walk model. The classification accuracy can mount up to 90% depending on the number of training categories and target complexity (Gallager, 2016).

- **VPR Video Plankton Recorder II**

The VPRII (Fig. 9) is an instrument with a one-megapixel camera, a small diameter tow cable allowing for relatively fast towing on coastal ships (up to 12 knots), a 3-axis motion for automatic movement targeting organisms from 100 µm and up to few centimetres. As it is connected with a fibre optic tow cable, automatic plankton identification is possible through a real-time software (Davis et al, 2005) and can take up to 30 pictures per second at the maximum speed of 12 knots (Aubert et al, 2017). The focal length of the VPRII needs to be short to obtain clear pictures, which implies smaller analysed volumes as increasing the focal length would only render pictures too blurry for analysis. This instrument can also be used on a benchtop configuration.

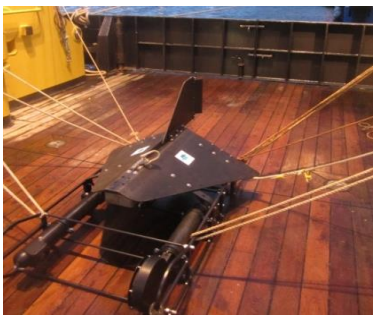

Figure 9 – The VPR II (source: VLIZ)

- **DAVPR Digital Autonomous Video Plankton Recorder** (SeaScan Inc)

Figure 10 – The DAVPR (source:

The Digital Auto Video Plankton Recorder (DAVPR, Fig. 10) is an autonomous underwater digital imaging system designed for rapid imaging and estimation of concentration and vertical distribution of plankton taxa (Davis et al., 2005). The DAVPR may be deployed as an additional sensor on an existing towed or vertical profiling platform or towed alone using the Seascan V-Fin Depressor (SeascanInc, 2021). It is mounted with a high resolution camera able to take 15 image frames per second. Camera field of view and resulting calibrated image volume can be adjusted. Plankton was detected in each image frame as a region of interest (Smith Jr et al., 2021).

2.1.3 Benchtop scanning imagers

- **ZooScan** (Hydroptic)

The ZooScan (Fig. 11) is a plankton scanner composed of waterproof panes to process liquid samples (Gorsky et al, 2010). It produces images with a pixel size of 10.6 µm, sample can be from net tows or bottles. The scanner recovers a high-resolution, digital image and the sample can be recovered without damage. These digital images can then be investigated by computer processing. This instrument has the advantages of evaluating different parameters such as abundance, biomass, size and taxonomic information as well as eliminating abiotic particles that can be very frequent in samples (silt, sediments) (Naito et al, 2019). While the resolution of the digitized zooplankton images is lower than the image obtained using a binocular microscope this technique has proved to be more than adequate for large sample sets. Identification of species is done by automated comparison of the image (vignette) of each individual animal in the scanned image with a library data set which may be built by the investigator for each individual survey or imported from a previous survey. The latest machine-learning algorithm allows high recognition levels and can be combined to manual sorting.

Figure 11 – The ZooScan (source: Hydroptic)

2.2 Single-cell optical characterization

In this section we address single-cell/particle optical analysis by "pulse shape-recording" automated flow cytometry for describing and characterizing the phytoplankton size/functional groups based on the fluorescence properties (pigment content) and scattering properties (size, structure, composition) of individual cells and/or colonies.

- **Automated flow cytometry: CytoSense and CytoSub instruments** (CytoBuoy b.v.)

Figure 12 – The CytoSense on a cruise (source: LOG)

The CytoSense (Fig. 12) and CytoSub – its submersible counterpart, are automated flow cytometers that record optical profiles. Before intercepting a laser beam, the sample is injected in a particle free fluid with the same optical properties (e.g. saline solution, ultra-pure water). It will then stretch out in a very thin laminar thread fluid in which every particle is separated (Dubelaar and Gerritzen, 2001). Every particle passes the laser beam individually and produces pulse shapes of optical properties. Five signals compose each pulse shapes: forward scatter, sideward scatter, red fluorescence, orange fluorescence and yellow fluorescence (Louchart et al., 2020). Particles are recorded above a defined scatter or fluorescence threshold and phytoplankton cells are separated from non-photosynthetic particles according to their fluorescence. An image-in flow device records pictures of preselected groups of cells, resolving cells at its best above 20 µm but able to collect pictures of 2 µm beads (with lower resolution) (Karlson et al., 2017). Each picture is coupled with an optical scattering and fluorescence profile, increasing the classification efficiency (Lombard et al, 2019). The CytoSense sensors are adaptable on ships of opportunity and scientific vessels, whereas the submersible version (CytoSub) fits in fixed stations and buoys, running samples from a subsampling dedicated system isolating sea water from a continuous flow of pumped sea water (Karlson et al., 2017).

- **Automated flow cytometry: CytoPro** (Cytobuoy b.v.)

The CytoPro is a joint project between CNRS-MIO and Cytobuoy to target smaller organisms than typically targeted by the Cytosense (Silovic et al., 2017). It is coupled to a staining module to extend the capabilities of the CytoSense (Cytobuoy) technology allowing for high resolution and high frequency recording of heterotrophs. It can be installed on ships during oceanographic missions and even be controlled at distance (Silovic et al., 2017). Sheath fluid (filtered fresh or seawater) focuses the flow of the particles and the planktonic microorganisms are analyzed individually, with or without a prior staining with one or several fluorescent dyes. Each organism is individually analyzed, and can be imaged (for size > 15-20 µm). Autofluorescence of the organisms is recorded to discriminate autotrophs from heterotrophs. The data and images can be analyzed with advanced data and image analyses and machine learning algorithms.

- **Other automated flow cytometers**

Two other automated cytometry technologies exist, even though not used by JERICO members. Firstly, the Flow Cytobot (FCB), mounted with a 520 nm laser, measures light scattering and fluorescence, and targets smaller particles such as Synechococcus (around 1µm) and cells up to 10µm. Its system is automated, allowing for the instrument to be used *in situ* or unattended (Olson et al, 2003).
More recently, the SeaFlow allows for automated small phytoplankton analysis, including *Prochlorococcus.* It has the particularity of not using a sheath fluid allowing it to continuously sample a direct seawater stream on a ship (Swalwell et al, 2011).

2.3 Bulk bio-optical instrumentation

In this section we describe in vivo bulk approaches LED/multispectral or variable/active fluorometry for analysing the phytoplankton community based on bulk properties: fluorescence or absorption of a large number of cells. Multi wavelength approaches allow differentiating pigment groups of microalgae, whereas variable/active fluorometry addresses photosynthetic parameters and potential primary productivity.

- **LED Fluorometers**

There is a large variety of LED (single-wavelength) fluorometers depending on the studied parameter targeted (here, pigmentary groups), the sensitivity of the instrument and the technical information. For information you can refer to JericoNEXT Deliverable 2.2 from which the following table is sourced.

Table 1: Examples of LED fluorometers (Modified from Petersen et al, 2017)

Chla Sensor	**Manufacturer**	**Excitation/emission wavelength**
ECO FL	WETLabs	470/695 nm
C3/C6P	Turner Designs	460/696nm or <635/>695nm
MicroFlu-chl	Trios	470/685 nm
ECO-Triplet	SeaBird	470/695 nm
Phycoerythrin Sensor		
Eco FL	WETLabs	518/595 nm
C3/C6P	Turner Designs	525/>590nm
ECO-Triplet	SeaBird	518/595 nm
Phycocyanin Sensor		
Eco FL	WETLabs	630/680 nm
C3/C6P	Turner Designs	590/>645nm
MicroFlu-blue	Trios	620/655 nm
ECO-Triplet	SeaBird	630/680 nm
fDOM Sensor		
ECO FL	WETLabs	370/460 nm
C3/C6P	Turner Designs	365/470 nm
MicroFlu-cdom	Trios	370/460 nm
ECO-Triplet	SeaBird	370/460 nm

Figure 14 – The OSCAR (source: Trios)

- **Hyperspectral cavity absorption sensors**

Several cavity absoption sensors are used by JERICO members. Firstly, the PSICAM (Point -Source Integrating Cavity Absorption Meter) is an instrument that measures the absorption over the entire visible light spectrum in water. It consists of a cavity with reflective walls and a central light source (Wollschläger and Grunwald, 2011). This principle allows to measure the real absorption spectra without the use of many assumptions (Oscar, 2021).

Secondly, the OSCAR (Fig. 14) is a new high-end absorption meter, following the principle of the PSICAM. This instrument is suitable for laboratory use, but also for *in situ* profiling and moored applications. Internal data logging function and low power consumption make the sensor suitable for autonomous measurements (Oscar, 2021).

Finally, the Hyperspectral Absorption Sensor is a custom-made sensor and a modified version of the manual or semi-automated PSICAM. The HyAbs is the result of combining the advantages of the PSICAM approach and the high resolution of continuous measurement to overcome obstacles linked to light absorption and scattering (Wollschläger et al, 2016). It measures the absorption coefficients of the water constituents, taking advantage of an integrating cavity for this purpose. Parameters measured are absorption coefficient spectra,

CDOM, Phytoplankton biomass (chlorophyll-*a*), SPM, algal groups Absorption coefficient spectra in the range of the visible light (400-710 nm) with a resolution of 2 nm. Calibration was changed from dye-based to solid standard (Wollschläger et al, 2016). At the moment, the instrument is custom made and not commercially available.

Multi-Spectral fluorometers

- **Multiexciter** (JFE Advantech Co, Ltd)

The Multiexciter (Fig. 15) is a 9-wavelength LED fluorometer (375, 400, 420, 430, 470, 505, 525, 570 & 590 nm), with additional capacity to measure turbidity, temperature and depth. It is an *in situ* instrument and may be equipped with a mechanic wiper to prevent biofouling. Instruments provide data output as raw fluorescence data (arbitrary units) or as µg Chla L^{-1} for those taxonomic groups included in the spectral library of the software (Petersen et al., 2017). It estimates phytoplankton group composition using the observed excitation spectra as they are quantified and identified through mathematical processing from recorded fingerprints or cultivated algae. This instrument can also reduce the noise-effect caused by the reflectance of suspended particles in the water (Yoshida et al, 2011).

Figure 15 – The Multiexciter (source: JFE Advantech)

- **FluoroProbe and AlgaeOnlineAnalyzer** (bbe Moldaenke GmbH)

Figure 16 – The Fluroprobe (source: LOG)

The FluoroProbe (Fig. 16) and AlgaeOnlineAnalyzer (AOA) are highly sensitive benchtop (AOA, Fluoroprobe) or submersible (Fluoroprobe) spectrofluorometers designed to measure total phytoplankton chlorophyll *a* concentration and to discriminate four spectral algal groups: brown algae (mainly Heterokontophyta and Dinophyta but also some signal from Haptophyta), cyanobacteria (cyanobacteria with phycocyanin as major pigment), green algae (Chlorophyta but also some signal from Haptophyta, see Houliez et al., 2012, cryptophytes (Cryptophyta, Rhodophyta, cyanobacteria with phycoerythrin as major pigment). They use 5 LEDs (Fluoroprobe: 470, 525, 570, 590, 610 nm / AOA: 430, 470, 525, 590, 610 nm) for sequential light excitation of the phytoplankton accessory pigments located in photosystem II antenna. An additional LED (370 nm) is used for measurement of chromophoric dissolved organic matter (CDOM or yellow substances). "The complement of accessory pigments varies between phytoplankton, differences in differences in the wavelengths at which Chla fluorescence is stimulated and/or differences in the emission spectra have been used to infer taxonomic structure" (MacIntyre, Lawrenz & Richardson, 2011). LED excitation provide data output such as raw fluorescence data and µg Chla L^{-1} for the taxonomic groups listed above (Karlson et al., 2017). The Fluoroprobe can be deployed in three different operating modes.

Firstly, in laboratory using a docked workstation for discrete samples measurements in a 25 mL optical glass cuvette and ambient light excluded from the optics by a cover.

Secondly, an *in situ* as a profiler can be used for water column measurements at several depths. It can be deployed with a cable and can be lowered into the water by hand or by using a winch (maximum of 100m) or by using an "autostart" plug.

Thirdly, a specially designed Flow-Through Unit (inlet and outlet connections) allows the sample water to be transported using an external pump. The AOA can be deployed in laboratory using a specially developed

workstation, in high frequency measuring stations or associated with other sensors in a pocket ferry box or a ferry box, see Lefebvre & Poisson Caillault, 2019.

Instruments for measuring variable/active fluorescence

- **FRRF Fast Repetition Rate Fluorometry**

Figure 17 – Act 2 (source: Chelsea Technologies)

Fast Repetition Rate fluorometers measure phytoplankton photosynthetic activity. Instruments used by JERICO partners are the machines from Chelsea Technologies. There are other models of FRRF fluorometers including FiRE from Satlantic. These instruments work with short intense light flashes cumulatively causing single closure (Single Turnover) of PSII reaction centres. From the fluorescence induction curve it is possible to fit different parameters, like functional absorption cross-section of PSII and connectivity parameter (Kolber et al 1998). FRRFs are usually equipped with a blue light, which preferentially excites PSII antenna of algae but underestimates species with low PSII cross section in the 400-500 nm region such as cyanobacteria. FRRFs can be equipped with two or three excitation wavebands (multi-spectral approach) in order to encompass a larger range of phytoplankton (Houliez et al, 2017). JERICO members use the FastOcean Fast Repetition Rate (FRRf) system (Chelsea Technology Group). It is designed for profiling systems (APD system, Ambiant Plus Dark sensor) and moorings but when coupled with an Act2 system (Fig. 17), it can be used in laboratory or in flow through applications, allowing automated measurements of Fluorescence Light Curves (FLCs). The inlet depth varies with ship, but is in general between 3-4 m, so the photosynthetic activity of the phytoplankton in the surface mixed layer is measured (Artigas et al, 2019).
A new variable fluorescence technology has been recently developed, the LabSTAF (Chelsea Technologies). This instrument is also a Single Turnover Active Fluorometry (STAF) for measuring phytoplankton primary productivity (PhytoPP) and photophysiology, which is a fully automated acquisition allowing for continuous measurements without manual intervention (Chelsea Technologies, 2021).

2.4 Overview of available automated sensors for plankton observations implemented in JERICO S3

Out of the many instruments used by JERICO S3 partners, we can distinguish a shared amount of *in situ* tools and fixed station tools (laboratory or onboard). The *in situ* automated sensors are the following: the Imaging FlowCytoBot, the CytoSub, the LISST-Holo, the UVP5 and UVP6-LP, the CPICS, the VPR, most LED fluorometers, the FluoroProbe, the Multiexciter, the FRRfs. While the "benchtop" sensors (which can be used on-board research vessels for manual or automated pumping and recording, the latter more suitable for fixed stations) are: the FlowCAM, the ZooCAM, ZooScan, the Planktoscope, the CytoSense, the CytoPro, the AOA, the PSICAM, OSCAR and HyAbS, the Fast Ocean FRRf Act2 and LabSTAF. After adapted configuration, the following instruments can also be used "benchtop": the IFCB, the CytoSub, most LED fluorometers, the Fluoroprobe and the Multiexciter. This diversity in deployment is useful as it can cater to different spatial and temporal needs, as well as target different type of organisms and environments.
Automated flow cytometers are less diverse compared to plankton imagers. Imagers vary in their functioning as inflow instruments (automated or not), underwater imaging or holographic imaging. They collectively cover a large range of particles from small phytoplankton (picoplankton) if coupled to automated flow cytometers, to nano- and microplankton with adapted inflow systems as the CytoSense/sub (from 1µm to 800µm) and inflow imagers (CytoSense as well and IFCB up to 150 µm), to bigger phytoplankton (big colonial stages), micro-aggregates including marine snow and zooplankton with underwater *in situ* imaging profilers.
Regarding bulk bio-optical instrumentation, there is a great diversity of single LED (mono-spectral) instruments, some of which are highlighted in this report. In some cases, the combination of multiple LED fluorometers can result in an effective multi-spectral analysis. Multispectral fluorometers are fewer in number and some of them still

need to be confirmed as fully functional in marine open ocean oligotrophic waters, such as the Fluoroprobe. These tools originated in the need for multispectral excitation of different pigments in order to be able to differentiate different phytoplankton groups and for example to address environments in which cyanobacteria form a good share of the phytoplankton biomass, as they are usually underestimated by instruments only using blue excitation light (Raateoja et al., 2004). FRRfs are adapted to a wide range of marine systems from oligotrophic open ocean to eutrophic coastal systems.

Within the JERICO S3 consortium, automated observation potentialities and effective deployments are not distributed equally amongst partners (Table 2) neither amongst Integrated Research Sites (IRS) nor Pilot Super Sites (PSS) across European coastal systems. Imaging inflow benchtop techniques, mainly devoted to phytoplankton and microzooplankton, are well expanded especially from Baltic to the NW Mediterranean (FlowCAM) including Norwegian Sea and Faroe Islands, or only deployed in the Baltic, Norwegian Sea, Skagerrak/Kattegat and North Sea (IFCB). *In situ* imagers (targeting both colonial phytoplankton, zooplankton and aggregates) are well represented in the Baltic, North Sea, English Channel and NW Mediterranean whereas benchtop scanning imagers are mainly used by partners of both the North Sea and English Channel as well as NW Mediterranean. Single-cell optic characterization of mainly phytoplankton (automated flow cytometry) is deployed from the Baltic to the North Sea and English Channel, as well as NW Mediterranean, Adriatic and Cretan Seas. Hyperspectral cavity absorption sensors are mainly deployed in the Baltic, Norwegian and North Sea.
LED fluorometers are used in all IRS and PSS (wide range of machines associated to profiling CTDs or continuous recording systems), sometimes associated together in order to perform a multi-spectral approach. On the contrary, multi-spectral machines are less expanded in the JS3 consortium, even though they are mostly deployed from the Baltic to the North Sea and English Channel (excepting some joint research activities between PSS and IRS). Finally, variable/active fluorometers are deployed mainly in continuous recording systems from buoys/fixed stations to research vessels (mainly) in the Baltic, North Sea and English Channel, whereas the profiling system is only deployed in the English Channel.

Table 2: Biological sensors, usage and availability (based on questionnaires and JS3 MS25; Artigas et al., 2021)

Sensor	**Variables measured**	**Detection limit range**	**Availability**	**JERICO acquisition and usage**
Automated imaging systems				
Imaging in flow (in situ or ex situ, automated or manually handled)				
Imaging FlowCytobot	Phytoplankton (and microzooplankton) cell abundance, diversity (species identification based on morphology) and biomass (based on cell volume)	Cell size : 5-150 µm	https://mclanelabs.com/imaging-flowcytobot/	NIVA, SYKE, SMHI, IMR
FlowCAM	Particle densities, phytoplankton and microzooplankton abundance, diversity (species identification based on morphology) and biomass (based on cell volume).	Particle size range: 2 µm to 1000 µm. Sample volume > 100 µl. Need to change flow chambers and microscopic objectives to address a defined size-range at good definition	https://www.fluidimaging.com/	CNRS-LOG, CNRS-LOV, CNRS-MIO, Hereon (ex HZG), VLIZ, FAMRI, IMR, SYKE
ZooCAM	Imaging Zooplankton samples for addressing abundance, diversity (species identification based on morphology) and biomass (based on cell volume)	Metazooplankton organisms and fish eggs larger than 300 µm ESD		IFREMER, CNRS-LOV

LISST-Holo2	Holographic images of plankton	~25-2500µm size range	https://www.sequoiasci.com/product/lisst-holo/	Hereon (ex HZG), SYKE
In situ imagers				
Underwater Vision Profiler UVP5 + UVP6 LP	The raw data is an image of *in situ* marine particles or plankton. Many morphological properties are measured and the object are usually classified into categories of size or taxa.	Marine Particles > 100µm Plankton > 500µm	http://www.hydroptic.com/	CNRS-LOV CNRS-LOG
CPICS Continuous Plankton Imaging and Classification System	Underwater *in-situ* images of Phytoplankton, Zooplankton and particles that can be directly associated with hydrography measurements (e.g. temperature, pressure, salinity, oxygen concentration, and fluorescence)	Plankton and particles in the size range between 35µm and several mm	https://coastaloceanvision.com/home-original/products/	Hereon (ex HZG)
VPR Video Plankton Recorder II and DAVPR	Underwater in-situ images of Phytoplankton, Zooplankton and particles and accompanying Hydrography	Plankton and particles in the size range between 100 µm and several mm	Seascan Inc.	Hereon (ex HZG), VLIZ, FAMRI
Benchtop scanning imagers				
ZooScan	The raw data is an image containing ZooPlankton organisms. Many morphological properties are measured and the object are usually classified into categories of size or taxa.	ZooPlankton possible identification > 50µm	http://www.hydroptic.com/	CNRS-LOV, CNRS-LOG, CNRS-MIO, IFREMER, BL, VLIZ
Planktoscope	Single cell resolution imaging of phytoplankton and zooplankton	Phyto and zooplankton	https://www.planktoscope.org/discover	CNRS-LOV
Single-cell optical characterization				
CytoSense and CytoSub instruments	Phytoplankton at the single cell/colony level. Recording of the pulse shapes of fluorescence emitted by the pigments and the scatter emitted by the cell itself when the cell intercepts the laser beam. Resolve phytoplankton functional groups abundance and their average sizes, as well as their optical characteristics including proxy of pigment content.	Sizes= from <1 up to 800 µm in width and up to 4 mm for chains Up to 10000 particles s^{-1} > 1000 images for > 15 µm with a resolution of 3.6 pixels/µm	https://www.cytobuoy.com/product/cytosense/	CNRS-MIO, CNRS-LOG, CNRS-BOREA, VLIZ, IFREMER, CEFAS, RWS, SYKE, IRB, HCMR
CytoPro	It is an automated flow cytometer coupled to a staining module to extend the capabilities of the CytoSense technology to the detection of heterotrophs (prokaryotes, flagellates, ciliates) and address the physiology of auto- and heterotrophs (viability, activity)	Heterotrophs (prokaryotes, flagellates, ciliates)	Commercially available (www.cytobuoy.com)	CNRS-MIO
Bulk bio-optical instrumentation				
Cavity absorption sensors				
PSICAM	Absorption over the entire visible light spectrum in water	Spectral absorption coefficients in a range of 400–710 nm	https://sunstonesci.com/product/psicam	NIVA

OSCAR	Absorption meter, following the principle of the PSICAM	256 channels, 360 to 750 nm, 3.3 nm/pixel	https://www.trios.de/en/oscar.html	NIVA, SYKE
HyAbS	Absorption coefficient spectra, CDOM, Phytoplankton biomass (chlorophyll-a), SPM, algal groups	Absorption coefficient spectra in the range of the visible light (400-710 nm) with a resolution of 2 nm	Custom made and not commercially available.	Hereon (ex HZG)
LED fluorometers (mono-spectral)				
Seapoint Chlorophyll Fluorometer (SCF)	*In situ* measurements of chlorophyll	Excitation WL: 470 nm CWL, 30 nm FWHM Emission WL: 685 nm CWL, 30 nm FWHM	http://www.seapoint.com/scf.htm	IH
WetLabs ECO FLNTU fluorometer (Chl a), TriOS microFlu-blue fluorometer (PC), TriOS microFlu-223 CDOM fluorometer, other LED-fluorometers	Chlorophyll a, Pycocyanine (PC), PhycoErytrhine (PE), CDOM	470/695, 620/655, 370/460 440/680, 530/570, 630/650, 360/430	https://www.seabird.com/eco-flntu/product?id=60762467722 https://www.trios.de/en/nanoflu.html	SYKE
WetLabs ECO FLNTU fluorometer (Chl a), other LED fluorometers	Chl a, PE	470/695, 518/595	https://www.seabird.com/eco-flntu/product?id=60762467722	HCMR
In vivo LED fluorometers	Chl *a*, PE, PC	470/695, 518/595, 630/680		IRB
ECO Triplet-w	Biogeochemical measurements of chlorophyll *a* and FDOM fluorescence and red backscattering	470/695, 518/595, 630/680, 370/460	https://www.seabird.com/eco-triplet-w/product?id=60762467721	OGS, CNR
Multiparametric probe model YSI 6600V2-4	Chl *a*, PE, PC	470/695, 518/595, 630/680	https://www.xylem-analytics.asia/productsdetail.php?6600V2-Sonde-24	SOCIB
Multi-Spectral fluorescence				
Multiexciter	This instrument is a 9-wavelength LED fluorometer (375, 400, 420, 430, 470, 505, 525, 570 & 590 nm), with additional capacity to measure turbidity, temperature and depth. Data output such as raw fluorescence data in the chlorophyll *a* emission spectrum, and µg chl*a* L^{-1} estimated for the taxonomic groups included in the spectral library of the software.	Phytoplankton (pigmented organisms) studies	https://www.jfe-advantech.co.jp/eng/ocean/tahachou/	SYKE

FluoroProbe	Chlorophyll *a* fluorescence intensity is measured at 690-700 nm, after excitation at 470, 525, 570, 590, 610 and 370 nm (relative units). Total chlorophyll *a* concentration estimated (µg chl *a* L^{-1}) for the taxonomic groups included in the manufacturer's library through an algorithm: Brown algae = Cyanobacteria Green algae, Cryptophytes, CDOM concentration (arbitrary unit), Water temperature (°C), Transmission (%), Depth (m)	Estimated range: 0.5 - 200 µg chl *a* L^{-1} Resolution chl determination: 0.1 µg chl *a* L^{-1}	https://www.bbe-moldaenke.de/en/products/chlorophyll/details/fluoroprobe.html	CNRS-LOG
AlgaeOnlineAnalyzer	Fluorescence intensity after excitation at 470, 525, 570, 590, 610 and 370 nm (relative units). Total chlorophyll a concentration estimated (µg chl a. L^{-1}), for the taxonomic groups included in the manufacturer's library through an algorithm: Brown algae, Cyanobacteria, Green algae, Cryptophytes, CDOM concentration (arbitrary unit), Water temperature (°C), Transmission (%), Depth (m)	Estimated range: 0.5 - 200 µg chl *a* L^{-1} Resolution chl *a* determination: 0.1 µg chl *a* L^{-1}	https://www.bbe-moldaenke.de/en/products/chlorophyll/details/algaeonlineanalyser.html	IFREMER, CNRS-LOV, CEFAS
Multispectral Variable (active) fluorescence				
FastOcean Act2 Laboratory System	This fast repetition rate fluorometer measures phytoplankton photosynthetic activity. The instrument works with short (microseconds) intense light flashes cumulatively causing single closure of PSII reaction centres.	low chl *a* (<0.1 µg L^{-1}) Excitation LED Channels: 3 x 6 custom-made LEDs, centred at 450, 530 and 624 nm. LED filtering: 630 nm short-pass filter (>OD6 between 655 and 750 nm).	https://chelsea.co.uk/products/act2/#main	CNRS-LOG, CNRS-BOREA, NIOZ
FastOcean APD	Primary productivity assessment, understanding phytoplankton physiology, nutrient stress analysis, algal health assessment	Custom LEDs with emission centred at 450, 530 and 624 nm 630 nm short pass filter blocks LED emission within the fluorescence detection waveband	https://chelsea.co.uk/products/fastocean-apd/#main	CNRS-LOG, VLIZ, RWS, SYKE
LabSTAF	Monitoring phytoplankton primary productivity using latest in Single Turnover Active Fluorometery (STAF) technology. Fully automated acquisition allowing for continuous measurements without manual intervention.	Excitation wavebands: 452, 472, 505, 417, 534, 594, 622 nm Detection limit: can resolve Fv with an amplitude equivalent to 0.001 mg m-3 of chlorophyll a	https://chelsea.co.uk/products/labstaf/#main	CNRS BOREA

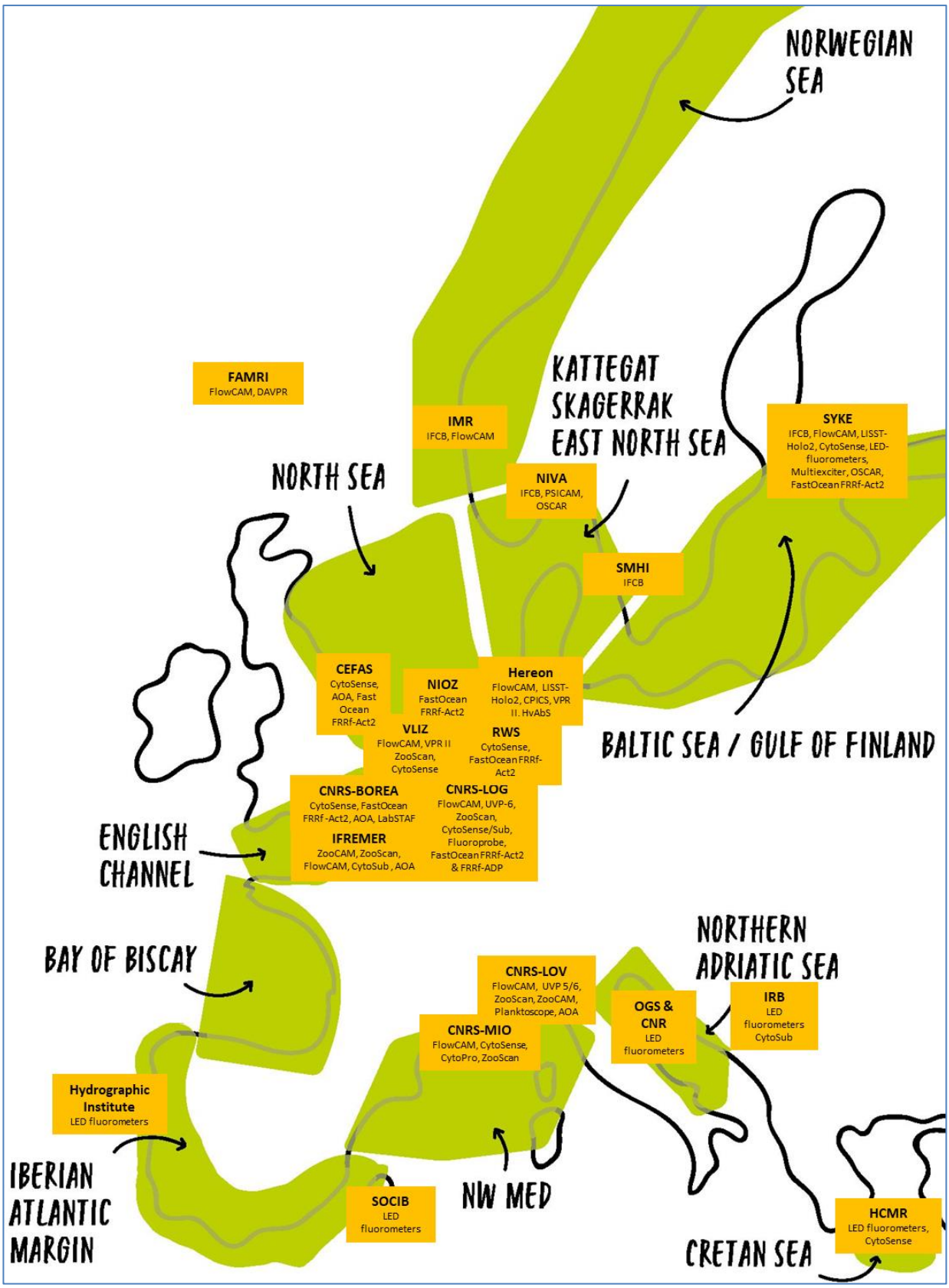

Figure 18 – Map of the localisation of sensors potentially or effectively deployed by JERICO S3 participants in the different Integrated Research Sites (IRS) and Pilot Super Sites (PSS) of European Coastal systems (based on JS3 partner's information and JS3 MS25; Artigas et al., 2021).

3. OUTREACH, DISSEMINATION AND COMMUNICATION ACTIVITIES

Release of questionnaires on current operational practices

In November 2020 and April 2021, three questionnaires on current operational practices were released with the aims to continue the 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. The objective was to improve the readiness of ship-based and autonomous platform observing networks by guaranteeing their robustness, reliability, and long-term sustainability.

The first questionnaire was on *in vivo/in situ* automated flow cytometry and resulted on a two-part workshop (April 9 and 12, respectively) discussing the answers to the survey and the best practices, as well as presenting the new vocabulary for flow cytometry and clusters associated. The two other ones released in April 2021 were on the topic of plankton automated imagery and *in vivo* fluorometry. Two workshops will be organized to discuss results and best practices for both approaches in September/October 2021. Link to other expert and user networks on both techniques will be made as a conclusion of these three series of workshops.

Workshop on the harmonisation of the use of biological sensors

On April 22, during the JERICO week, a workshop on the harmonisation of biological sensors was organized by Julien Mader and Felipe Artigas as part of the WP5 tasks, in coordination with Véronique Créach for the data issues (WP6). A summary of the discussions held during this workshop were released in the JERICO S3 MS25 on WP5 Subtask 5.3.3 "State of the art capturing and analysing gaps in Best Practices for implementing and operating biological data acquisition in coastal observatories" (Artigas et al., 2021).

Communication in international Workshops, Seminars and Meetings

- *COCAS workshop International Coastal Buoys Network Workshop "From the Buoy to the data":* oral presentation on phytoplankton automated observation for fixed stations, 23-24 Nov, 2020.

-*2021 FerryBox and High-Frequency Radar online workshops:* 6 oral presentations (SYKE, CNRS LOG, CNRS MIO, IFREMER) on continuous recording coupled to FerryBox or Pocket FerryBox systems, 17-18 March 2021.

- *9th EuroGOOS International Conference:* a dedicated oral presentation on phytoplankton automated observation, 3-5 May 2021.

- *MBON meeting with JERICO S3* in order to explore links between the Marine Biodiversity Ocean Network and JERICO work on biological automated observations, 15 Feb, 2021

- *ASLO International Aquatic Sciences Meeting (ASM) 2021:* organisation of two special sessions (SS28 on Aquatic microbial community structure and dynamics: new insights from non-destructive high throughput automated single-cell analysis and SS66 on Coastal Ocean Observing Systems to understand and predict changes of the coastal ocean and participation in at least 6 oral presentations, 22-27 June 2021.

- *I/ITAPINA Imagine/Imaging The Atlantic– A Pelagic Imaging Network Approach*: oral presentation, 28 and 29 June, 2021.

Outreach activities

Boulogne-sur-Mer "Maritime Fair": some sensors were presented to the general public, 7-11 July, 2021.

Dedicated academic activities

- *Theoretical and practical lessons (dedicated Course) at the Universté du Littoral Côte d'Opale and the University of Lille (B.Sc. and M.Sc. degrees):* automated plankton analysis from the field to data analysis and interpretation.

Joint research field activities:

- One international cruise was conducted (March 2021) into two Norwegian Fjords, led by Klas Ove Möller (Hereon, ex HZG) and with contributions by LOV (Villefranche). The overall goal was to compare different *in-situ* imaging instruments and to harmonize the data output including CPICS, UVP5, UVP6-LP, UVP6-HF, LOKI, PELAGIOS, LISST-Holo, LISST 200 and self-developed imaging systems.
- Other international joint cruises were organized to compare different sensors, but unfortunately not held because of the pandemia (as the VLIZ JERICO-LifeWatch spring cruise in May 2021, inviting partners from North Sea and English Channel) postponed to 2022.
- Preparation to install many sensors on a North Sea Ferryline are being carried out as a collaboration between NIVA and RWS.
- Measurements of opportunity were carried out by both automated flow cytometry and multispectral fluorometry in the Atlantic Margin crossing Celtic Seas, Baay of Biscay and Iberian Margin until the Canary Islands (AMATLANTE-H1 cruise, May 2021, CNRS LO).
- Deployment of these two techniques coupled to a FerryBox in the NW Mediterranean by CNRS MIO and collaborators (MOOSE cruise, June 2021)
- Deployments of different sensors were and are carried out by some partners in regular monitoring and field stations (as the Utö observatory by SYKE in the Baltic, the MAREL Carnot and SMILE Buoys by CNRS LOG-BOREA and IFREMER in the English Channel), amongst other deployments in the different JS3 IRS and PSS.

4. CONCLUSION

As technology develops it allows for more precise detection, characterisation, counting, imaging and processing of plankton variables, better estimation of plankton biomass, better definition of either functional or taxonomic groups as well as assessment of photosynthetic parameters and estimation of primary productivity.

For years, past and present JERICO members have tested, inter compared and deployed automated instruments and techniques, many of which are used in several different research facilities, and have developed protocols, usage optimization and operational practices. However, there is a need for gathering current operational practices and to follow it by discussing common practices that will become best practices after clear definition and agreement by partners within and out of the JERICO S3 consortium. This report, the many outreach questionnaires, workshops and activities within WP5-Task 5.3-Subtask 5.3.3, including the JS3 MS25 (Artigas et al., 2021) aim to survey different uses and practices and to harmonize them between the different actors of JERICO-S3, who are deploying the sensors in the great variety of European coastal systems.

5. REFERENCES

Artigas F., Créach V., Houliez E., Karlson B., Lizon F., Seppälä J., Wacquet G. (2019). JERICO-NEXT WP3 D3.2: Novel methods for automated in situ observations of phytoplankton diversity and productivity: synthesis of exploration, inter comparisons and improvements. 85 pp.

Artigas L.F., Créach V., Gallot C., Hubert Z. & Thyssen M. (2021). JERICO-S3 MS25 - WP5 Subtask 5.3.3 State of the art capturing and analysing gaps in Best Practices for implementing and operating biological data acquisition in coastal observatories. 6 pp.

Anderson E., Wilson C., Knap A., Villareal T. (2018). Summer diatom blooms in the eastern North Pacific gyre investigated with a long-endurance autonomous surface vehicle. PeerJ. 2018 Aug 15; 6:e5387.

Aubert, A., Rombouts, I., Artigas, F., Budria, A., Ostle, C., Padegimas, B. and McQuatters-Gollop, A. (2017). Combining methods and data for a more holistic assessment of the plankton community. EcApRHA Deliverable WP1.2. ISBN: 978-1-911458-22-7 - Publication Number: EcApRHA1.2/2017, OSPAR, London, UK, 42 pp.

Karlson B., Artigas F., Créach V., Louchart A., Wacquet G., Seppälä J. (2017). JERICO-NEXT WP3 D3.1: Novel methods for automated in situ observations of phytoplankton diversity. 69 pp.

Coastal Ocean Vision (2021). CPICS – Continuous Particle Imaging and Classification System. Available at : https://coastaloceanvision.com/home-original/products/ (accessed May 25, 2021)

Colas F., Tardivel M., Perchoc J., Lunven M. , Forest B., Guyader G., Danielou M-M., Le Mestre S., Bourriau P., Antajan E., Sourisseau M., Huret M., Petitgas P., Romagnan J-B. (2018). The ZooCAM, a new in-flow imaging system for fast onboard counting, sizing and classification of fish eggs and metazooplankton. Progress in Oceanography September 2018, Volume 166, Pages 54-65

Davis C., Thwaites F., Gallager S., Hu Q. (2005). A three-axis fast-tow digital Video Plankton Recorder for rapid surveys of plankton taxa and hydrograph. Limnol. Oceanogr.: Methods 3, 59–74

Gallager, S. M. (2016). The Continuous Plankton Imaging and Classification Sensor (CPICS): A Sensor for Quantifying Mesoplankton Biodiversity and Community Structure. American Geophysical Union, Ocean Sciences Meeting 2016, abstract #IS52A-07

Guidi L., Jackson G. , Stemmann G., Miquel J. , Picheral M., Gorsky G. (2008). Relationship between particle size distribution and flux in the mesopelagic zone. Deep-Sea Research I 55 (2008) 1364– 1374.

Gorsky G., Ohman M., Picheral M., Gasparini S., Stemmann L., Romagnan J., Cawood A., Pesant S., Garcia-Comas C., Prejger F. (2010) Digital zooplankton image analysis usingthe ZooScan integrated system. Journal of Plankton Research, Volume 32, Issue 3, 285–303.

Graham G. W., Smith W.a.M. (2010). The application of holography to the analysis of size and settling velocity of suspended cohesive sediments. Limnol. Oceanogr. Methods 8, 1-15

Haëntjens, N., Della Penna, A., Briggs, N., Karp-Boss, L., Gaube, P., Claustre, H., & Boss, E. (2020). Detecting mesopelagic organisms using biogeochemical-Argo floats. Geophysical Research Letters, 47.

Houliez, E., Lizon, F., Thyssen, M., Artigas, L.F., Schmitt, F.G. (2012) Spectral fluorometric characterization of Haptophyte dynamics using the FluoroProbe: an application in the eastern English Channel for monitoring Phaeocystis globosa. J. Plankton Res. 34: 136-15

Houliez E., Simis S., Nenonen S., Ylöstalo P., Seppälä J. (2017). Basin-scale spatio-temporal variability and control of phytoplankton photosynthesis in the Baltic Sea: The first multiwavelength fast repetition rate fluorescence study operated on a ship-of-opportunity. Journal of Marine Systems. Volume 169, May 2017, Pages 40-51

Ifremer (2015, 7 novembre). Le ZooCam https://wwz.ifremer.fr/L-ocean-pour-tous/Nos-ressources-pedagogiques/Suivez-nos-campagnes/Campagne-EVHOE-2015/Le-ZooCam

Kolber ZS, Prásil O, Falkowski PG (1998) Measurements of variable chlorophyll fluorescence using fast repetition rate techniques: defining methodology and experimental protocols. Biochimica et Biophysica Acta 1367:88-106

Kraft K., Seppälä J., Hällfors H., Suikkanen S., Ylöstalo P., Anglès S., Kielosto S., Kuosa H., Laakso L., Honkanen M., Lehtinen S., Oja J., Tamminen T. (2021). First Application of IFCB High-Frequency Imaging-in-Flow Cytometry to Investigate Bloom-Forming Filamentous Cyanobacteria in the Baltic Sea. Front. Mar. Sci., 25 March 2021

Lefebvre A., Poisson-Caillault E. (2019). High resolution overview of phytoplankton spectral groups and hydrological conditions in the eastern English Channel using unsupervised clustering. Marine Ecology Progress Series January 2019, Volume 608 Pages 73-92

Lisst-Holo2 (2021). - Sequoia ScientificSequoia Scientific. Available at: http://www.sequoiasci.com/product/lisst-holo/ (accessed May 25, 2021)

Lombard F, Boss E, Waite AM, Vogt M, Uitz J, Stemmann L, Sosik HM, Schulz J, Romagnan J-B, Picheral M, Pearlman J, Ohman MD, Niehoff B, Möller KO, Miloslavich P, Lara-Lpez A, Kudela R, Lopes RM, Kiko R, Karp-Boss L, Jaffe JS, Iversen MH, Irisson J-O, Fennel K, Hauss H, Guidi L, Gorsky G, Giering SLC, Gaube P, Gallager S, Dubelaar G, Cowen RK, Carlotti F, Briseño-Avena C, Berline L, Benoit-Bird K, Bax N, Batten S, Ayata SD, Artigas LF and Appeltans W. (2019) Globally Consistent Quantitative Observations of Planktonic Ecosystems. Front. Mar. Sci. 6:196

Louchart A., Lizon F., Lefebvre A., Didry M., Schmitt F., Artigas F. (2020). Phytoplankton distribution from Western to Central English Channel, revealed by automated flow cytometry during the summer-fall transition. Continental Shelf Research Volume 195, 15 April 2020, 104056

MacIntyre H., Lawrenz E., Richardson T. (2011). Taxonomic Discrimination of Phytoplankton by Spectral Fluorescence. In DJ Sugget et al. (eds.) Chlorophyll a Fluorescence in Aquatic Sciences: Methods and Applications, Developments in Applied Phycology 4; 129-169.

Naito A., Abe Y., Matsuno K., Nishizawa B., Kanna N., Sugiyama S., Yamaguchi A. (2019) Surface zooplankton size and taxonomic composition in Bowdoin Fjord, north-western Greenland: A comparison of ZooScan, OPC and microscopic analyses. Polar Science, 19: 120-129.

Natunen K., Jukka Seppälä J., Koivula R., Pellinen J. (2017). Monitoring cell-specific neutral lipid accumulation in phaeodactylum Tricornutum (bacillariophyceae) with nile red staining – a new method for flowcam monitoring cell-

specific neutral lipid accumulation in *Phaeodactylum tricornutum* (bacillariophyceae) with nile red staining – a new method for flowcam. J. Phycol. 53, 396–404 Phycological Society of America

Olson R., Shalapyonok A., Sosik H. (2003). An automated submersible flow cytometer for analyzing pico- and nanophytoplankton: FlowCytobot. Deep-Sea Research I 50 (2003) 301–31.

Olson, R. J., and H. M. Sosik. 2007. A submersible imaging-in-flow instrument to analyze nano- and microplankton: Imaging FlowCytobot. Limnol. Oceanogr.: Methods 5: 195–20.

Oscar (2021). Trios. Available at : https://www.trios.de/en/oscar.html (accessed July 28, 2021).

Petersen W., Möller K. (2017). JERICO-NEXT WP2 D2.2: Report on the status of sensors used for measuring nutrients, biology-related optical properties, variables of the marine carbonate system, and for coastal profiling, within the JERICO network and, more generally, in the European context.

Planktoscope (2021). Available at https://www.planktoscope.org/how-it-works (accessed May 19, 2021)

Pollina T., Larson A., Lombard F., Li H., Colin S., de Vargas C., Prakash M. (2020). PlanktonScope: Affordable modular imaging platform for citizen oceanography. bioRxiv preprint doi: https://doi.org/10.1101/2020.04.23.056978

Raateoja M.,Seppälä J., Ylöstalo P. (2004). Fast repetition rate fluorometry is not applicable to studies of filamentous cyanobacteria from the Baltic Sea. Limnol. Oceanogr., 49(4),1006–1012

SeascanInc (2021). Digital Autonomous Video Plankton Recorder (DAVPR) Available at: https://www.seascaninc.com/products/p/davpr (accessed May 25, 2021)

Silovic T., Grégori G., Dugenne M., Thyssen M., Calendreau F., Cossart T., Kools H., Dubelaar G., Denis M. (2017). A new automated flow cytometer for high frequency in situ characterisation of heterotrophic microorganisms and their dynamics in aquatic ecosystems. IMEKO International Conference on Metrology for The Sea Naples, Italy, October 4-6, 2017

Smith Jr., W. O., Zhang, W. G., Hirzel, A., Stanley, R. M., Meyer, M. G., Sosik, H., et al. (2021). A regional, early spring bloom of *Phaeocystis pouchetii* on the New England continental shelf. Journal of Geophysical Research: Oceans, 126

Swalwell J., Ribalet F., Armbrust V. (2011). SeaFlow: A novel underway flow-cytometer for continuous observations of phytoplankton in the ocean. Limnol. Oceanogr.: Methods 9, 2011, 466–477

Wollschläger, J and Grunwald M. (2011). PSICAM - A novel probe for continuous absorption determination in water. Helmholtz-Zentrum Geesthacht, Institute of Coastal Research.

Wollschläger, J.; Röttgers, R.; Petersen, W.; Zielinski, O. (2016). The Hyperspectral Absorption Sensor - Advantages and challenges of continuous, in situ absorption coefficient measurements. American Geophysical Union, Fall Meeting 2016, abstract #OS24A-03

Yoshida M., Horiuchi T., Nagasawa Y. (2011). In situ Multi-Excitation chlorophyll fluorometer for phytoplankton measurements. JFE Advantech Co., Ltd.