Operational Sensing Life Technologies for Marine Ecosystems

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D3.1 – List of parameters to be extracted from CytoBuoy and bio opt imaging

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Preface

This document is a deliverable for the ANERIS project, funded under the European Union's Horizon Europe Research and Innovation Action under grant agreement No. 101094924.

This document presents a list of parameters that can be extracted from the Imaging in Flow Cytometer, CytoSub, made by CytoBuoy and the bio opt imaging made by OsloMet.

Summary

ANERIS focuses on the concept of Operational Marine Biology (OMB), which is a biodiversity information system for systematic and long-term routine measurements of the ocean and coastal life, and their rapid interpretation and dissemination. The systematic and long-term routine measurement is done with the next generation of scientific instrumentation tools and methods for sensing marine-life.

The design of the new instruments and methods will integrate different types of marine life-sensing technologies: genomics, imaging-biooptics and participatory sciences. The technologies will be implemented in a co-design framework, involving all the interested stakeholders: academia, industry, civil society and government. The production of FAIR Operational Marine Biology data will be carried out in a distributed IT infrastructure built from edge and cloud compute nodes, to be connected with the European Open Science Cloud (EOSC).

The parameter list provided in this document are from the CytoSub and Bio Opt Imaging. The CytoSubs will be deployed and working in operational mode in EMSO-SMARTBAY and EMSO-OBSEA. The Bio Opt Imaging developed and tested at the Oceanlab of OlsoMet, including field tests and validation, will be deployed and working at CNRS.

All data will be made available through the cyber-infrastructure by QUANTA and EGI.

List of Abbreviations

EGI – European Grid Infrastructure

EOSC –European Open Science Cloud

FAIR - Findability, Accessibility, Interoperability, and Reuse

OBSEA – Expandable Seafloor Observatory

OMB – Operational Marine Biology

1. Introduction

This document will describe the parameters that can be extracted from the CytoSub and the Bio Opt Imaging. In the chapters A all the parameters will be described from the CytoSub and in the chapters B all the parameters from the Bio Opt Imaging can be found.

A.1. Parameters CytoSub

The CytoSub is the waterproof single cell analysis and imaging flow cytometer of large numbers of individual particles. Unique is the fully automated extremely rapid analyses, enabling large sample sizes. The CytoSub ‘’Shallow’’ that will be used in ANERIS project, can be used for operations in surface water up to max 25m of depth.

This list of parameters is clustered by the sort of information it contains. The specific setting is specified, the value is described and if needed a description is given.

File Information		
Setting	**Value**	**Description**
File name	xxxx.cyz	
Date modified	day-month-year hour:minute	
Date created	day-month-year hour:minute	
Last accessed	day-month-year hour:minute	
File size	MB	
Folder	Name	
Folder path	C:\Name	Where should the measurement file be stored. Press the folder button to open a folder selection dialog.
User remarks	Tekst	A simple text field where you can enter some remarks that will be visible in CytoClus later when analyzing the data.

Measurement Results		
Setting	**Value**	**Description**
Detector noise levels	Forward Scatter Left: xxmV, Forward Scatter Right: xxmV, Sidewards Scatter: xxmV, Fl Yellow: xxmV, Fl Orange: xxmV, Fl Red: xxmV	
Concentration	E2 p/µL	
Pictures	YEAR	
Pumped volume	µL	

Analysed volume	µL	
Particles (smart triggered)	AMOUNT	
Particles (downloaded)	AMOUNT	
Particles (counted)	AMOUNT	
Duration	s	
Start	day-month-year hour:minute	

Auxiliary Sensors		
Setting	**Value**	**Description**
Sheath temperature	°C	
Laser temperature	°C	
System temperature	°C	
PMT temperature	°C	
External power supply voltage	Volt	
External pressure	Bar	
Absolute pressure	mB	
Differential pressure	mB	

Measurement Settings		
Setting	**Value**	**Description**
Measurement name	Tekst	The name of the measurement, this is used as the base for generating the name of the data file. Instead of just a simple name you can actually include folders in here as well, e.g. Folder1\Demo2. In that case Folder1 will be created in the save location if it does not exist yet. This is especially useful when automatically transferring files to a remote location since the Folder1 part will be transferred as well.
Stop after seconds	s	
Stop at # of particles	-	
Stop at analysed volume	-	
Stop at pumped volume	-	
Stop at # images	AMOUNT	
Force Flush	TRUE	
Max time out	Auto	
Pre-concentration	TRUE	

Measure noise levels	TRUE	This will measure noise levels in the system with the measurement. This can be very useful when troubleshooting and checking if the instrument is still functioning correctly. This is on by default and should be left on, in the future we will probably remove this option completely because there is no reason not to do this.
ROI Name	Tekst	
Smart Trigger		

Measurement Instrument Settings		
Setting	**Value**	**Description**
Sample pump speed	µL/s	
Configured Sample pump speed	µL/s	
Limit particle rate	TRUE	
Maximum particle rate	NUMBER	
Enable minimum auto speed	FALSE	
Minimum auto speed	µL/s	
Trigger level	mV	The trigger level determines the minimum value that the signal must have before it is considered a particle (And it must have this value for at least 4 samples). The lower you set this value the smaller the particles that will be detected and vice versa. If you set it very low then you will start getting lots of (electrical noise) that are not particles. For old instruments this would happen around 5 to 6 mV, for newer instruments that are very clean you can go as low as 1 to 2 mV sometimes. In general 30mV is a nice value to start with.
Trigger channel	Sidewards Scatter	The trigger channel determines which detector CytoUsb will use to decide if a signal is part of a particle or not. In most cases the Sidewards Scatter is used for that as that is the most sensitive channel and it will give you the complete particle.
Enabled BST module	FALSE	

PMT levels	Sidewards Scatter: 60 (16x), Fl Yellow: 90 (1754x), Fl Orange: 90 (1754x), Fl Red: 100 (4341x)	The sensitivity of the Photo detectors. For all but the forward scatter detectors the sensitivity can be controlled. There are some preset settings for Low, Medium and High that can be used as a starting point. (The 3 buttons at the top right, L, M and H). If you are looking for small particles you may need to increase the sensitivity to be able to see them. There is a limit to this because increasing the sensitivity may also increase the noise levels. On the other hand if you have a high sensitivity set and a large particle passes to the laser beam the detector will get saturated. It will see there is a particle there but it is not able to distinguish any details. In that case you need to reduce the sensitivity for that channel.
PMT preset	User sensitivity	
Beads Measurement	FALSE	
Backflush Time	s	
Biocide Module Enabled	FALSE	
GPS Enabled	FALSE	

IIF Settings		
Setting	**Value**	**Description**
Enabled IIF	TRUE	Enable or disable taking of images
IIF parameter file	C:\Users\Cyto\Documents\My CytoSense\Image In Flow\E.iif	
Selected IIF mode	Smart Grid IIF or target all	
IIF smart grid	Sidewards Scatter, Fl Red	
IIF Restrict Fws Range	FALSE	
IIF FWS Ratio Min	AMOUNT	
IIF FWS Ratio Max	AMOUNT	
Photograph Large Particles	FALSER	
Optical Magnification	xxx times	
Camera Pixel Size	xxx µm	

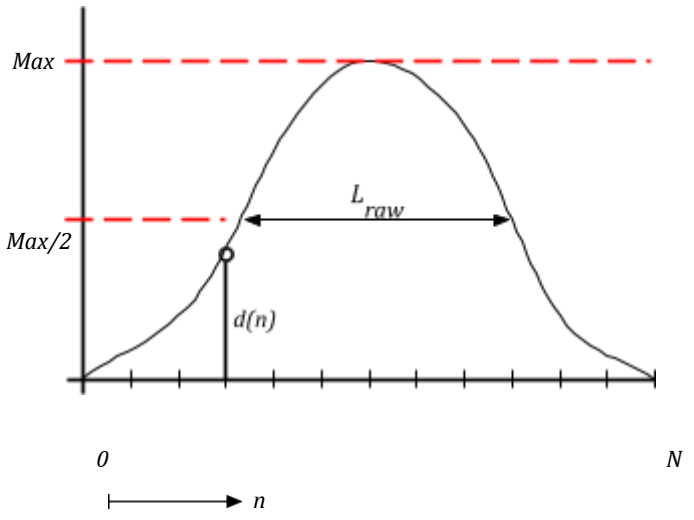
Instrument Info		
Setting	**Value**	**Description**
Serial number	NUMBER	
Hardware number	NUMBER	
Sample Core Speed	m/s	

Sheath Speed	NUMBER	
Laser beam width	µm	
Machine	TEKST	
Samplepump calibration date	day-month-year hour:minute	
Biocide Concentration	AMOUNT	The concentration of the active ingredient of the biocide in parts per million.
Sample since biocide	AMOUNT	
Biocide Left (ml)	AMOUNT	The amount of biocide left in the container, in milliliters.
Beads Left (ml)	AMOUNT	The volume of beads left in the syringe.
Number Of Lasers	AMOUNT	
Lasers	TEKST	

Version Information		
Setting	**Value**	**Description**
CytoUSB version	TEKST	
Date of dll	day-month-year	
Date of Hardware	day-month-year hour:minute	
PIC Firmware	NUMBER	

A.2. List of Equations CytoSub

A.2.1. Introduction



A.2.2. Total

The total is the sum of the sample values:

$$total = \sum_{n=o}^{n=N} d(n)$$

A.2.3. Average

The average is the total divided by the number of samples:

$$average = \frac{total}{N+1}$$

A.2.4. Maximum

The maximum is defined by the sample with the largest value.

A.2.5. Centre of gravity

The center of gravity determined as follows:

$$CG = \frac{\sum_{n=o}^{n=N} n \cdot d(n)}{total}$$

The center of gravity gives an idea of the location where the signal content is concentrated.

A.2.6. Asymmetry

The asymmetry is determined as follows:

$$AS = \left| \frac{2 \cdot CG}{N+1} - 1 \right|, 0 \leq AS \leq 1$$

Asymmetry determines the extent to which the particle is not symmetric with respect to the central sample.

A.2.7. Fill factor

The fill factor is determined as follows:

$$FF = \frac{total^2}{(N+1)\sum_{n=o}^{n=N} d^2(n)}$$

The fill factor gives another indication of the profile's shape; a square profile, i.e. where all samples are equal, has a fill factor of 1. If all samples are zero, the fill factor is 0.

A.2.8. Inertia

The inertia is determined as follows:

$$I = \frac{\sum_{n=o}^{n=N}(n^2 d(n)) - CG^2 \cdot total}{\frac{1}{12}(N+1)^2 \cdot total}$$

A.2.9. Number of cells

The number of cells is determined as follows:

$$NC = \frac{N+1}{2\pi}\sqrt{\frac{\left(\sum_{n=1}^{n=N}(d(n)-d(n-1))^2\right)}{\left(\sum_{n=0}^{n=N} d^2(n)\right) - \frac{total^2}{N+1}}}$$

The number of cells gives an estimation of the number of peaks in the profile. Since these peaks can be wide or narrow (consisting of lower and higher frequencies), this equation gives an average corresponding to the most dominant component. In CytoClus, there is an alternative cell counting mode, in which simply the peaks of the signal are counted. Only the peaks where the signal drops below 70% of the maximum in between are considered.

A.2.10 Length

The length is determined on the basis of the signal length at half maximum. So, we start by determining the number of samples *l* between the point where the signal crosses *Max/*2 for the first time and where it crosses *Max/*2 the last time. (Note: in CytoClus, the locations of crossing Max/2 are interpolated, as these locations are usually between two samples.) The core speed in combination with the sampling frequency determines the distance between samples.

This distance is (nominal corespeed is 2*m/s*):

$$dx = \frac{corespeed}{4} \ (\approx 0.5 \ \mu m)$$

The raw length is now found as,

$$L_{raw} = ldx$$

This raw length must be corrected by multiplying it with a correction factor as follows,

$$L = L_{raw} \frac{70}{70 + u + 0.5u^2 + 1.5u^4},$$

Where $u = \left(\frac{beamwidth}{L_{raw}}\right)^2$. The correction is made to compensate for the fact that the measured signal is a convolution between a particle and a laser beam of finite width. This means that even an infinitesimally small particle will result in a signal with a length of at least the beamwidth of the laser.

Figure 2 shows the result of a convolution between a Gaussian laser beam and a spherical particle of several lengths. It is seen that for larger particles, the relation approaches $L = L_{raw}$.

The curve is inaccurate for particles smaller than 1.0 *µm* diameter, however, for such particles the length is hard to determine since very few samples are available. Furthermore, such small particles always display a Gaussian profile.

A.2.11. First

The first data value of a pulse shape.

A.2.12. Last

The last data value of a pulse shape.

A.2.13. Min

The minimal data value of a pulse shape.

A.2.14. Variable Height Length

The length parameter described in Section 2.9 works well for most particle shapes, but not for all. E.g. If you have longer chains where some of the cells have a gas vacuole that produces a large spike in forward scatter then the length at 50% of the maximum will result in only the length of the spike. For these cases we added the *Variable Height Length* parameter. It works exactly the same as the normal length parameter, except you can configure at which height the length should be determined, this way you can adjust the calculation to better match the particles you are measuring. In the settings for CytoClus and CytoUsb you can configure the height as a percentage of the maximum, ranging from 1% to 100%. (The default value is 50%, which is the same as the normal length parameter.)

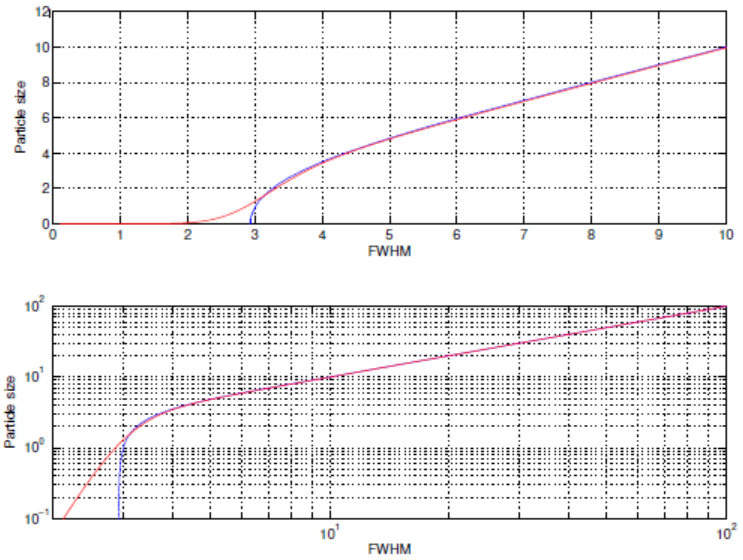


Figure 2. Analytically determined relation between L and Lraw. Linear (top) and logarithmic

B. 1. Parameters Bio Opt Imaging

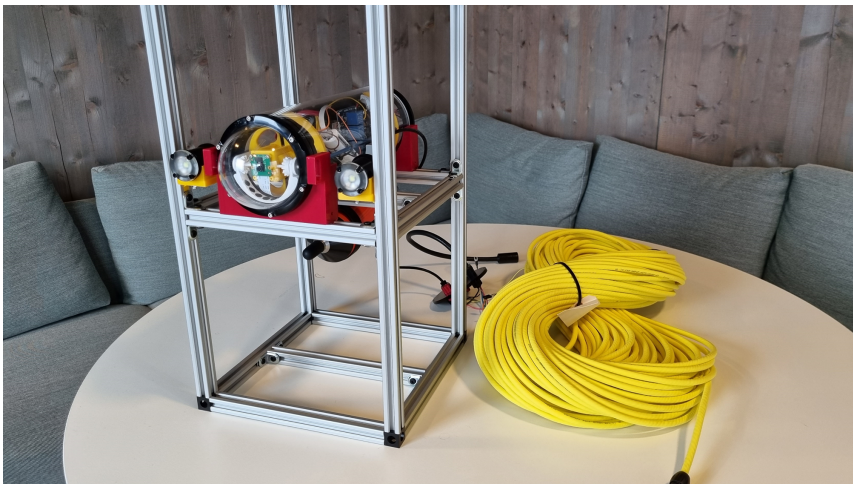


Figure 3. ANERIS EMUAS preliminary prototype

1.1 Still images

1.1.1 Resolution

Images generated by the biooptics imaging systems may be used for different purposes. If the objective is to store the images to be used by an AI/ML pipeline for the generation of data products then resolution is probably best kept limited. However images may be also used for science dissemination purposes, in which case a greater resolution is desirable.

First EMUAS prototype used a raspberry-pi camera module. This is an affordable solution but with clear limitations in terms of image quality.

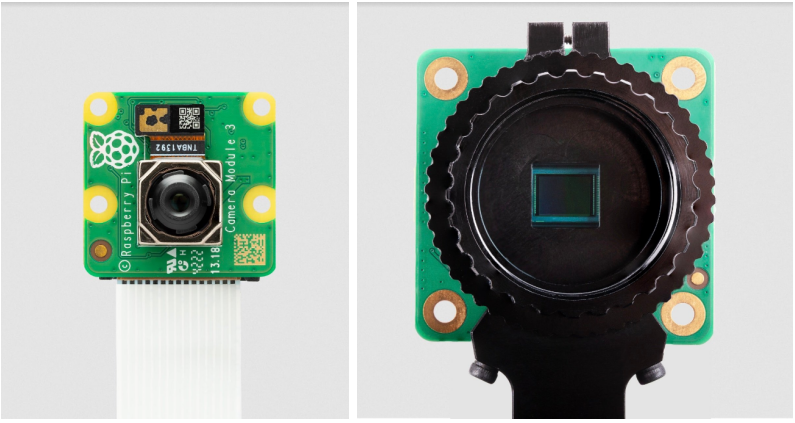


Figure 3. Raspberry-pi camera modules

Specification	Camera Module V1	Camera Module V2	Camera Module HQ	Raspberry Pi High Quality Camera
Resolution	5MP	8MP	12.3MP	Depends on Lens
Sensor Type	OmniVision OV5647	Sony IMX219	Sony IMX477	Sony IMX477
Frame Rate (Max)	30 fps	30 fps	30 fps	30 fps
Field of View	54° horizontal	62.2° diagonal	77.6° diagonal	Depends on Lens
Connectivity	Ribbon Cable	Ribbon Cable	Ribbon Cable	C-Mount
Focal Length	Fixed	Fixed	Interchangeable	Interchangeable
Aperture	f/2.9	f/2.0	Depends on Lens	Depends on Lens

Focus	Fixed	Fixed	Manual	Manual
Dimensions	25mm x 20mm x 9mm	25mm x 23mm x 9mm	38mm x 38mm x 30mm	38mm x 38mm x 14mm
Compatibility	Raspberry Pi Models A/B	Raspberry Pi Models A/B	Raspberry Pi Models A/B	Raspberry Pi Models A/B
Low-Light Performance	Moderate	Improved	Enhanced	Depends on Lens
ISO Sensitivity	N/A	N/A	N/A	Depends on Lens

1.1.2 Timestamps

Images are timestamped to millisecond accuracy ideally using a high accuracy clock reference, either through use of GPS receiver or Network Time Protocol.

1.1.3 Frequency

Frequency of still images will be dependent on storage capability. Typical intervals include sampling periods of 1-10s.

1.1.4 Format

RAW, JPEG

1.1.5 Stereo / Multiple camera configurations

In case of multiple camera deployments, a priori information on the relative position and orientation of the cameras will be provided. This can be in the form of a homogeneous transformation matrix, or a displacement vector and set of camera angles.

1.1.6 Metadata

Metadata including deployment depth, deployment estimated coordinates (latitude, longitude).

1.2 Video

1.2.1 Resolution

In a similar manner as for still images, the resolution of the produced videos may be different according to the end use. When the purpose is to run AI/ML algorithms such as YOLOv8 then video image resolution is usually kept low to speed up processing. On the other hand if the videos are also to be used for general science dissemination a higher video quality is desired.

1.2.2 Format

MPEG-4

1.2.3 Stereo / Multiple camera configurations

In case of multiple camera deployments, a priori information on the relative position and orientation of the cameras will be provided. This can be in the form of a homogeneous transformation matrix, or a displacement vector and set of camera angles.

1.2.4 Metadata

Metadata including deployment depth, deployment estimated coordinates (latitude, longitude).

References

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