JERICO WP10

D10.2 - Development of set of software for image analysis

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1. Document Description

1.1. REFERENCES

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2. Executive Summary

The Jerico Project is the first step of a pan-European coastal Infrastructure, open to all providers and users. Work package 10 is dedicated towards improvement of existing and emerging technologies on coastal observatories The WP description identified three issues which if addressed would result in a quality improvement of a European Observatory of coastal ecosystems.

- Dealing with the improvements and the development of tools and sensors,
- Dealing with the use and the development of platforms
- Dealing with the establishment of a sound implementation strategy for long-term coastal observatories.

The 10.2 'Set of Software' deliverable is primarily linked to Task 10.1 from the Jerico Description of Work - **Development of new tools and strategies for the monitoring of key biological compartments and processes.**The idea behind this deliverable is to harness existing imaging and biology expertise within different fields to develop and test new software designed to process the following data:

1. **Sediment Profile Images.**
   SpiArcBase provides an excellent tool for the analysis of Sediment Profile Images (SPI).
2. **Mobile platform recorded video.**
   AviExplore allows the treatment of video imaging of the water sediment interface acquired using a ROV (or other mobile carriers) in order to infer the abundance of epibenthos (suprabenthos).
3. **Fixed platform recorded video.**
   AviExplore is also used to analyse video imaging by fixed cameras. The main purpose is to allow the survey of recruitment on substrates, as well as the growth characteristics of fouling organisms. Image analysis is used to track the animals settling on the substrate, measure their interactions and growth rates.

   It is to be noted that for the convenience of final users, **a single software (AviExplore) is proposed for video data originating from fixed and mobile platforms** giving access to the different modules depending on the desired analysis.
4. **Phytoplankton and Zooplankton images**. Zooprocess - an integrated analysis system for acquisition and classification of digital zooplankton images from preserved zooplankton and phytoplankton samples

The Deliverable will analyse the functionality and performance of these software systems under the following headings.

- Description of the rationale for the software development
- High level description of software and it functionality
- Information on Manuals and set-up of software
- How to get Delivery/download of software
- Conclusions

3. Rationale

The need for assessment of the Ecological Quality Status of European marine waters is increasing due to policy requirements associated with European directives (Marine Strategy Framework Directive (MSFD, (2008/56/EC) and Water Framework (WFD, 2000/60/EC). Both the WFD and the MSFD require the realization of periodic monitoring surveys, which enhances the need for automatic, or at least semi-automatic, assessments of biological compartments and processes. Image analysis techniques has proven to have a big potential regarding this particular point since they have for example already been used to: (1) automatize the taxonomic classification of phytoplankton (Sosik and Olson, 2007), (2) facilitate the acquisition and classification of zooplankton images (Gorsky et al., 2009), (3) study coastal dynamics by video observations (Almar et al., 2009) and satellite images (Sánchez-Carnero et al., 2011)

One of the aims of Jerico is to strengthen the use of image analysis techniques to monitor biological compartments and processes that are recorded either at high frequency and/or over large spatial scales using automated or semi-automated procedures. This section highlights the reasons for the developments proposed for each compartment:

3.1. Sediment Profile images (SPI)

The analysis of benthic macrofauna is used in both WFD and MSFD and several biotic indices have been recently developed to infer sound ecological quality assessments (Borja et al., 2000; Borja et al., 2003; Muxika et al., 2007; Rosenberg et al., 2004). Macrofauna analysis is time consuming and tedious and only provides little information regarding the functioning of benthic ecosystems, which makes its use problematic within the MSFD and to a lesser extent in the monitoring part of the WFD. Sediment Profile Imaging was developed in the late 1960s (Rhoads and Cande, 1971; Rhoads and Young, 1970; Young and Rhoads, 1971). It consists in collecting *in situ* 2D images of vertical profiles of the sediment column most often using a specifically designed piece of equipment (i.e., a sediment profiler). The so-collected sediment profile images (SPIs) constitute 2D “optical cores” of the upper part (typically 30cm) of the sediment column (Germano et al., 2011). Sediment Profile Imaging can provide an efficient tool for rapid and cost-effective assessments of both the structure and the functioning of benthic habitats (Rosenberg et al., 2009) providing that its performances are well established (see (Grémare et al., 2009; Josefson et al., 2009; Teixeira et al., 2010) for a similar problem regarding macrobenthic indices) for example through comparisons with other approaches (Labrune et al., 2012; Rosenberg et al., 2003; Rosenberg et al., 2000).

Sediment Profile Imaging has been applied to a variety of problems and disciplines (see review by Germano et al. 2011). Corresponding applications are numerous and first relate to the assessment of the ecological quality of benthic habitats (Diaz et al., 2004) based on the secondary succession models describing the response of benthic macrofauna to organic enrichment (Pearson and Rosenberg, 1978) and physical disturbance (Rhoads and Germano, 1982).

The information contained in SPIs is a rich combination of complex biological, physical and chemical processes that are often difficult to analyze. Moreover its interpretation requires

specific knowledge relative to processes taking place at the sediment-water interface (Germano et al., 2011). (Diaz and Schaffner, 1988), proposed a list of parameters to be measured from SPI. From a biogeochemical standpoint, the information contained in SPIs is mostly indicative of:

(1) the presence of benthic macrofauna (living organisms and/or tubes),

(2) macrofauna activity through the presence and the vertical positioning of several kinds of biogenic structures such as: feeding pits, feeding mounds, burrows and oxic voids,

(3) the interaction of a large variety of biogeochemical processes taking place at the sediment-water interface, which is documented through differences in color between surface and subsurface sediments The corresponding parameter is the thickness of the layer of red-brown colored surface sediments overlying the apparent Redox Potential Discontinuity (aRPD; (Lyle, 1983) later called Mixing Depth (MD, (Teal et al., 2010).

Recent years have seen the development of computer-assisted procedures for SPI analysis. However, most of them consist in using standard image processing software such as Adobe Photoshop® (Rosenberg et al., 2003) or Image Analyst® (Solan and Kennedy, 2002) or Image J® (Birchenough S.N.R., 2012; de Moura Queirós et al., 2011; Godbold and Solan, 2009). Overall, the lack of specificity of such generic software and the absence of a well-established standard procedure limits the usefulness of Sediment Profile Imaging (Germano et al., 2011). To our knowledge, there has been no attempt to automate the drawing of the sediment water-interface and of (most) biogenic structures. Conversely several semi-automated procedures have been proposed to assess the aRPD (e.g., (de Moura Queirós et al., 2011; Ghita et al., 2004; Teal et al., 2010) and burrows (Ghita et al., 2004). However, all these procedures are based on fixed thresholds applied either to a greyscale image (Teal et al. 2010) or to the red layer of the image (de Moura Queirós et al., 2011). One major limitation of this approach is that the threshold value is strongly depending on sites (see de Moura Queiros et al., 2011) and is not accounting for the fact that light distribution is not even within each individual SPI.

The **SpiArcBase** software is specifically designed to process SPIs. Its main original features include: (1) automatic drawing of the sediment-water interface using two algorithms based on two types of edge detection filters, (2) automatic computation of the aRPD using several algorithms based either on a split-merge (Cheevasuvit et al., 1986; Horowitz and Pavlidis, 1974) or a growing seed approach (Adams and Bischof, 1994), (3) semi-automatic detection, analysis and classification of biogenic structures based on the study of grey-scale gradients, and (4) a database for storing all SPI variables.

3.2. Video Imaging Software - AviExplore

In the marine environment, the use of benthic infauna as indicators of environmental quality status has become widely used (Leonardsson et al., 2009; Rosenberg et al., 2004) . However, epibenthos video and image analysis provides a complementary and yet more holistic description of the habitat than benthic sampling (Roberts et al., 2004).

The epibenthos community comprises the flora and fauna living on the surface of the seabed. The reasons for using epibenthos for environmental assessment can be summarized: (1) the epibenthos and the suprabenthos are major food resources for fishes and birds (Bernardo-Madrid et al., 2013), (2) the epibenthos is the only benthos present on mostly rocky areas, (3) in

soft bottoms, sedimentation and re-suspension rates are considerably modified by the epifauna and (4)in general the biomass (bio-volume) of epibenthic organisms is higher than the one of endobenthic organisms.

Benthos and epibenthos sampling strategies at deep seabed present some difficulties (Desbruyères, 2010): (1) the use of multitube sediment corers will not allow megafauna to be sampled, (2) the use of heavy grabs may penetrate too much into the sediment and miss the fauna living at the sediment-water interface, (3) the use of trawls and dredges makes difficult to estimate the sampling surface. Furthermore the epibenthos sampling tools are numerous and substrate-dependent (i.e. beam trawls for soft bottoms or suction samplers for hard bottoms). None of the sampling engines are recommended as a standard for a quantitative epibenthos assessment across all substrate type (Rees and Service, 1993). Moreover, some species from the epibenthos community are very mobile, making them difficult to capture when sampling. In this context, video and image analysis has proven to be an alternative *in situ* tool adequate to assess the epibenthos at all substrate types in a qualitative and quantitative manner.

Another advantage of video/image analysis is that it is a non-destructive and non-selective technique that allows saving recordings and considering all the visible organisms. Biological information to extract from video/image analysis include abundance, composition and activities of species inhabiting the sediment-water interface, in general the most suitable targets are relatively big epibenthos organisms (i.e. Benthic Crustaceans) and bacterial mats (Aguzzi et al., 2011; Matabos et al., 2012).

Depending on the objectives of the study, imaging devices for epibenthos surveys can be carried on different platforms types (Smith and Ruhmohr, 2005): static platforms like benthic landers (Roberts et al., 2005) or mobile platforms like Remote Operated Vehicles (ROV) and Autonomous Underwater Vehicles (AUV). Each type of platforms provides imaging recordings that may deal with different difficulties. Static platforms produce long-series of images acquired under different light conditions (especially for shallow waters), different water turbidity produced by sediment suspension and different degrees of biofilm development. Those parameters reduce directly the visibility and affect the quality of images. Apart from the visibility reduced issues, image analysis of mobile platforms need also to take into consideration the position and speed of the platform so that the exact position of observed organism is located.

The AviExplore software is designed to analyse videos. As main original features, it: (1) provides image selection tools on recorded videos to analyse, (2) allows automatic extraction of targeted information, (3) proposes solutions for long-term series and (4) allows for real time acquisition and in some cases real time analysis.

3.3. Images derived from laboratory experiments designed to process and assess phytoplankton and zoo plankton

The goal of this work is to develop a sampling and an analytical protocol for the end to end pelagic ecosystem monitoring based on sample collection followed by image analysis for the semi-automatic recognition of different plankton groups that could be used as indicators. The protocol will be used to analyse bottles and net samples taking benefit of current development of imaging instruments such as the FlowCam (for microplankton) and the Zooscan (for meso and macrozooplankton). These two instruments are widely used by the planktonologist community. This task has been divided in two components: (1) the software development (which consists of the deliverable D10.2), and (2) the application of the protocol on a one year time series of observation.

At the start of the project, each instrument works with its suite of softwares (Zooprocess/Plankton Identifier for Zooscan and ZooImage for Flowcam) while operational deployment requires an integrated and compatible methodologies.

At the end of JERICO, the following has been completed.

The software: this work started in 2011. A first step in the software development for different instruments was achieved by releasing a first version zooprocess 7.09 (in February 2012). The software is still in development and release zooprocess 7.16 (December 2013) has been released on the zooscan web page and is fully operational. The current version is 7.18 (http://www.obs-vlfr.fr/LOV/ZooPart/ZooScan/). The software has been used on a data set consisting of millins of images from Flowcam, Micrsocopy and zooscan. This work has started in summer 2011 by collecting plankton with different gears (bottles and nets) and stopped in December 2012. Samples have been collected weekly with Niskin bottles and different nets (50, 100, 200 and 680 µm meh size) to collect protozoa and metazoa. The samples have been analysed using the FlowCam and Zooscan (Figure 1). The full analysis has been completed in summer 2012 and a consistent data set has been produce and now published (Romagnan et al., 2015).

Romagnan, J.B., Legendre, L., Guidi, L., Jamet, J.L., Jamet, D., Mousseau, L., Pedrotti, M.L., Picheral, M., Gorsky, G., Sardet, C., Stemmann, L. (2015) COMPREHENSIVE MODEL OF ANNUAL PLANKTON SUCCESSION BASED ON THE WHOLE-PLANKTON TIME SERIES APPROACH. Plos One (accepted).

Detailed description is given in the next section.

4.High Level Description

4.1. SpiArcBase Description

SpiArcBase presents a graphical user interface designed to enhance the selection of features observed on SPIs (**Fig. 6**) and to facilitate storage of SPI data via a Microsoft® Access format database, which also includes the meta-information related to individual SPI. The list of images stored within a database is available for selecting the image to work with. SpiArcBase then displays the selected image in order to proceed with the analysis, which includes: (1) drawing of the sediment-water interface, (2) detection and analysis of biogenic structures, and (3) drawing of the aRPD. The user has the possibility of visualizing the sediment-water interface, the aRPD and biogenic structures through overlays on the original SPI. By doing so, the user can check and whenever necessary modify the variables automatically assessed by the software. The final values for all variables are then stored within a data base and can be exported as standard text files (**Fig. 7**).

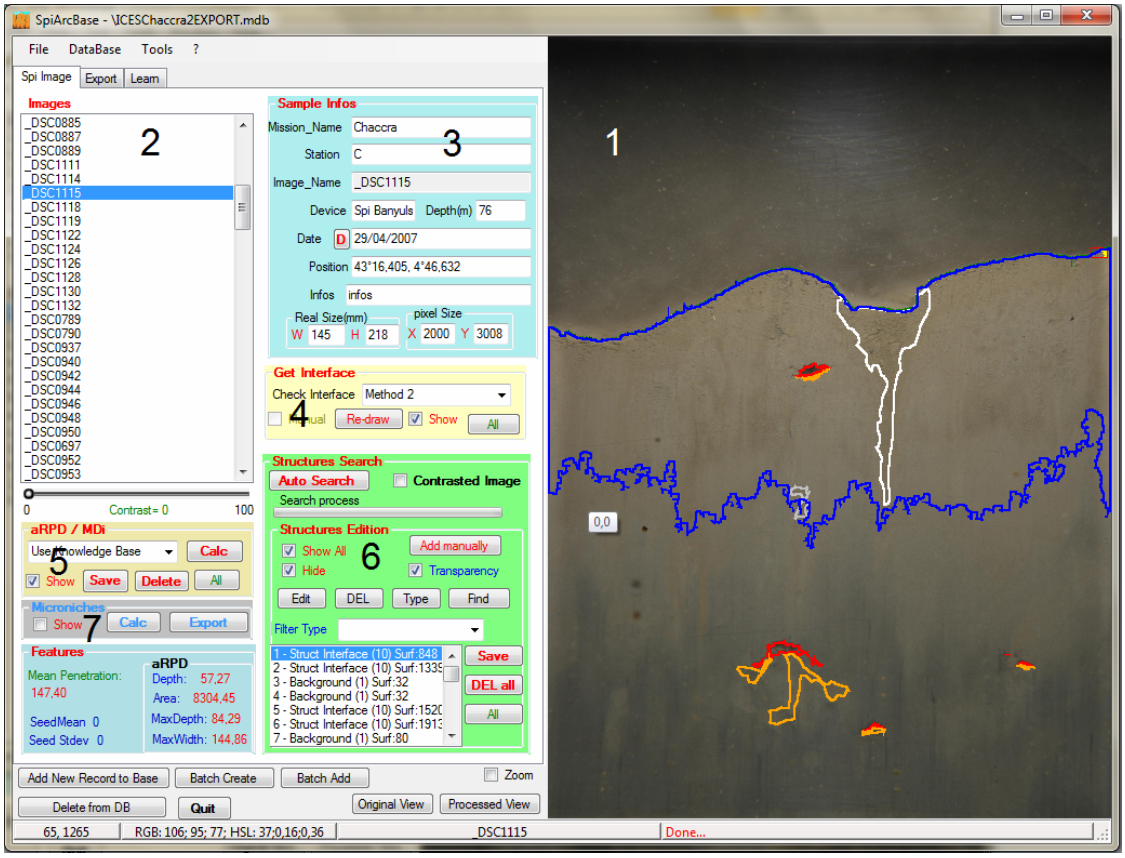

Figure 1. SpiArcBase: Main window. (1) Selected image with overlays of the computed sediment-water interface (in blue), the aRPD (in blue) and biogenic structures ("oxic voids" in red, "associated with oxic voids" in orange and "feeding structure" in white), (2) List of images in the current database, (3) Meta-information related to the acquisition of the selected image, (4) Sediment-water interface drawing/computation, (5) aRPD drawing/computation, (6) Biogenic structure computation and list

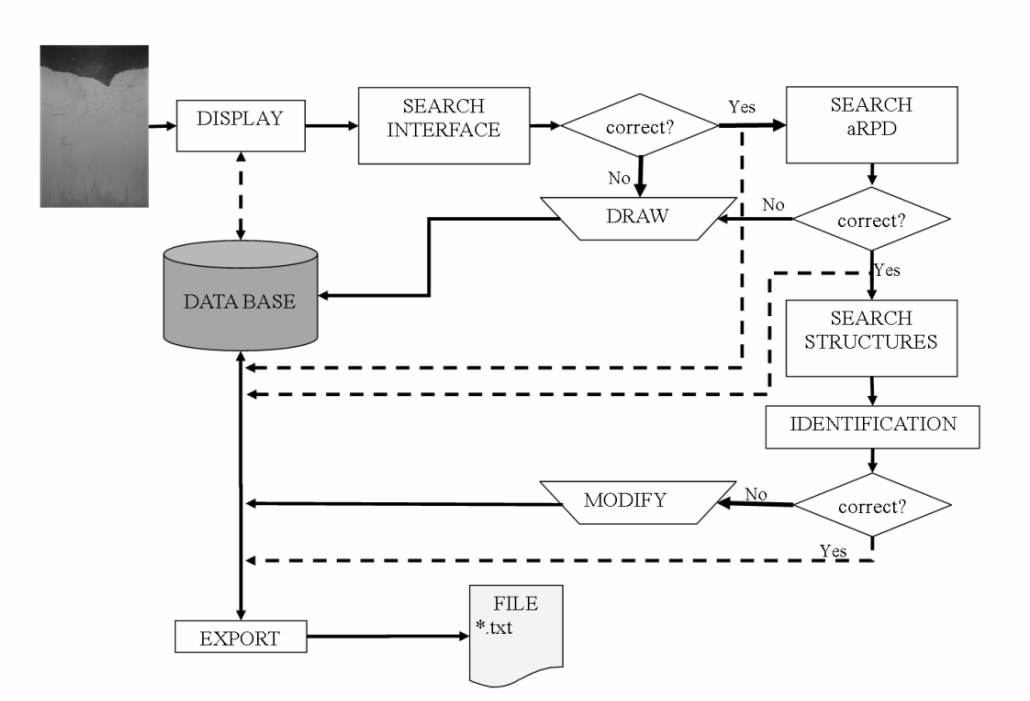

Figure 2. Overall flowchart

4.2.1 Drawing of the sediment-water interface

The first step consists in locating and drawing the sediment-water interface. The location of the defined sediment-water interface along each vertical pixel column is then used as an origin to other detected features. SpiArcBase uses two algorithms to draw/compute the sediment-water interface automatically (**Fig. 8**). The first one is based on the difference computed between the original and a 80% contrasted image as the interface is normally better perceived over a contrasted image. The resulting image is segmented using the mean value of the blue layer of the top two centimeters of the image (assuming that they correspond to overlying water) as a threshold. The so-obtained binary image is used to draw the interface, which is defined as the line constituted by the uppermost rows with a pixel value differing from 0 within all pixel column. The interface is then finally flattened using a moving average filter computed on 20 pixel columns.

The second algorithm is composed of four steps: (1) the original image is reduced to decrease computation time, (2) the reduced image is smoothed using a Kuwahara filter to attenuate the noise without affecting the sharpness of the edges (Papari et al., 2007), (3) a high pass filter (Myler and Weeks, 1993) is applied to the smoothed image to enhance its sharpness, and (4) the resulting image is processed with a threshold maximizing the entropy of the grey level histogram of the filtered image. Here again, he so-obtained binary image is used to draw the interface, which is defined as the line constituted by the uppermost rows with a pixel value differing from 0 within all pixel column. A linear interpolation is used between adjacent columns for pixel columns where no pixel value differs from 0. The interface is then finally flattened using a moving average filter computed on 20 pixel columns.

If none of these procedures proves appropriate, the user can draw the sediment-water Interface manually.

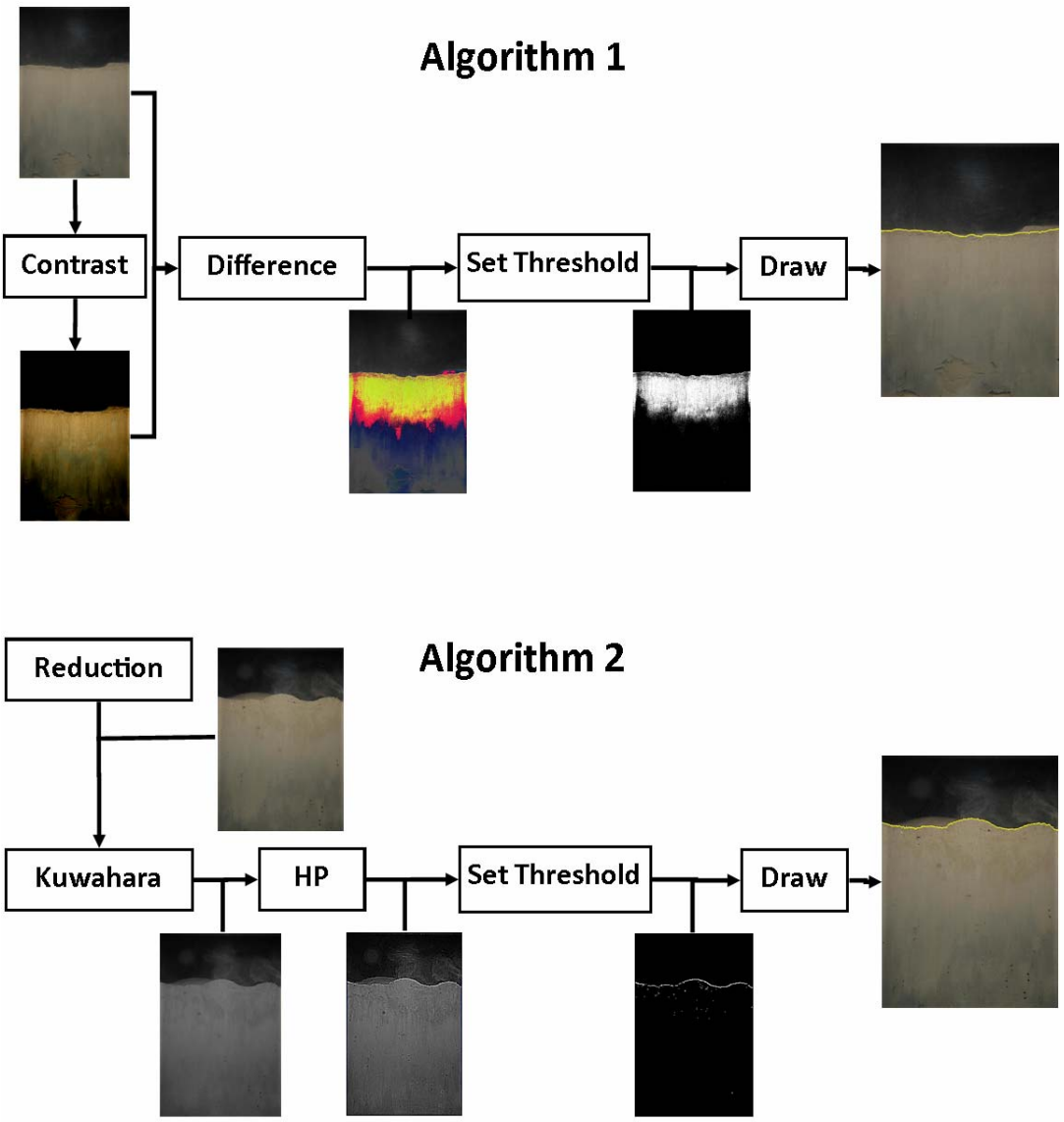

Figure 3. Flowcharts of the 2 algorithms used to draw the sediment-water interface. Examples of image processing at each intermediate step are provided for both algorithms

4.2.2 Detection and analysis of biogenic structures

The search of biogenic structures (**Fig. 9**) starts by highlighting all areas within the SPI presenting traces of biological activity. Corresponding structures are then automatically drawn, identified and automatically classified into different types, namely: unknown, background, burrows, oxic voids, infauna, structures at the sediment-water interface and structures associated with oxic voids. The user then checks these outputs and if necessary can: (1) redraw and/or modify the classification of each biogenic structure, (2) add new or remove existing biogenic structures, and (3) even create new categories of biogenic structures.

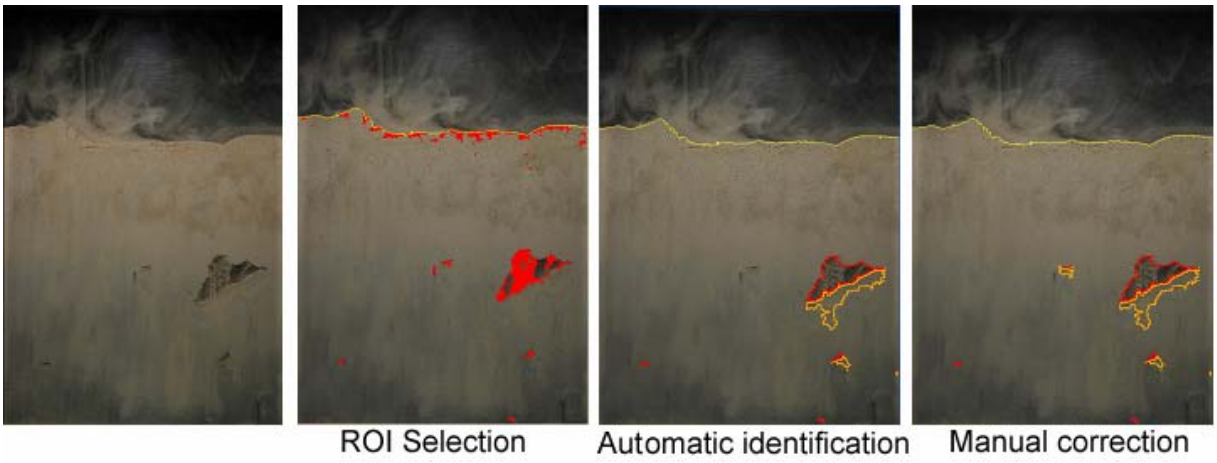

Figure 4. Example of image processing at each intermediate step constitutive of the analysis of biogenic structures

The search of biogenic structures is composed of two main algorithms. The first one selects the regions of the sediment part of the image where biogenic structures are likely to be present. To do so, the algorithm applies a Canny edge detector filter (Canny, 1986; Gonzalez and Woods, 1992) to a reduced (by a factor 4) image with an enhanced sharpness (coef. 20). The resulting image is processed with a threshold maximizing the entropy of the grey level histogram of the filtered image. Regions with steep gradients are isolated, resized (by a factor 4) and selected as Regions of Interest (ROI). The second algorithm analyzes each of these ROI for automatic classification according to different variables including color ranges, depth within the sediment column and saturation. The drawing of the biogenic structures is achieved using seeded region growing algorithms (Hojjatoleslami and Kittler, 1998) with each ROI acting as a seed. Biogenic structures are finally included within a structure list and saved into the database. The first algorithm is only executed over the sediment part of the image; some biogenic structures are therefore not automatically detected. This is for example the case of faecal pellets, tubes and epifauna. The user can choose to draw them manually.

For each biogenic structure, the following parameters are automatically computed and saved within the database: (1) barycenter (average X and Y position of the structure) (2) surface (3) perimeter, (4) width, height and XY position of the minimum bounding rectangle of each structure and (5) Mean RGB values.

4.2.3 Drawing of aRPDs

To identify the aRPDs, SpiArcBase has two working modes based on supervised machine learning algorithms The first mode is used to train the software and to elaborate a learnt system that is then used to assess aRPDs within the second mode. The user can build a new or modify an existing aRPD learning set (**Fig. 11A**). The learning set is saved in a separate file so that it can be used independently from the database containing analyzed images. The training process starts by selecting a subset of SPIs. Eleven features (see list in **Tab. 1**) are extracted from each of these images. This information is saved in: (1) a test file, which allows for reading; and (2) a binary file (binary file 1), which consists in a working matrix with an empty column for codes (**Fig. 11B, Step 1**). The training process includes the use of 6 different algorithms to calculate possible aRPDs, which are saved in another binary file (binary file 2) also containing the images (**Fig. 11B, Step 2**).

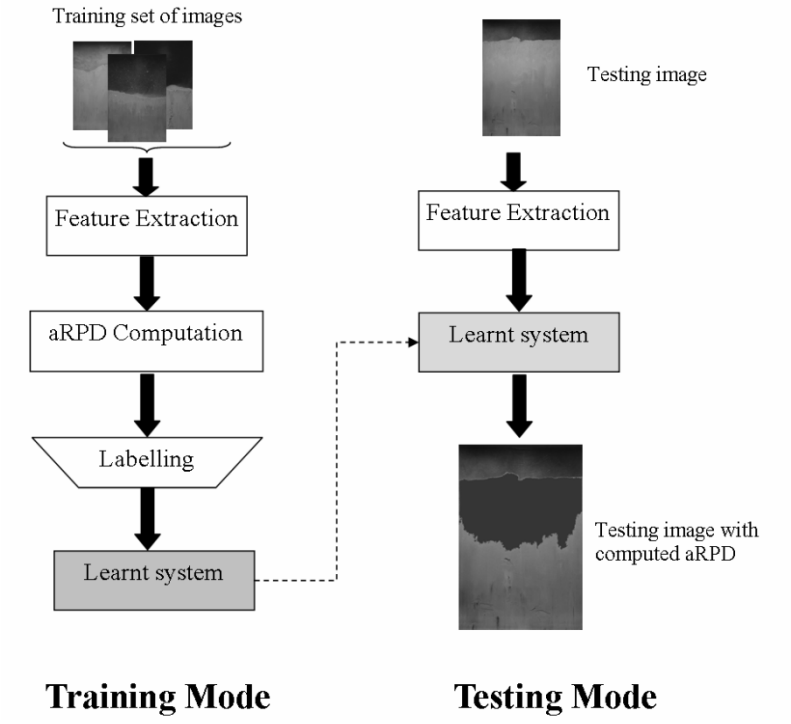

Figure 5. Machine learnt structure for the computation of the aRPD. A learnt system is built during the training mode and then used during the testing mode.

Table 1. List of the 11 features extracted from SPIs during the drawing/computation of aRPD.

List of extracted features
Maximum red value (*)
Maximum green value (*)
Maximum blue value (*)
Peak of the best fitting Gaussian distribution at the red histogram (*)
Peak of the best fitting Gaussian distribution at the green histogram (*)
Peak of the best fitting Gaussian distribution at the blue histogram (*)
Width of the best fitting Gaussian distribution at the red histogram (*)
Width of the best fitting Gaussian distribution at the green histogram (*)
Width of the best fitting Gaussian distribution at the blue histogram (*)
Slope of the average gray level per depth within the upper part (200 pixels) of the sediment column (**)
Slope of the average gray level per depth within the lower part (200 pixels) of the sediment column (**)
(*) *Extracted features from Red, Blue and Green histograms from the deepest part of the picture*
(**) *Extracted feature from gray level histogram*

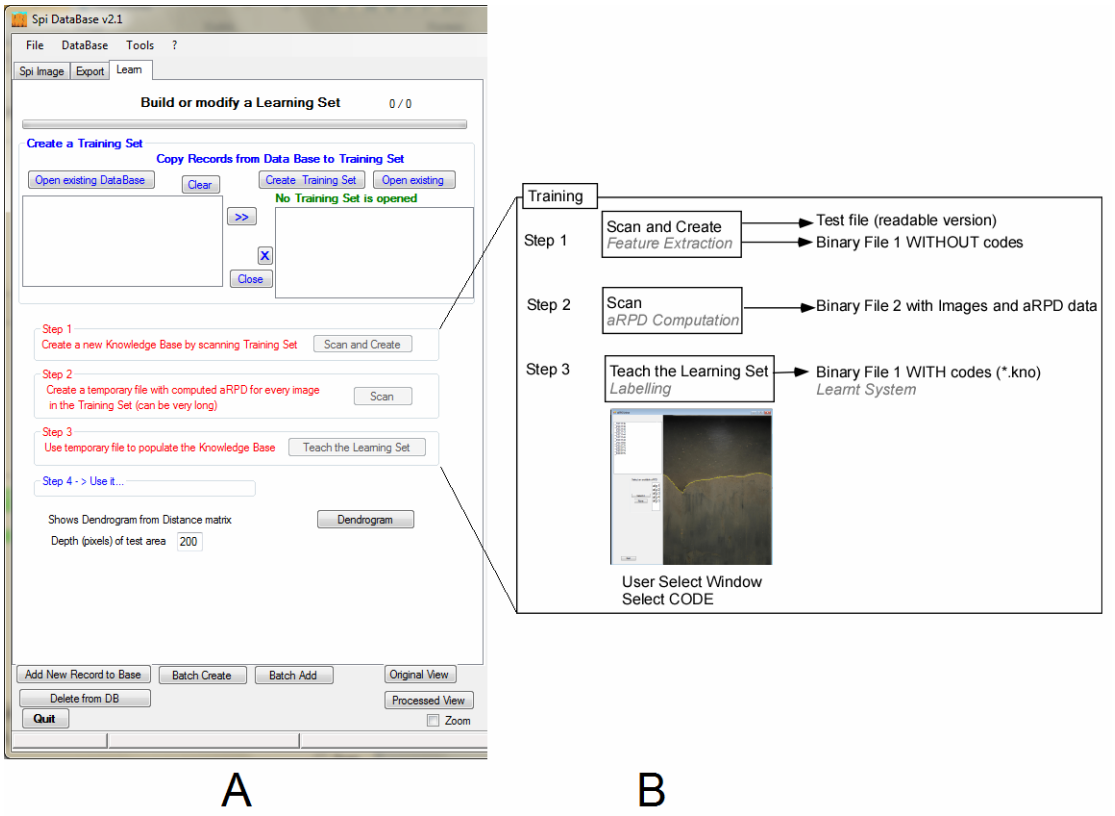

Figure 6. A: Screenshot of the tab used to build or modify a learning set for the automatic computation of the aRPD, B: Steps to follow to create a learnt system and relationships with the machine learnt structure used for the drawing/computation of aRPD.

The 6 different algorithms are based on different image segmentation procedures applied only to the sediment part of the image: (1) Split and merge (Cheevasuvit et al., 1986) (2) RGB Histograms, (3) Thresholding over mean and standard deviation of RGB values, (4, 5) Seeded region growing (Adams and Bischof, 1994), (6) Gray-level histogram (Orlando and Rui, 2002). The segmentation is followed by an algorithm to cover possible patches located close to the interface and fill possible holes.

For each image of the training set, the user selects the algorithm that fits the best with its own visual assessment of the aRPD. This is done within a specific window (**Fig. 11B, Step 3**) where images and possible aRPDs can be superposed. This allows filling the column for codes within binary file 1, which results in a labeled learnt system, which can then be used within the testing mode. The 11 features mentioned above are then extracted from each analyzed SPI and compared with those of the SPIs of the training set using a normalized Euclidean distance. SpiArcBase then applies to the analyzed image the aRPD algorithm selected for the most similar image within the training set. If this procedure does not prove appropriate, the user can draw the aRPD manually.

4.2.4 Data storage and exportation

All identified variables (interface, aRPD and biological structures) are saved to the database together with their metadata which can then be retrieved and exported within standard text files (*.txt) for further analyses (**Fig. 12**). A complete set of search parameters regarding SPI acquisition allows the user to easily select a set of images whose features are to be exported. The user can also define the variables to be exported. A pre-set possibility is the number, the surface and the position of each biogenic structure; and all extracted variables including the surface and the position of each biogenic

structure (pre-set as “large”). The corresponding “*.txt” file constitutes a summary report of the analyzed image (**Tab. 2**). Together with the aRPD variables (i.e., surface and average thickness), the mean and maximal penetration depths are also reported.

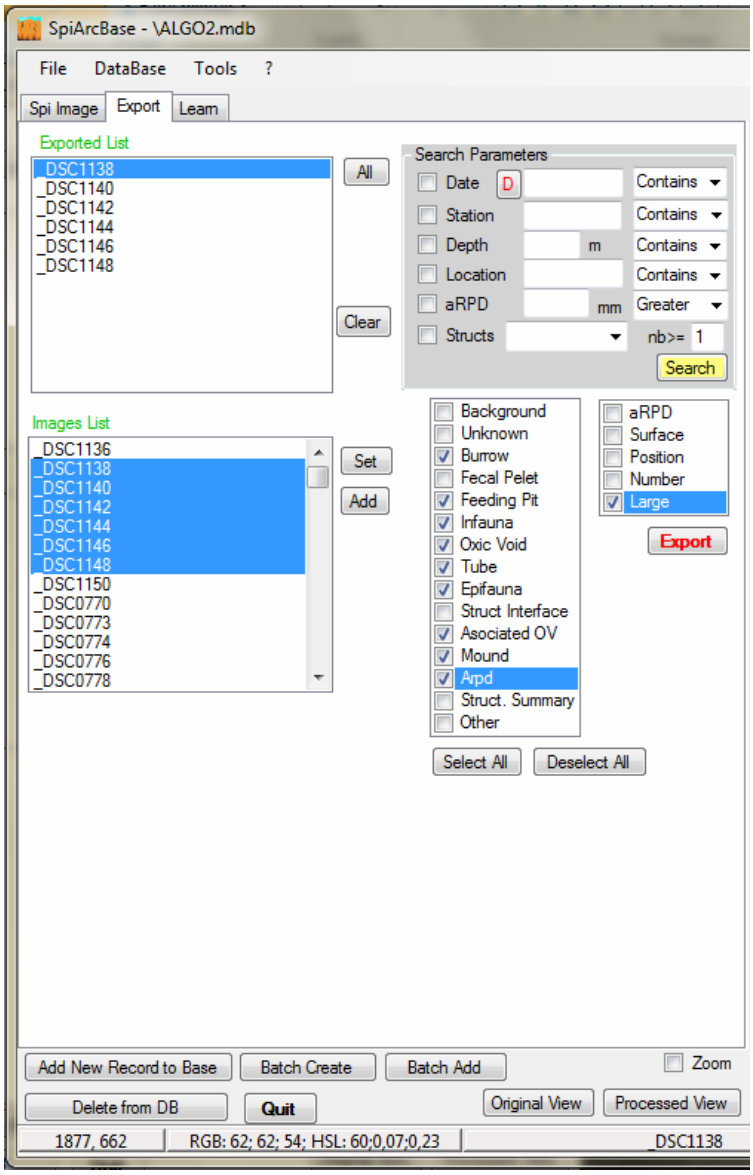

Figure 7. Screenshot of the data exportation tab.

Table 2. SpiArcBase: Example of data output. Data originating from the SPI are summarized in a "*.txt".

Sediment Profile Analysis Export
24/02/2012
12:06
LARGE
DSC_1115

aRPD (mm^2)	8304.44	Depth (mm)	57.27	Mean Penetration Depth (mm)	147.39	Max Penetration Depth (mm)	159.44
Image Width (mm)	145	Img height (mm)	218				

Name	Surf (mm^2)	Width (mm)	Height (mm)	Depth Barycenter (mm)	Max Depth (mm)
Oxic Void	3619	9.49	4.05	26.81	28.77
Associated Oxic void	1798	8.91	3.91	28.12	29.71
Burrow	5546	0.72	0.43	60.37	60.59
Oxic Void	8488	18.70	8.48	105.88	111.97
Oxic Void	131	1.88	0.72	110.30	110.67
Oxic Void	604	3.84	1.81	113.86	114.72
Oxic Void	863	5.94	2.53	116.75	118.20
Associated Oxic void	1906	6.16	2.68	118.20	119.65
Bellow Feeding Pit	81152	25.07	61.02	17.17	55.44
Associated Oxic void	28251	10.43	8.84	109.87	113.42
Associated Oxic void	982.5	5.15	1.96	114.94	116.10

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4.2. AviExplore Description

AviExplore is the software that allows acquiring and analyzing standardized AVI videos. AviExplore presents a graphical user interface (**Fig. 13**) that allows to access three different modules:

(1) AviExplore - Mobile. This module allows the extraction of information on films taken with moving sensors and cameras. This module has two working modes: Real time and recorded video.

(2) AviExplore - Fixed. This module allows for the survey of activity on surfaces by using films taken with a fixed camera. This module has two working modes: Real time and recorded video.

(3) ScriptEdit. This module can be seen as a tool that allows writing and testing scripts that will be used in the other two AviExplore modules. It is however a standalone module that can be used to compute data from videos or images.

At the starting window the main system characteristics are displayed (**Fig. 13-4**), this can be especially useful for memory allocation. Although AviExplore is not open-source software, it has been designed so that new functionalities can be included. This can be done using extension files (*.dll) written in C++ language. This way, the software will evolve in time.

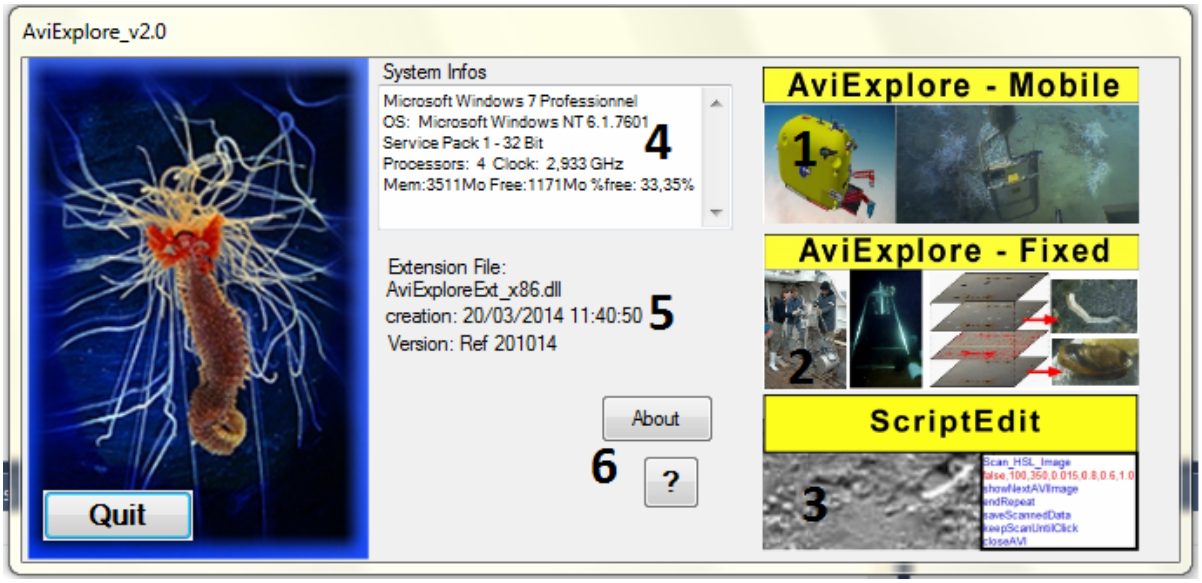

Figure 8. AviExplore: Starting window. (1) access to the module related to videos coming from mobile vehicles, (2) access to the module related to videos coming from fixed platforms, (3) access to the module for editing scripts, (4) summary of system characteristics, (5) extension file for evolutive versions of AviExplore and (6) software manual and information

AviExplore offers a wide range of possibilities of working, there can be different interaction between modules of AviExplore. On the schematic example of **Fig. 14**, during the acquisition time, the user may consider AviExplore real time acquisition mode (it is present for both fixed and mobile modules), and obtain a preliminary set of results or a video to analyze. During the testing time, the user can test different functions over a video with the AviExplore ScriptEdit module and obtain a script. AviExplore ScriptEdit offers the opportunity of saving and loading scripts that have already been tested via a database. The selected script may be used during the analyzing time for the video in order to obtain

a set of results.

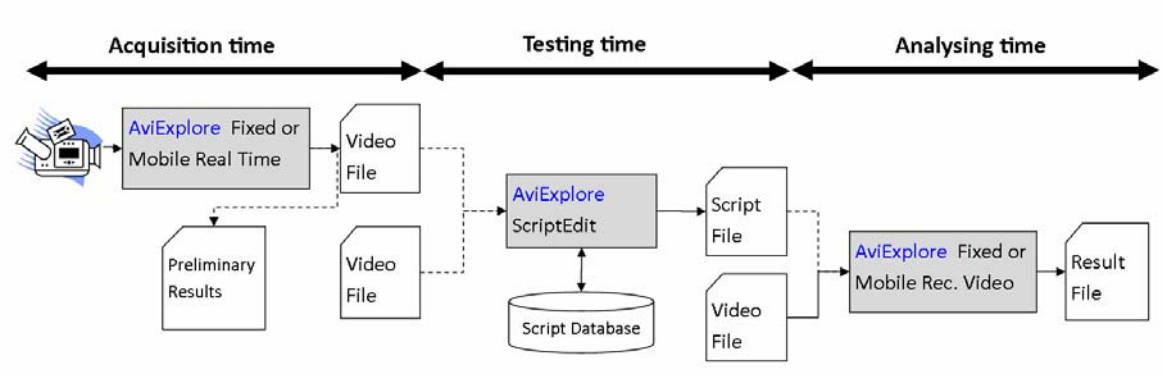

Figure 9. Example of interactions between different modules and modes of AviExplore.

4.2.1. Description of common features

The three modules of AviExplore use a similar internal structure (**Fig. 15**). In general, images are extracted from video sources; each incoming image is called 'Origin Image'. The result of applying an operation (for example contrast enhancement) on an 'Origin Image' is saved on the 'Work Image' structure. If the user needs to apply a second operation, he can either use the 'Secondary Work Image' or rewrite on the 'Work Image'. There is also the possibility of working with grayscales images by using the structure called 'Gray Image', grayscale operations can be faster. Operations can be bidirectional; this means that a 'Work image' can become the 'Origin Image', in this case having the raw source back becomes impossible.

Binary hexadecimal masks are also use as internal structures to store intermediate information, this masks can be drawn by the user or computed with a script. They can be saved and loaded using an external file (*.msk). Other Regions of Interests (ROIs) can also be drawn by the user and saved on a different external file (*.roi). These ROIs structures can be converted in internal binary regions for computations.

External companion files are also a common feature within the three modules. The program creates, uses and maintains external files that gather different type of information (**Fig. 16**):

- The AVI input file (main input)
- Script files to process images (processing)
- ROI files to store working areas (service)
- Mask files to get fast mask loading (service)
- Data and companion files to store intermediate results (information)
- Time and position files which can be of interest for image tracking of mobile recorders (information)
- Export information on text files.

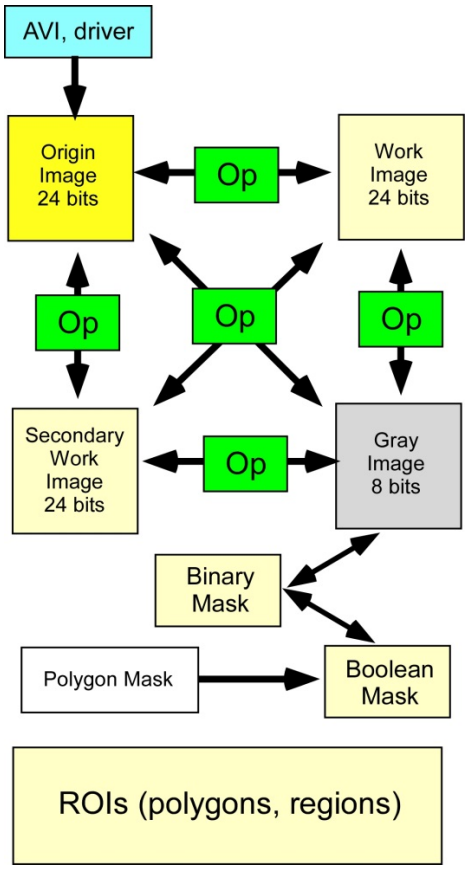

Figure 10. Flowchart of the internal structure of AviExplore. Origin, Work and Secondary Work are 24 bit Bitmaps for color pictures and video frames. The Gray image is a 8 bit image which has connexions with a 8 bit buffer (Mask) used to store intermediate measures. ROIs are polygonal structures saved in external files which can be loaded upon demand

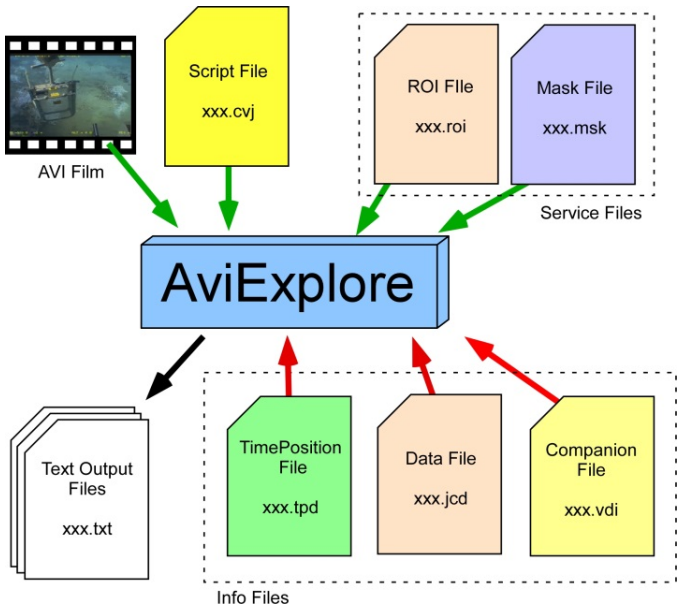

Figure 11. Summary of external companion files related to AviExplore. Green arrows refer to files that need to be loaded by the user. Red arrows refer to file automatically loaded by the software if they exist.

The graphic user interfaces have also a common structure within the three modules (**Fig.17**): (1) displayed windows, (2) strip menu, (3) Scrollbar for video timing. Depending on the module, the functionalities that appeared on the left side of the window (**Fig.17-4**) may differ.

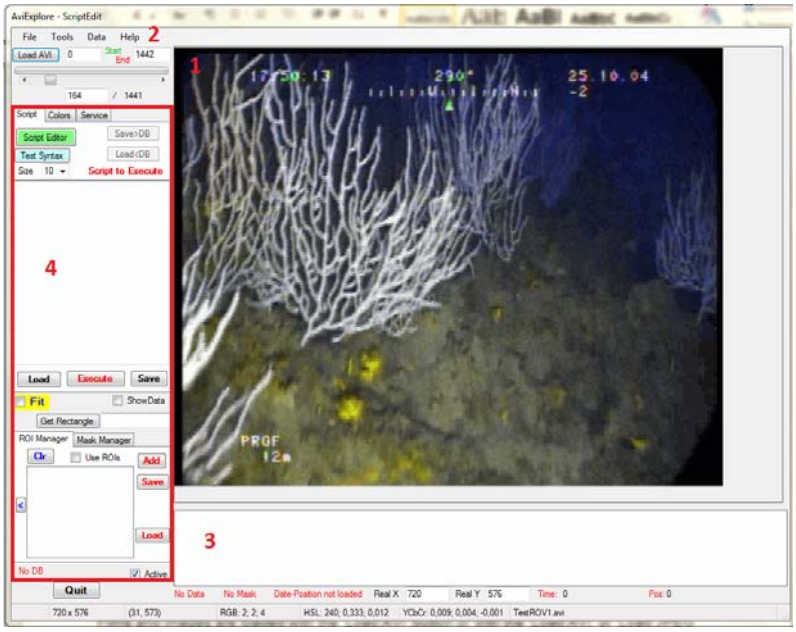

Figure 12. AviExplore Graphic user interface. Common structures: 1) video display window, 2) strip menu and 3) video time Scrollbar. The red rectangle (4) differs between modules

4.2.2. AviExplore Mobile Description

The AviExplore mobile module graphic interface (**Fig. 18**) allows choosing between two different working modes: (1) Real time acquisition (details at 4.2.4) or (2) Input from recorded Video.

In the context of mobile recorded videos (**Fig. 19**), AviExplore takes the video to analyse and a geolocalization file if available. The geolocalization file is used to situate in time and geographically an identified object. Very sophisticated ROVs produce this file when acquiring the information but other mobile acquisition tools not, if the user has the information concerning the original location of its video he can generate a geolocalization file, if not the user can however proceed with the analysis of the video although identified objects will not be located. The first step when analysing a video is to extract each image. It is recommended to proceed with a global scene analysis; this process extracts features from the images and generates a companion file with the relevant metadata. This companion file is used to compare images in a very fast manner. The process of image comparison is an interesting tool that allows identifying common properties and objects between images. It can be used to make shorter videos to analyse, for instance analyse certain images where we believe there might be a specific object. Object detection can be done using different tools and functionalities present in the module mainly: filtering, masks, image segmentation algorithms, detection of edges, scripts. If the companion file has been created it can also be used to detect and/or identify objects. On the example showed in **Fig.18** two different dedicated searches have been done to identify two different species detected on the seafloor: *Eunicella stricta* and *Axinella polypoides*. Depending on the objective of the analysis, a count up process is launched to produce a result file that contained the demanded information. For example the number and surface of *Eunicella stricta* found per

image.

Figure 13. User interface for the module dedicated to AviExplore Mobile. 1) Select Real Time acquisition, 2) Load video file.

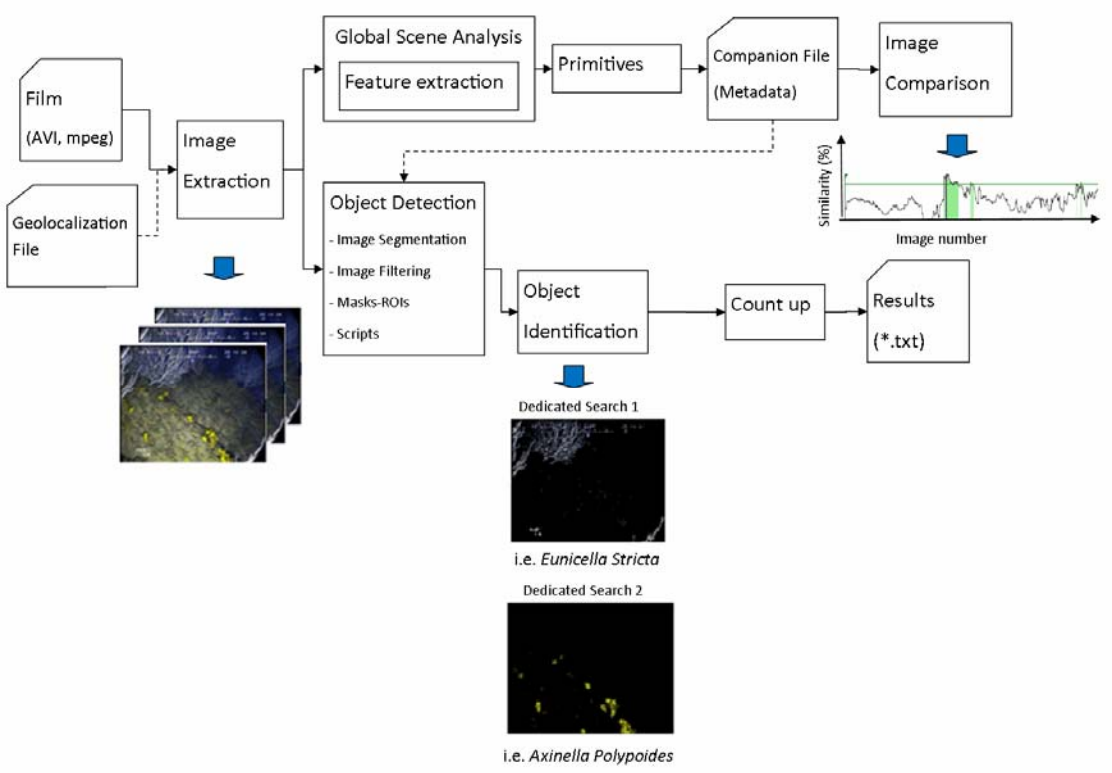

Figure 14. Flowchart of main steps in the analysis of mobile-sourced videos with some examples after processing.

4.2.3. AviExplore Fixed Description

The AviExplore fixed module graphic interface (**Fig. 20**) allows choosing between two different working modes: (1) Real time acquisition (details at 4.2.4) or (2) Input from recorded Video

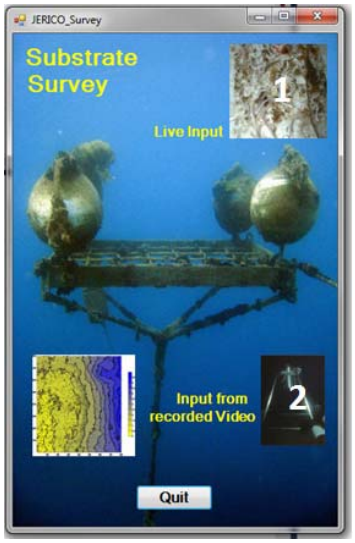

Figure 15. User interface for the module dedicated to AviExplore Fixed platforms. 1) Select Real Time acquisition, 2) Load video file

This module allows survey of activity on defined objects by using films taken with a fixed camera. AviExplore fixed module is based on the subtraction of images (**Fig. 21**). The first step when analyzing a fixed video, starts by extracting the images. The user may choose to focus on some areas of the images by drawing a Region of Interest (ROI) or a mask. When a video analysis is launched, features are extracted and images are subtracted. When regions and/or masks are used the subtractions of images only consider the selected areas and not the whole image. As a result from the video analysis a 'Data File' is created. In order to obtain a file with the selected results, the user may choose the information to export on a text file.

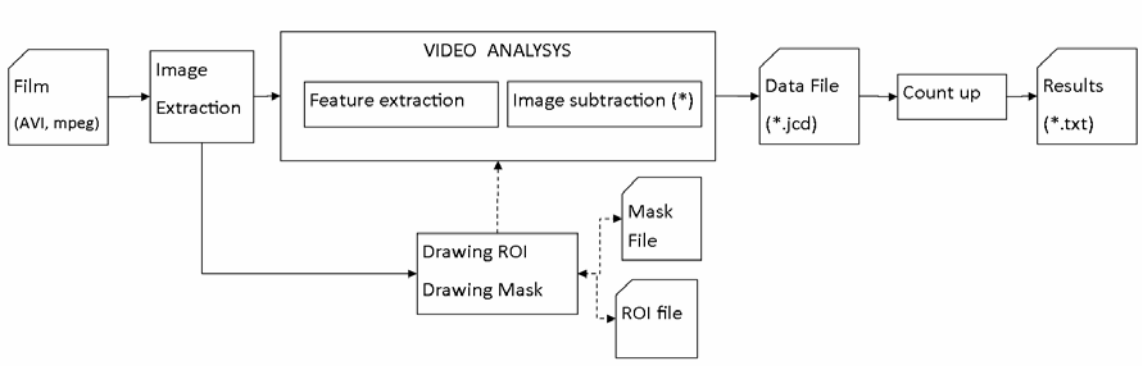

Figure 16. Flowchart of fixed video analysis

The key operation when analysing fixed videos is image subtraction. AviExplore proposes different types of subtractions namely (**Fig.22**): previous, gap and referent image. If the previous type is selected, the subtraction is done between consecutive images. For the gap type, the subtraction is done between two images that are separated by a fixed number of images. If the referent image is selected, the subtraction is always done between an image and the selected referent image (usually the beginning of your experience). Subtraction results can be expressed in three different modes: (1)

absolute value of the difference, (2) positive values and (3) negative values. There is also the possibility of choosing a minimum between pixels to be considered, this may be useful to remove differences due to lighting conditions. The user interface (**Fig.23**) allows choosing those parameters.

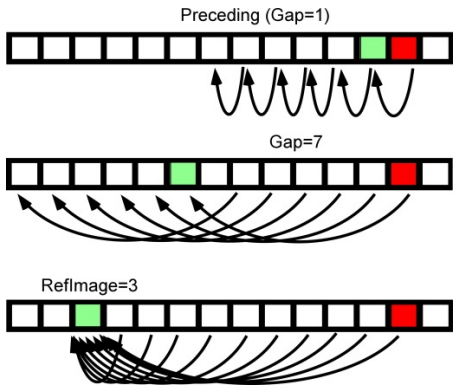

Figure 17. Schematic of different image subtraction modes

Figure 18. User interface for AviExplore Fixed Module for video analysis.

4.2.4. AviExplore Real Time acquisition

Real time acquisition can be done for both mobile and fixed platforms. Although the user interface and the way of accessing is not the same, the main idea behind the development is. The advantages of using AviExplore Real time acquisition are: (1) absolute control on your frame-rate (time-lapse), (2) immediate video treatment and analysis. The main real time video treatment concerns the possibility of applying different filters to the incoming images. The main real time video analysis allows the use of masks and the selection of objects.

There is however a timing limitation for the immediate video analysis, applied operations

cannot last very long so that the flux of images coming from the camera is not interrupted. As a consequence the use of loops is forbidden for the real time modes. This can be overpassed by saving the video and analysing it afterwards.

4.2.5. AviExplore Script Edit Description

The ScriptEdit module (**Fig.24**) is a tool that allows writing and testing scripts that will be used in the other AviExplore modules. However this module is also a standalone program that can proceed with the complete analysis of a video providing a result file. This module allows as well to edit videos from images and to extract images from videos.

The actual version of the software includes examples of object detection and identification routines (needed for the mobile module) to help the user to build their scripts and to give an idea of which kind of operations need to be done to obtain the required information.

Figure 19. User interface for the AviExplore ScriptEdit module

In order to avoid typing errors on scripts, the 'Script Editor' button, opens a new window (**Fig. 25**) that contains all the functions available in the program as well as the parameters you need to provide in order to use them. Furthermore, there is the possibility of saving the scripts on a Microsoft access database and access them easily.

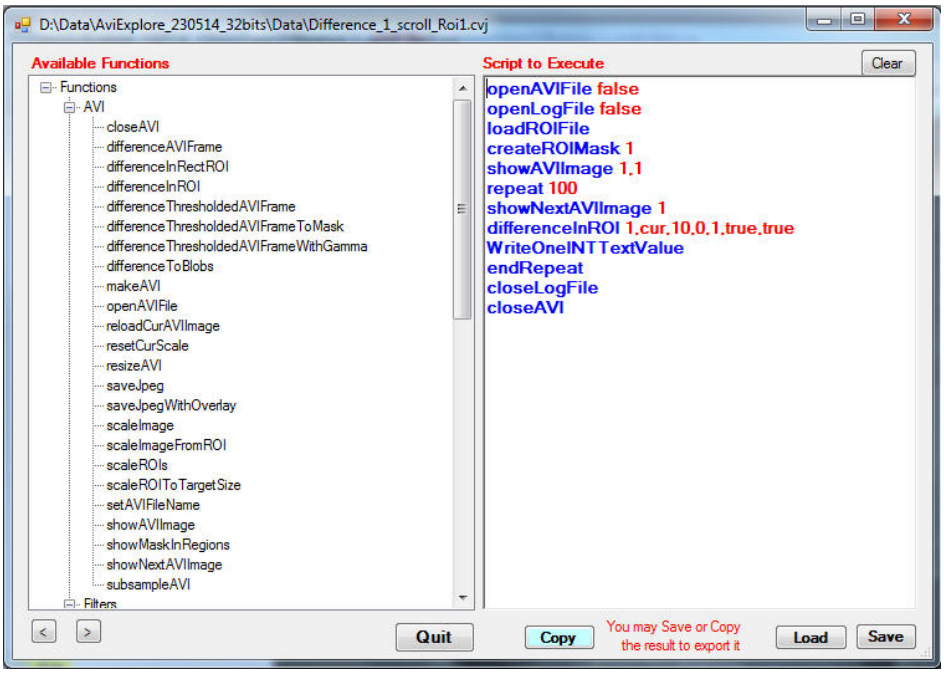

Figure 20. Script editor window to avoid typing errors on scripts.

4.3. ZooScan Description

ZooScan with ZooProcess and Plankton Identifier (PkID) software is an integrated analysis system for acquisition and classification of digital zooplankton images from preserved zooplankton samples.

Zooplankton samples are digitized by the ZooScan and processed by ZooProcess and PkID in order to detect, enumerate, measure and classify the digitized objects.

We present a semi-automatic approach that entails automated classification of images followed by manual validation, which allows rapid and accurate classification of zooplankton and abiotic objects.

We demonstrate this approach with a bi-weekly zooplankton time series from the Bay of Villefranche-sur-mer, France. The classification approach proposed here provides a practical compromise between a fully automatic method with varying degrees of bias and a manual but accurate classification of zooplankton. We also evaluate the appropriate number of images to include in digital learning sets and compare the accuracy of six classification algorithms. We evaluate the accuracy of the ZooScan for automated measurements of body size and present relationships between machine measures of size and C and N content of selected zooplankton taxa. The ZooScan system can produce useful measures of zooplankton abundance, biomass and size spectra, for a variety of ecological studies.

The software has been incrementally developed with a number of upgrades and enhancements since 2010.

The zooprocess suite of softwares can proceed to image acquisition (if necessary) or import images (Figure 2). All data and metadata of the samples are stored into a suite of folders in which all other results will be stored (Figure 3). The software can be operated on a servor on which all data will be stored. Hence several users can also access the same data (images, tables, metadata) collection (Figure 4). These images are then processed and analysed to extract the objects. Morphological and texture parameters are then obtained for each objects and used to build a learning set. The

classifcation algorithms is then tested on another independent set of images to assess the performance of the system. When the performance are not significantly improved all the objects are predicted into the plankton categories and validated by an expert.

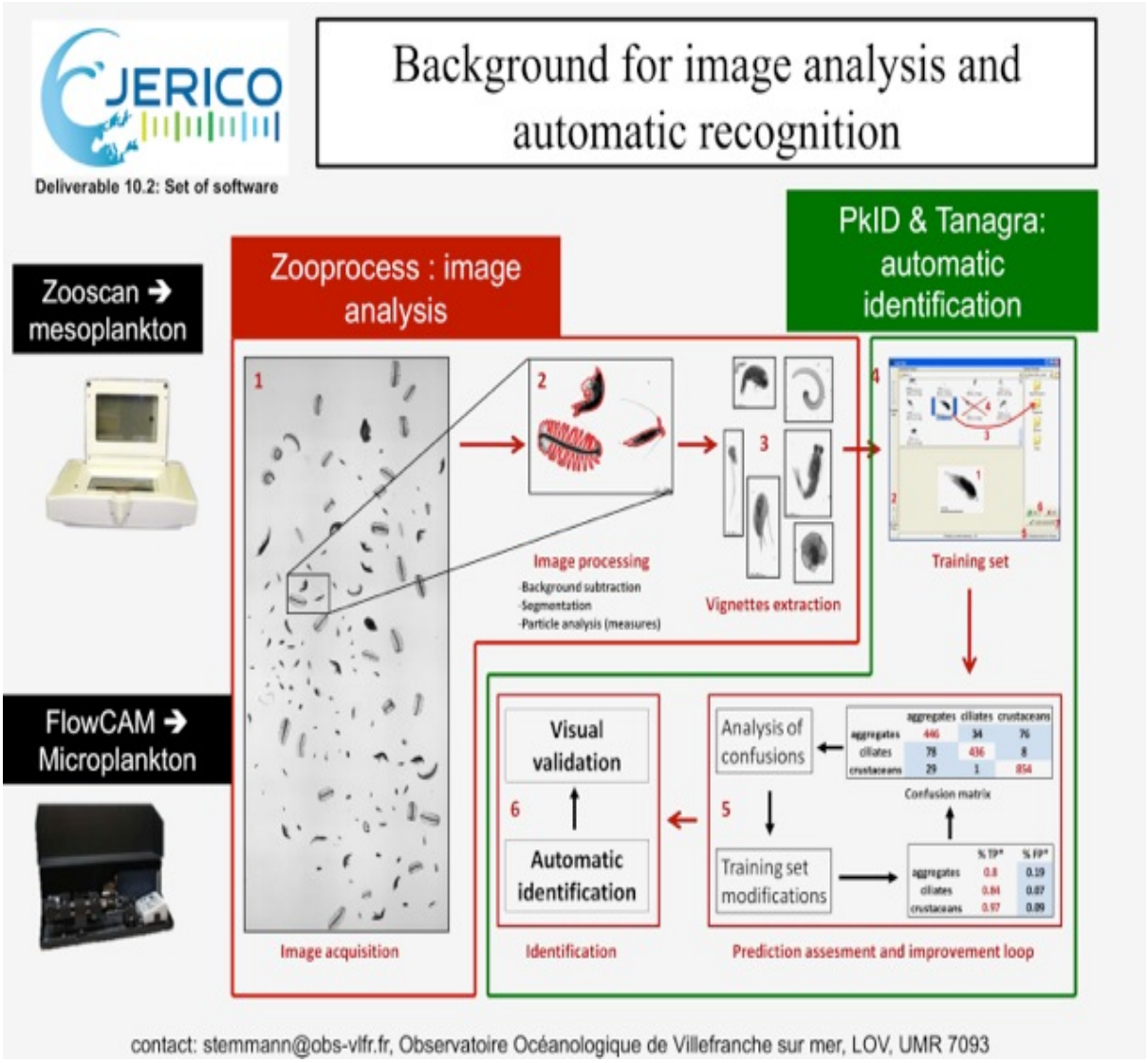

Figure 21 ***Flow Diagram illustrating Steps 1 to 6 how the Zooprocess image analysis and automatic recognition software identifies Zooplankton.***

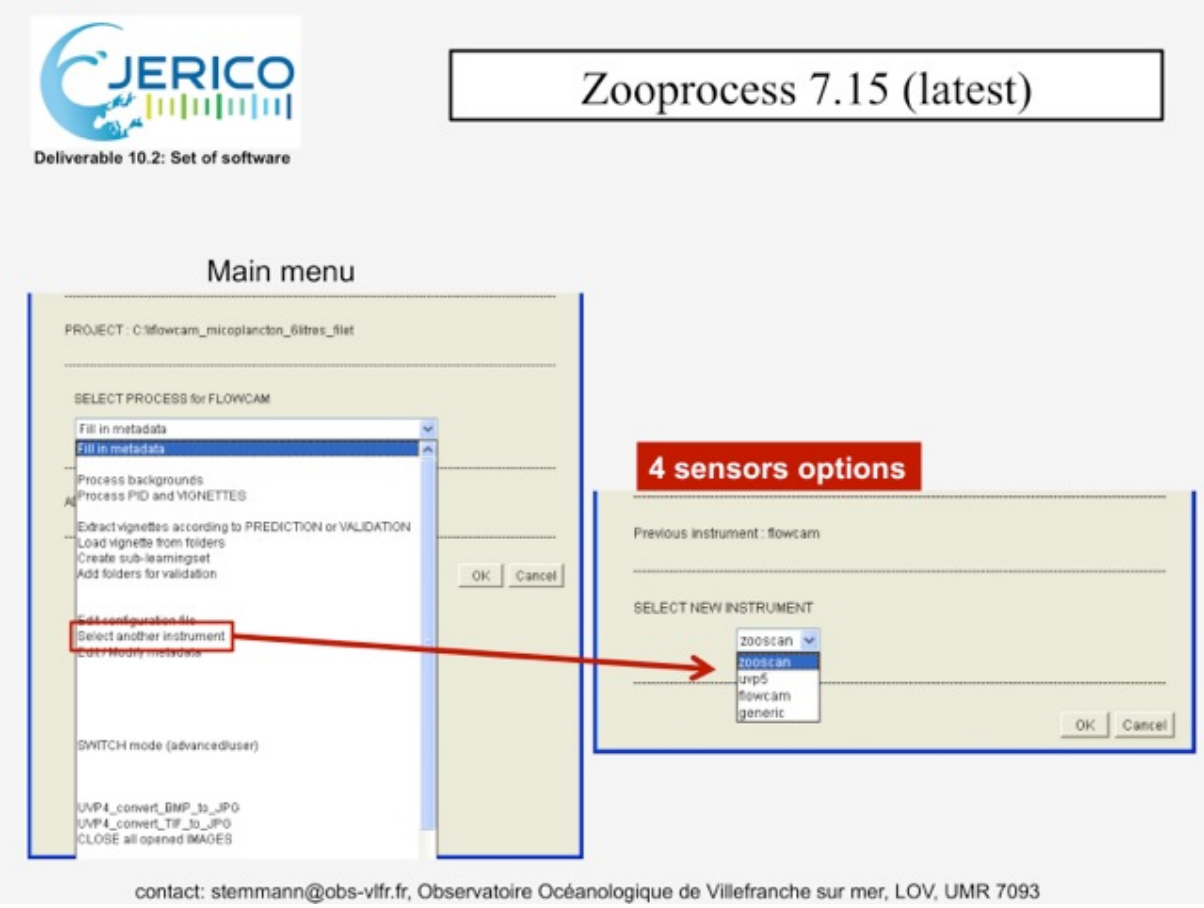

Figure 22 ***Instrument Selection screenshot from Zooprocess software.***

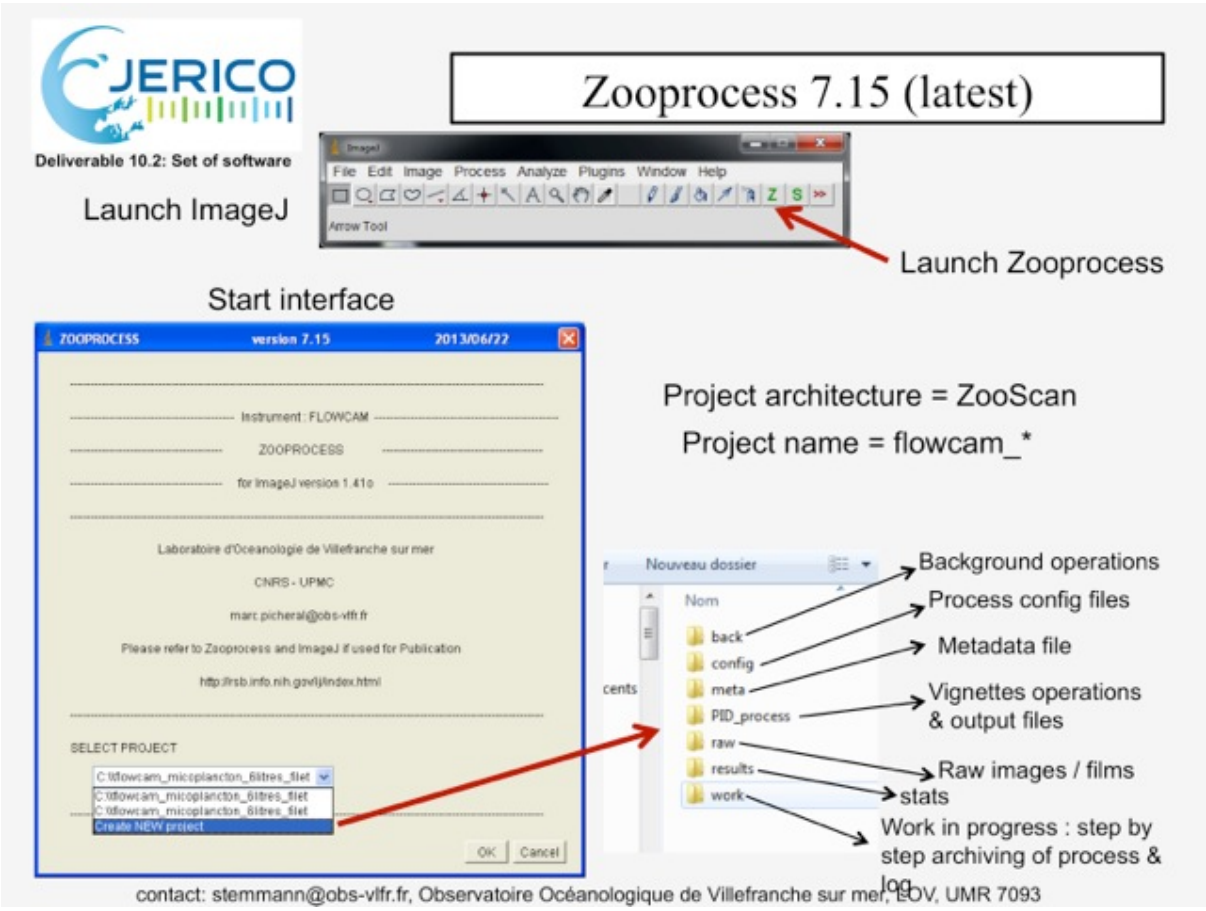

Figure 23 Zooprocess Start Interface screen and the generation of storage folders for one sample or a collection of samples.

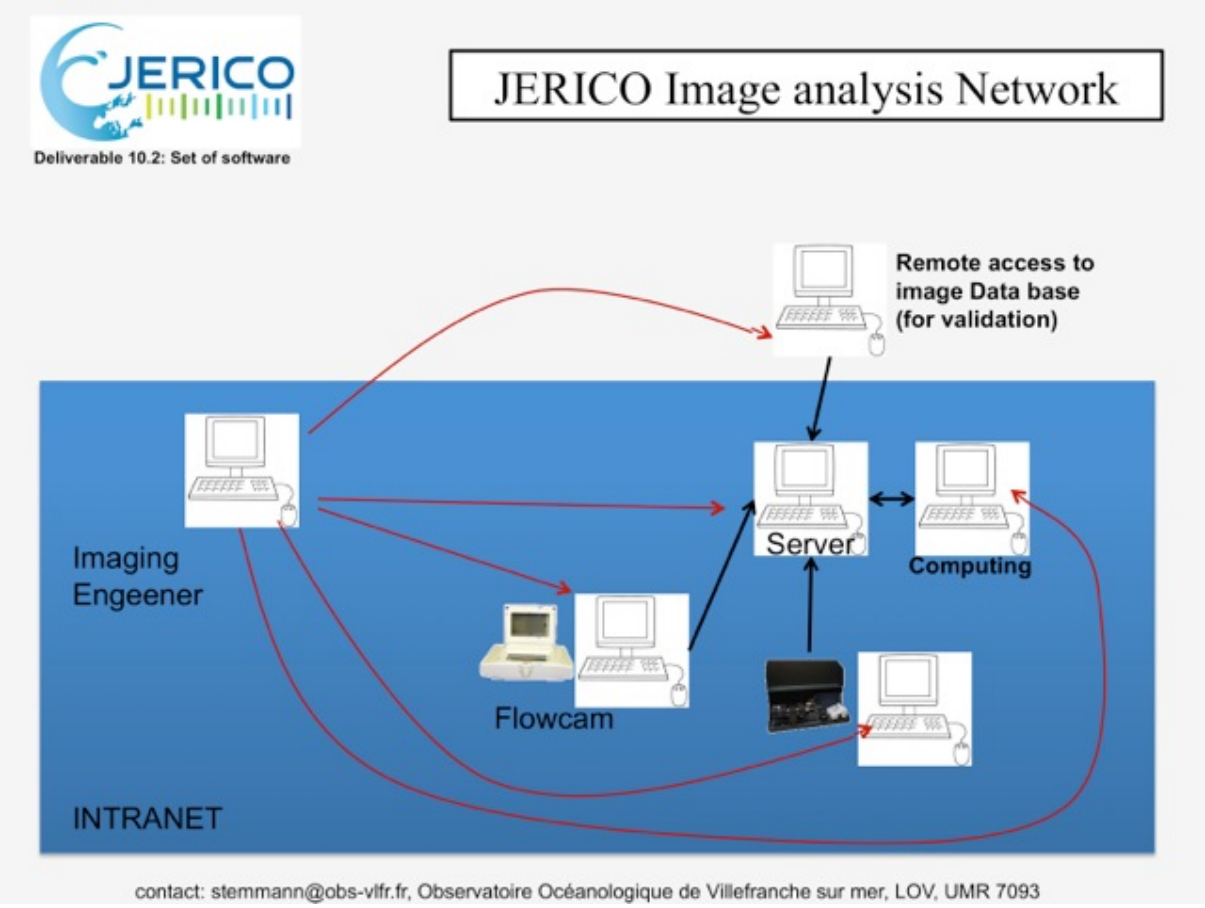

Figure 24 *Schema for Jerico Image Analysis Network using the zooprocess software.*

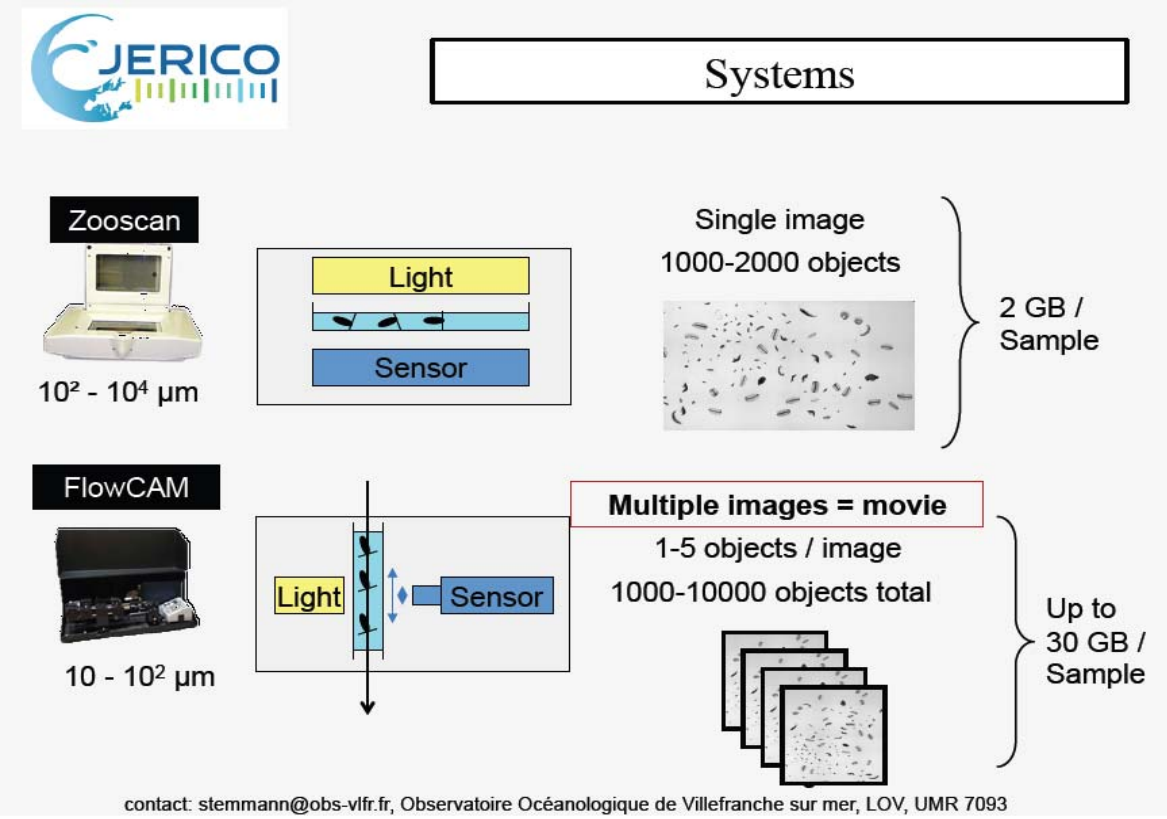

Figure 25 Overview of Zooplankton system

- Install ImageJ
  - *download and execute installation*
  - Check version
  - Set memory to 350 mB

- Install Zooprocess 7.14 set of macros
  - *download in c:/program_files/ImageJ/Macros folder*
  - *follow manual instructions*

- Download and extract the Zooscan_train_rectangle_discus_TP.rar archive on the root of a drive.

- Start Zooprocess and install it (for ZOOSCAN), fill the requested parameter using the Zooscan qualification sheet provided with the instrument! Select the above project when asked to create a project.

Train Processing samples with Zooprocess

- Start again Zooprocess pressing "Z" or launching ImageJ.

- Select the "train_rectangle_discus" project for all next steps.

- **SWITCH to ADVANCED** mode and **EDIT configuration file**
  - Open the only available file ("process_install_both_config.txt " created with the project)
  - Set ESD min to 4mm (instead of 0.3).
  - Press OK to save and replace file (do not rename!)

- **SWITCH BACK to USER** mode.

- **CONVERT & PROCESS Images and organisms in batch mode**
  - Use the only option that is available : "Convert AND Process Image AND particles (images in RAW folder)"
  - Keep all default parameters (Check Min ESD that must be 4 mm now!)

- Check Process
  - Check "YYYYMMDD_HHMM_suivi_log.txt" file from the "\Zooscan_train_rectangle_discus_carmen\Zooscan_config" folder
  - **CHECK process by viewing segmented images**
  - **VIEW vignettes** (optional)
    - "From a 2D graph…",
      - gamma = 1.3
      - Add Outlines,
      - Clear outside
        - X = ESD
        - Y = Mean,
    - Press EXIT green button and try another graph if you want!

  - Read sample_dat1.pid file using PID_viewer
    - Double click on the "rectangle_vlfr2_a_1_dat1.pid" file in "D:\Zooscan_train_rectangle_discus_carmen\PID_process\Pid_results\ target_d_1_dat1.pid" to open

- **SEPARATION from B/W msk image**
  - Keep all default parameters (except Gamma = 1)
  - Draw separation lines on the first vignette and divide some other rectangles even if not really necessary.
  - Go to the end or stop before (read instruction)
  - Do NOT reprocess immediately "Process LATER"

- **CONVERT & PROCESS Images and organisms in batch mode**
  You need to process again the images you separated just before.
    - Use the option: “Process again particles from processed images to include SEPARATION MASK (images in WORK sub- folders)” to reprocess images that have a separation mask recently created.
    - Read sample_dat1.pid file using PID_viewer to check that the separations have been included.
        - Click on the “rectangle_vlfr2_a_1_dat1.pid” file in “Zooscan_essai\Zooscan_scan_work\rectangle_vlfr2_a_1”
    - Check segmented image (*.sep) to see the white separation lines

- **STATS and GRAPHS**
    - Plot selected graph and histograms
    - Save all
    - From a selected sample option
        - X = ESD, Y = Median
        - Try another if you want!
    - All graphs will be closed by Zooprocess later on

- **Manual measurements on all vignetttes**
    - note that you can make up to 4 measurements by going back to a previous vignette and making another measurement (see manual).
    - set gamma to 1
    - Do measurements (try to measure length and width of rectangles)
    - Check measurements opening the “rectangle_vlfr2_a_1_length1.txt” file that has been created by the tool. The file is located in the sample directory of the “work” folder.
        - Click with right button on the file, select “ouvrir avec” (Open with), select Excel
        - Select the column A
        - “Données” (data), “converter” (convert), “délimité” (delimited), “point virgule“ (dot coma)
        - Look at the dimensions

- **EDIT and MODIFY metadata**
    - Open one of the available metadata file
    - Change date (respect format!)
    - Change Latitude
    - Change operator name

- Optional: Check Normalization of grey levels
    - **DIAGNOSTIC tools**
        - Select option “Check Normalization with OD discus”

Train Computer assisted sorting

Utilize *Computer_assisted_sorting_plankton_2013.doc*

Follow the manual to sort your vignettes in 5 categories

- Dark discus
- Clear discuss
- Dark rectangle
- Intermediate rectangle
- Clear rectangle

Create a Learning Set (LS) containing about 20 objects per category.

End doing a LS using the “SUBSET” tool and predicting again the samples using this new LS.

5. Access to download the software and User Manuals

5.1. SpiArcBase

5.1.1. Links to manual

The manual is available when pressing the button '?' on the software. A copy of the manual is here attached (**Annex 2**).

5.1.2. Access to software

The software is available to download http://spiarcbase.epoc.u-bordeaux1.fr/. In order to keep track of the people using it a form needs to be filled.

5.1.3. Computer Requisites

Hardware: Pentium PC or equivalent with at least 2GB of RAM

Software: Microsoft Windows XP, Vista or 7

5.2. AviExplore

5.2.1. Links to manual

The manual is available when pressing the button '?' on the software. A copy of the manual is here attached (**Annex 3)**.

5.2.2. Access to software

A site web to download the software is *under construction* (aviexplore.epoc.u-bordeaux1.fr). So far, AviExplore can be obtained by contacting one of the following members of the CNRS-UMR 5805-EPOC laboratory: a.romero-ramirez@epoc.u-bordeaux1.fr (Alicia Romero Ramirez), antoine.gremare@u-bordeaux1.fr (Antoine Grémare) or jc.duchene@epoc.u-bordeaux1.fr (Jean-Claude Duchêne).

5.2.3. Computer Requisites

Hardware: Pentium PC or equivalent with at least 2GB of RAM but 4GB is better.

Software: The AviExplore works under Microsoft Windows 7 or Windows 8. It needs to have Microsoft .NET Framework v4.5 to work; this framework is available to download on the Microsoft site (http://www.microsoft.com/fr-fr/download/details.aspx?id=30653)

A video codec needs to be present on the computer, the AVI MPEG4 Version 2.

5.3. ZooScan

The Zoo process software has extensive documentation to aid users in zooplankton identification.

5.3.1. Links to manuals

http://www.obs-vlfr.fr/LOV/ZooPart/ZooScan/Download%20Softwares/ZooProcess/ZooProcess_Manual_v716.pdf

5.3.1. Access to software

The latest version is available on a website:

http://www.obs-vlfr.fr/LOV/ZooPart/ZooScan/article.php3?id_article=214

5.3.2. Prepare computer

Utilize *ZooProcess_Manual_v714.doc to install it.*

Hardware: Pentium PC or equivalent with at least 8GB of RAM

Software: Microsoft Windows XP, Vista or 7

6. Conclusions

Introduction to conclusions

Add a few lines here……

SpiArcBase is a new software specifically designed for the processing of SPI's,

6.1. Sediment Profile Images

The spiArcBase software, developed in the frame of Jerico, allows enhancement of the interpretation of features observed on SPIs. Image analysis, coupled with use of database and learning processes facilitate image management and structures visualization, as well as inter-calibration of profiles coming from different areas.

Key features when analyzing sediment profile images are the drawings of the sediment-water interface and aRPD, and the assessment of biogenic structures. For each of these 3 features, SpiArcBase is aiming at providing an integrated set of tools allowing for: (1) carrying out the (often manual) currently most often used procedures, and (2) using new automated tools in view of facilitating and enhancing the objectivity of SPI processing.

Besides all the most often currently used procedures, it includes significant innovations such as: (1) possible automation of the drawing of sediment-water interfaces, (2) a new based on knowledge procedure for the drawing of aRPDs, and (3) the storage of SPIs variables within a database. By doing so, SpiArcBase is aiming at: (1) improving and standardizing the procedures used for SPI processing, and (2) enhancing the test and development of new SPI derived benthic habitat ecological quality indices. SpiArcBase will also hopefully contribute to the emergence of a wide community of users of Sediment profile Imaging, which may for example contribute: (1) to the creation of a "universal" learning set for the assessment of aRPDs, and (2) to the improvement of existing or to the development of new procedures through tight interactions with the software developing team.

More information concerning the development of SpiArcBase can be found in (Romero-Ramirez et al., 2013)(**Annex 4**). Reported use of SpiArcBase is so far limited to the Mediterranean Sea (Bonifácio et al., 2014; Labrune et al., 2012), however we know that it has been downloaded in other geographical areas and we are expecting to have more inputs.

6.2. Video Imaging

AviExplore provides a unique environment to analyse videos. AviExplore presents a user-friendly interface developed for dynamic image analysis on fast moving environments as well as long-time series of images.

AviExplore can work with images generated by any image acquisition system though its performance depends on the size of the images, the acquisition speed, and the characteristics of the acquisition frame.

One of the key features of AviExplore is the possibility of doing a fast image extraction from a video that leads to a straightforward image comparison. Image comparison is the core of AviExplore mobile module.

AviExplore provides with the ScriptEdit module and the real time acquisition working modes a wide range of video analysis possibilities which not only fulfil the initial objectives stated at the beginning of JERICO but oversteps them.

The use of AviExplore has been so far limited to the development research team, but we have used it within various and different environments and with satisfactory results. We hope for the the improvement of the existing tool by tight interactions with other users.

6.2.1. Mobile platforms

AviExplore mobile module has been specifically developed within the frame of Jerico to analyse films coming from underwater mobile vehicles like ROVs, AUVs, etc. The Script editor module allows building adapted scripts to analyse video and count not only epibenthos organisms (**Fig. 19**) but other objects present on or around the sea floor. One of these objects can be garbage (**Fig. 26**).

Filters, coupled with geo-localisation and image segmentation help extracting information on the presence of organisms and structures present on the sediment.

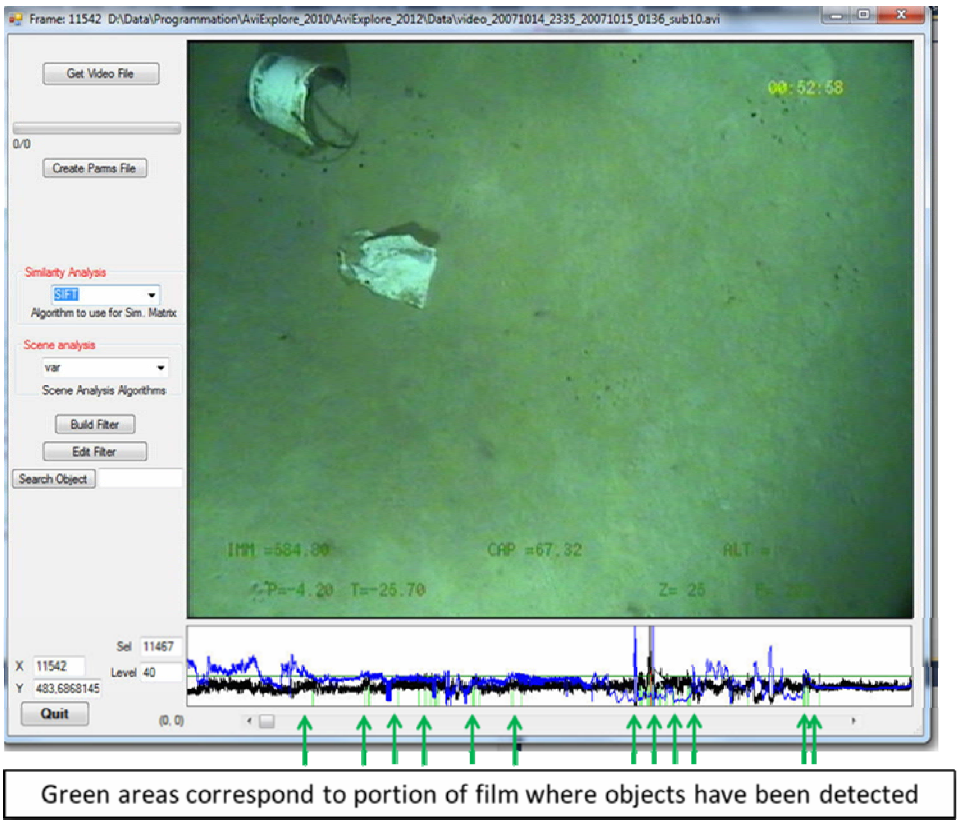

Figure 26. Example of the use of AviExplore mobile module for garbage location.

6.2.2. *Fixed platforms*

AviExplore fixed module has been specifically developed within the frame of Jerico to allow the survey of recruitment on substrates, as well as the growth characteristics of fouling organisms. Image analysis is also used to track the animals settling on the substrate, measure their interactions and growth rates (**Fig. 27**). The key feature of this module is image subtraction that can be combined with the selection of areas to analyse (**Fig. 28**). Based on our experience, and due to the fact that AviExplore has been optimised for fast image extraction, image subtraction can be first used to identify the regions where there is the most activity and a second time for more detailed information on those regions.

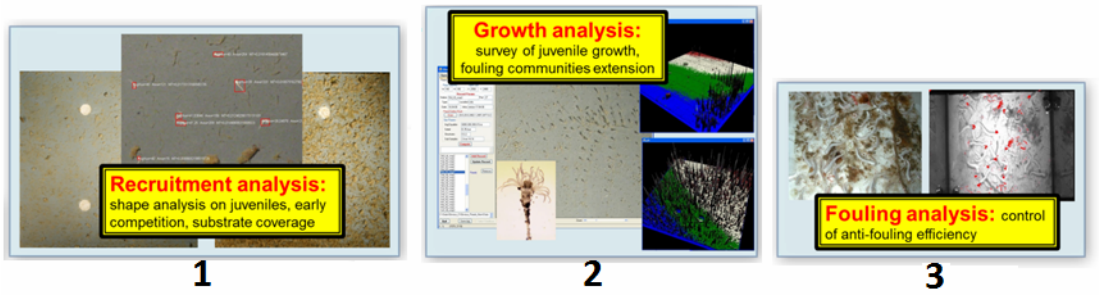

Figure 27. Examples of the use of AviExplore fixed Module.

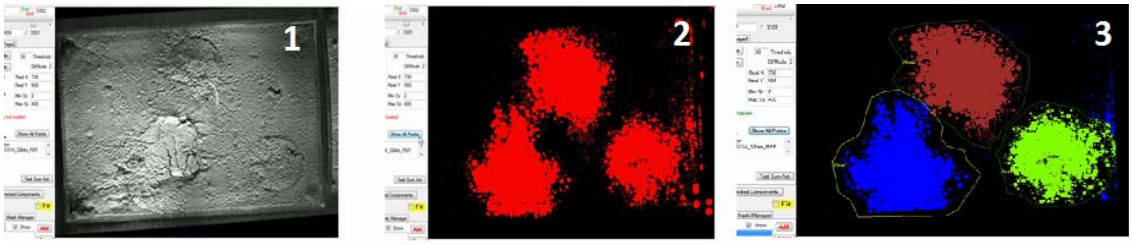

Figure 28. Example of use of AviExplore Fixed Module for the identification of regions whith high activity. 1) Gray-scale image, 2) Result of the overall addition of moved pixels, 3) Identification of three regions

Future developments of this module will include the possibility of using a 3D camera in order to have not only surface information but volume estimation.

6.3. Phyto and Zoo plankton

As part of Task 10.1 The Zooprocess Image analysis tool which has been in development over a number of years has been enhanced for zooscan and zooplankton analysis, adapted specifically to FlowCAM and Microplankton analysis and adapted to any kind of imaging device e.g Inverted microscopy tested and UVP tested. The software has facilitated a more holistic sampling strategy to get appropriate samples to test and a Holistic analysis of plankton dynamics based on zooprocess and combining 3 imaging instruments – Romagnan et. Al 2015. There are currently ongoing collaborations around imaging and the zooprocess software. We have build an agreement with the French MPA to analyse the zooplankton samples from their marine protected area. We have organised a meeting in Villefranche to build a community of users (http://www.obs-vlfr.fr/Rade/RadeZoo/RadZoo/Plankton_of_the_world/Plankton_of_the_world.html). We have also

organised training courses with the EU FP7 PERSEUS project to educate European scientists is the use of sampling protocols and Flowcam and Zooscan (http://www.obs-vlfr.fr/Rade/RadeZoo/RadZoo/Plankton_of_the_world/Plankton_of_the_world.html).

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