



Traceability and Quality Monitoring throughout the Fish Value Chain

## D3.2 Analysis methodology and performance







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## ACRONYMS LIST

TMF	Trace My Fish
R&D	Research and Development
UX	User Experience
ICT	Information and Communications Technologies
API	Application Programming Interface
SSO	Single Sign On
PCB	Printed Circuit Board
GUI	Graphical User Interface
AI	Artificial Intelligence

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## EXECUTIVE SUMMARY

In this Deliverable, the VIDEOMETER spectral imaging technology and data analysis methodology developed in the TMF project is presented. More specifically, descriptions of the two mobile Videometer instruments are provided, including information about the capabilities of the Videometer software and the Videometer workspace. Moreover, different applications are presented by the pilot partners, including the estimation of microbiological quality, changes in fisheye, gills, texture etc., while methodologies on data pre-processing and analysis of acquired images based on a specific problem/task are presented as well.

More specifically, NTNU partners examined two different analytical approaches for the estimation of salmon fillets color including direct analysis of the fish fillet and analysis of parts of the fillets with specific dimensions placed in a petri dish. In addition to that, the comparison of the performance of the two different Videometer instruments was tested.

UoI partners focused on the detection of parasites, along with texture, chemical and microbiological measurements.

AUA partners worked on the assessment of 1. the microbiological quality and freshness 2. Texture profile, 3. Any changes on fish head/eyes using the two imaging analysis instruments. Certain analytical methodologies are presented while a part of the analyses is still in progress.

The overall results will be presented in the final deliverables of WP6 at the end of the project.

## 1 INTRODUCTION

The initial goal of this Task was the development of a coherent data analysis methodology framework to properly analyse, segment, and classify data from the beginning to the final product. This data analysis can be performed in situ, in the cloud or both. During the TMF project, best practice methodology was defined and applied.

The three pilot partners (AUA, UoI, and NTNU) initially exploited the spectral imaging platform already being available in their laboratories, the VideometerLab, to provide information about the best configuration, choice of wavelength, measurement protocol, and more. After that, the first handheld mobile VideometerLite TMF prototype was available for them and was used in different experiments related to the fish quality throughout the value chain.

Then the second VideometerLite TMF prototype was developed, including different and more bands (at different wavenumbers) in order to test and evaluate the performance and usability throughout the BlueBio value chain by the partners.

Data from both mobile devices could be shared locally through wifi networks or directly to the cloud through 4G/5G.

In this Deliverable, a description of the two mobile Videometer instruments is provided, including information about the capabilities of the Videometer software and the Videometer workspace. Moreover, different applications are presented by the pilot partners, including the estimation of the microbiological quality, the changes in fish eye, gills, texture etc. A part of data analysis is still ongoing, so the results are still preliminary. Additionally, methodologies on data pre-processing and analysis of acquired images based on a specific problem/task are presented.

## 2 VIDEOMETER INSTRUMENTATION

Videometer's technology is based on LED band-sequential spectral imaging. Spectral imaging is a scientific methodology which combines imaging with spectroscopy, allowing for the measurement of different spectra within the same image (Carstensen, 2018).

Spectral imaging, in the Videometer case, is achieved by placing a camera on the top of an integrating sphere, with LEDs of different wavelengths placed around its equator. The integrating sphere comprises of a hollow sphere with a highly reflective coating, which allows for uniformity in the scattering and diffusing effects of the LEDs' light rays (Figure 1). The LEDs strobe sequentially to capture a stack of images recorded at different wavelengths. Consequently, each pixel in the captured image represents a reflectance spectrum. The range of the Videometer technology includes ultraviolet, visual, and near-infrared wavelengths. Additionally, fluorescence can be captured by placing different filters in front of the camera. (Carstensen & Folm-Hansen, 2006).

### 2.1 VIDEOMETERLAB

The VideometerLab is Videometer's flagship instrument. It is a high-performance spectral imaging instrument with applications for a broad range of industries. The unit integrates illumination, camera and computer technology with advanced digital image analysis and multivariate statistics.

Using the strobed LED technology, the VideometerLab combines measurements at up to 20 different wavelengths ranging from 365 nm to 970 nm into a single high resolution spectral image, with every pixel in the image representing a spectrum. The system offers customization possibilities, including the possibility to expand wavelengths to 1050 nm, or additional filters for multispectral fluorescence analysis.



Figure 1 VideometerLab Instrument

## 2.2 VIDEOMETERLITE

The VideometerLite is a portable, wireless, and cost-efficient solution. Its integrating sphere measures 130 mm in diameter, and it comprises of two easy-to-use and ergonomic buttons for boot-on, boot-off, and image capturing.

### 2.2.1 VideometerLite TMF Prototype 1

Prototype 1 was developed between month 1 and month 6 of the TraceMyFish project. The first prototype included 7 wavelengths in the spectrum of 405-850 nm i.e., 405 nm (Violet), 460 nm (Blue), 525 nm (Cyan), 590 nm (Amber), 621 nm (Red), 660 nm (Red), 850 nm (NIR).

The first prototype's development included its connectivity to the VideometerLab Software. A simple RESTful API for software connection and, hence, control and supervision via Wi-Fi or ethernet connection was made. Images can be captured in a matter of 7-10 seconds, allowing for rapid in-field analysis and motion control.

### 2.2.2 VideometerLite TMF Prototype 2

Prototype 2 of the VideometerLite includes more powerful LEDs, which would be suitable for the expansion of UV wavelengths to 365 nm and the addition of fluorescence filters. The wavelengths included in Prototype 2 are 11 and are: 365 nm (UV), 405 nm (Violet), 430 nm (Blue), 450 nm (Blue), 490 nm (Blue), 515 nm (Cyan), 590 nm (Amber), 630 nm (Red), 660 nm (Red), 690 nm (Red), 850 nm (NIR).

The VideometerLite's body was made to be modular, so to be able to add different mounts onto the instrument.



Figure 2 VideometerLite Instrument

Though not used in the pilots, but because of previous WP2 stakeholder requests, a VideometerLite Liq add-on was additionally developed to be able to analyze liquid products.

### 2.2.3 VideometerLite with Liq Add-on

The Liq mount serves as a convenient add-on for the VideometerLite. It can be attached to the device with just two screws. With this attachment, users gain the ability to capture instant and time-lapse images of 50 mL bottles containing liquid products.

The VideometerLite Liq Mount showcases a smart magnet lock lid, enabling opening and closing while effectively blocking out all external light sources. This guarantees a controlled environment for capturing precise and reliable spectral imaging data.

The analysis of liquid stability, turbidity and general color changes in liquids can be done with the Storyboards tool in the VideometerLab Software. The Videometer Storyboard is a separate Session feature which takes a folder of images as input. This folder can contain different samples each measured (imaged) at different timestamps to track color changes in the product/sample. The output, when generating this Storyboard, is then a transformed image, where the color change over time in the sample is visualized on a scale from blue to red, where red represents a big change in the product.



Figure 3 VideometerLite with Liq Add-on

## 2.3 DATA ANALYSIS AND PROCESSING

The VideometerLab Software is a complete image data analysis and processing platform, allowing users to fully evaluate and research their data.

Among the features and tools further developed and utilized in the TraceMyFish project, for the analysis of fish samples, are the following:

- a. Transformation Builder
- b. Pixel Classifier Builder
- c. Segmentation Builder (two versions)
- d. Segmentation Sequence Builder (two versions)
- e. Blob Tool

We, hereby, present the tools, their purpose and use.

### 2.3.1 Transformation builder

When you have a sample consisting of one or multiple objects, it is possible to perform different orthogonal transformations on the multispectral image which among others are used for discriminant analysis between defined classes. Meaning, that it is possible with the VideometerLab software to annotate certain parts (objects) of an image (or multiple images), and, by making a transformation, create a new image that separates classes/objects in the image(s) based on color differences/tendencies in the object(s). The resulting new image may have a different number of bands, and in many cases only one.

The transformation makes it possible to distinguish between objects based on color and surface chemistry, thus it can be used to separate certain classes and objects from the rest of an image, by associating objects to color trends.

There are three different scenarios associated with transformations:

- a. Known vs. known: here, users know how all possible objects in a sample might look like and on which basis they want to separate them from each other.
- b. Known vs. unknown: in this case, users know which objects they are looking for, but not the context they are inspecting them in.
- c. Unknown vs. unknown: in the last scenario, users do not know which features would make an optimal transformation. Therefore, a transformation is made based on the spectral noise in the various image bands, so to be able to investigate possible tendencies worth exploring.

Each of the three scenarios, in the VideometerLab Software, corresponds to a different type of transformation. Table 1 illustrates the correspondence.

Table 1 Transformation Scenarios

Scenario	Transformation
Known vs. known	nCDA
Known vs. unknown	nMahalanobis
Unknown vs. unknown	MNF/PCA

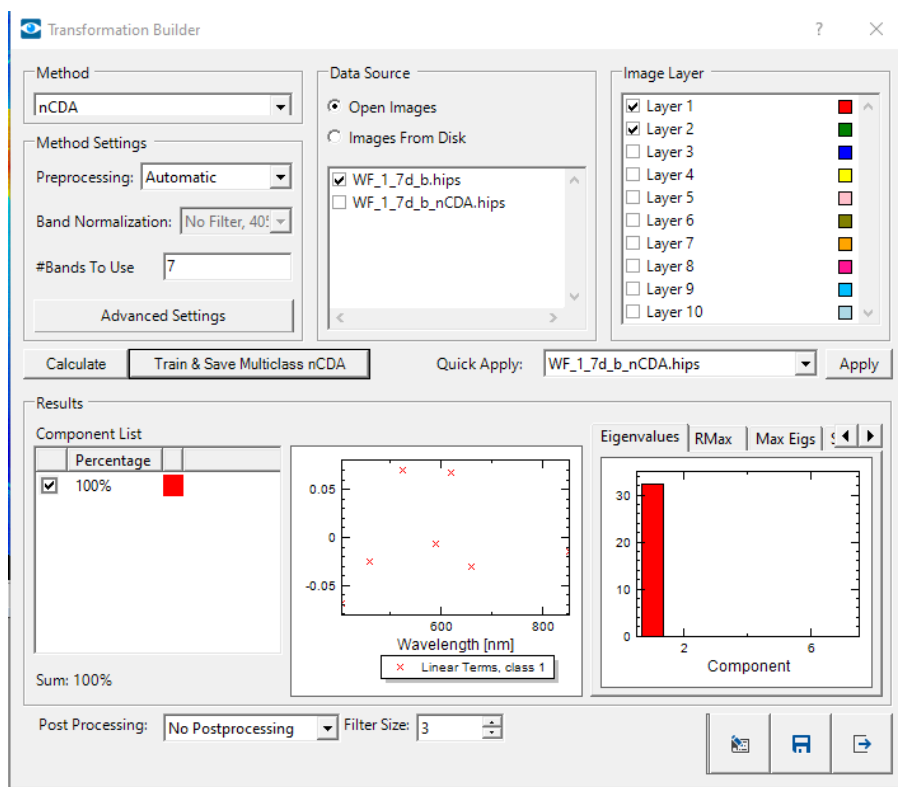


Figure 4 Transformation Builder Tool

### 2.3.1.1 Known vs. known – nCDA

The most applicable transformation is the normalized Canonical Discriminant Analysis (nCDA). This transformation is based on the known vs. known scenario. Before performing an nCDA, it is important to annotate the image with different color or paint layers. As an example, we take the sample consisting of a fish fillet in Figure 5. Here the “desired” class, the fish fillet, is painted red, and the “unwanted” class, the background, is painted green. The transformation based on this division of classes will create a new image with a new pixel range going from blue to red. Based on this new pixel range, it is possible to separate objects from the background or from other objects based on the annotation color difference (Figure 5).



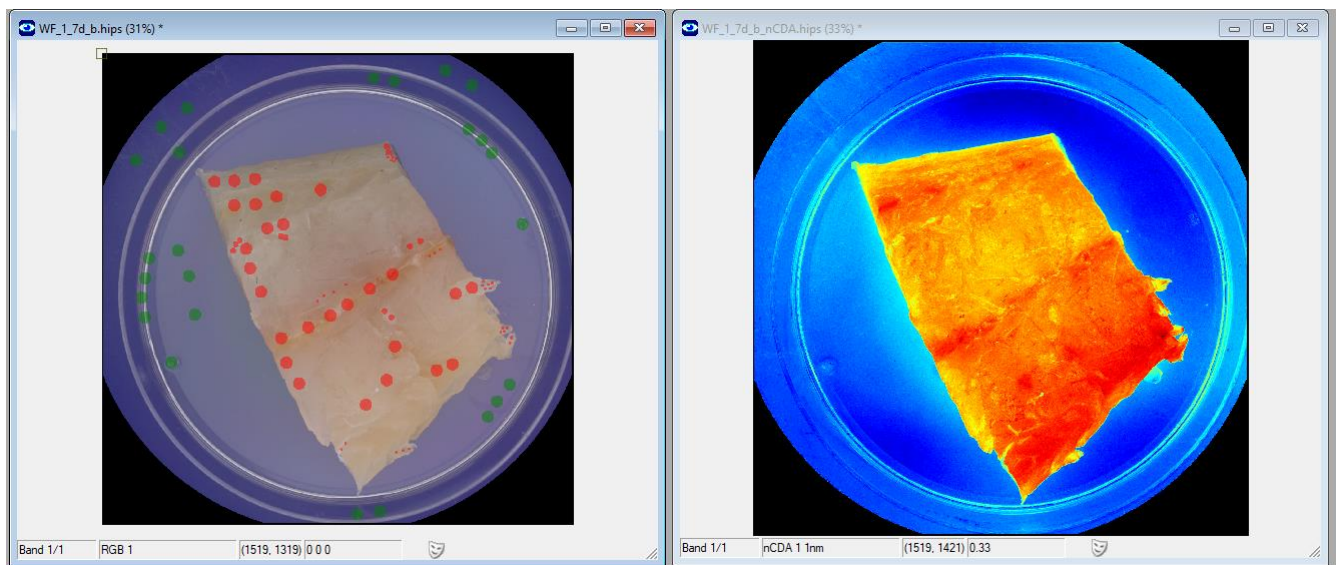


Figure 5 Foreground vs. background nCDA performed on white fish sample.

### 2.3.1.2 Known Vs. Unknown – nMahalanobis

In the case of a known vs. unknown scenario, it is possible to make a second discriminant analysis, based on how little all other objects look like the “desired” one – this is done with a Mahalanobis analysis.

In this case, you annotate your desired object in the image and make a transformation that identifies your “desired” object from the rest of the sample. An example can be seen in Figure 6.

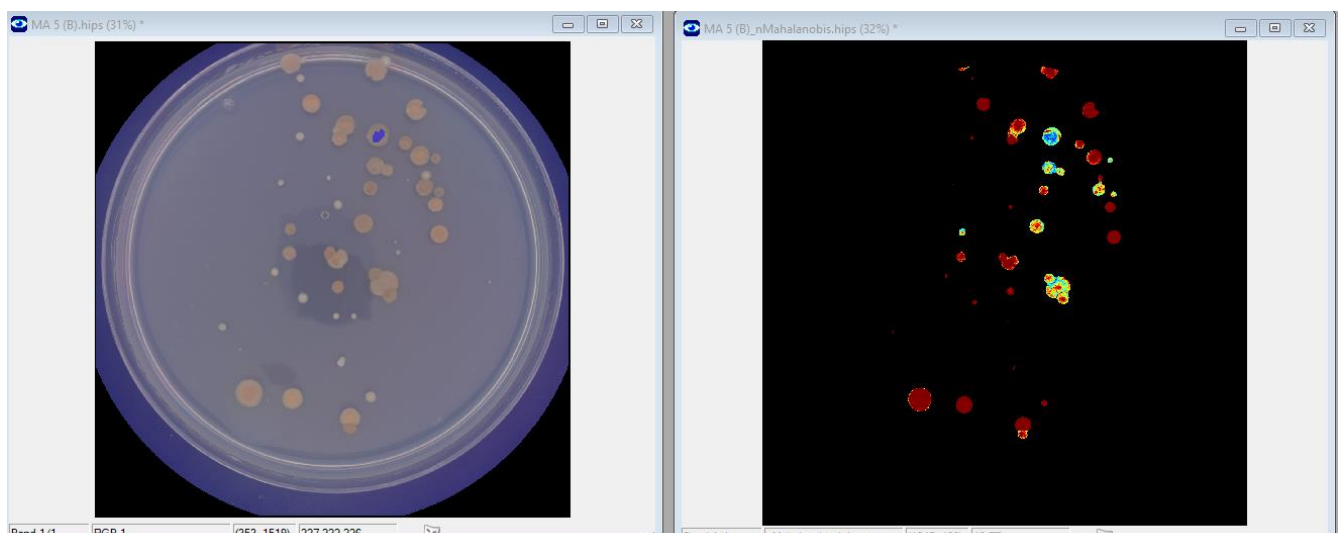


Figure 6 nMahalanobis performed on colony sample.

### 2.3.1.3 Unknown vs. unknown – MNF/PCA

The third scenario unknown vs. unknown is best analyzed with the MNF transformation. Once the MNF transformation has been applied, an output image consisting of the same number of bands as the reference

image is outputted. These bands each “express” a hidden feature in the image. The MNF estimates the noise pattern in the image and the orthogonal transformation is optimized in respect to signal versus noise. The first bands are the ones which best visualizes the most prominent differences in the sample image. The amount of expressed variation (distinction) in the following bands will gradually fade out, as all band “dimensions” are covered. Only a limited number of bands is often needed to visualize the differences in the sample. Figure 7 shows examples of an MNF transformation applied to a petri-dish with a fish fillet.

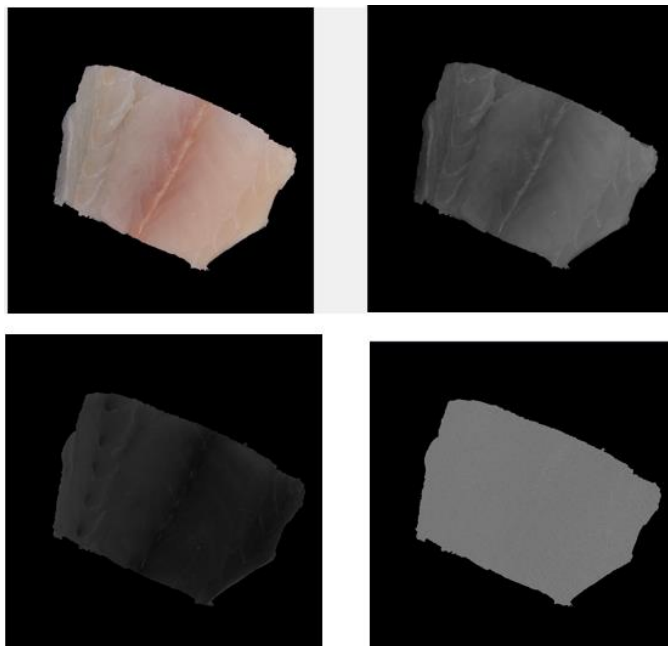


Figure 7 MNF performed on white fish fillet. From top left: Original image, MNF band 1, MNF band 2, and MNF band 7.

### 2.3.2 Pixel classifier Builder

The Pixel Classifier builder is a tool in the VideometerLab Software, that allows the user to assign a label, that corresponds to a certain class, to each pixel in an image. The number of classes is chosen manually by annotating the image with paint layers. The classification is, then, carried out thanks to the multidimensional nCDA

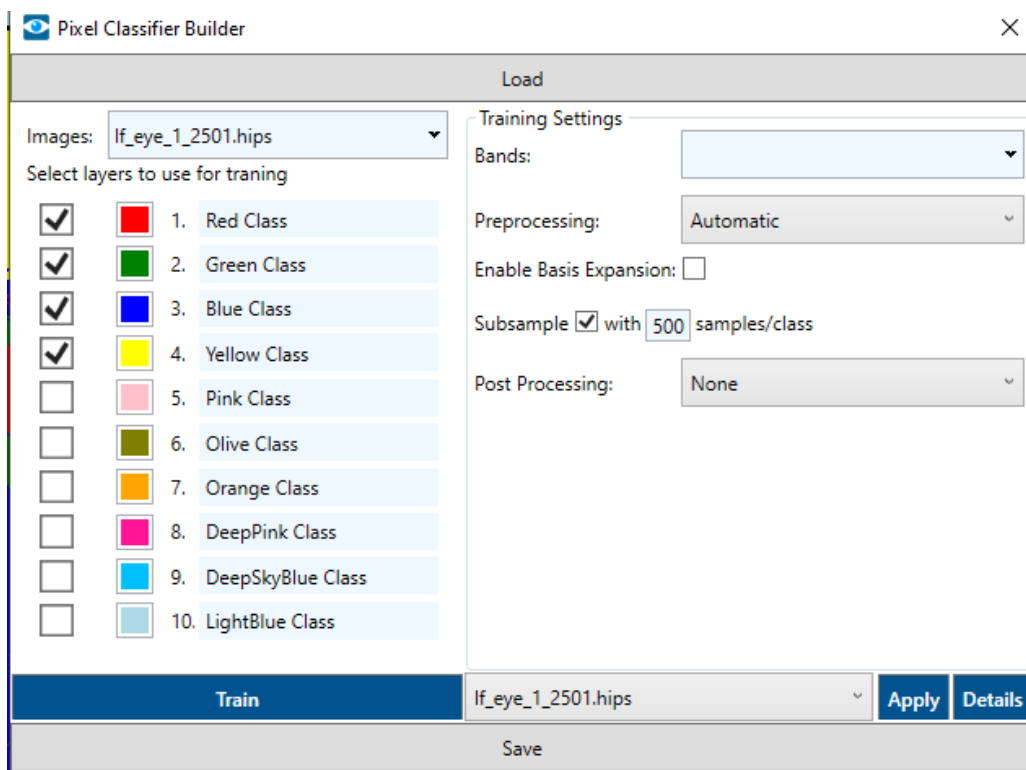


Figure 8 Pixel Classifier Builder

transformation, in fact, this tool combines a series of nCDAs so to discriminate between multiple classes. Finally, an image segmented into the chosen number of classes is given as the output.

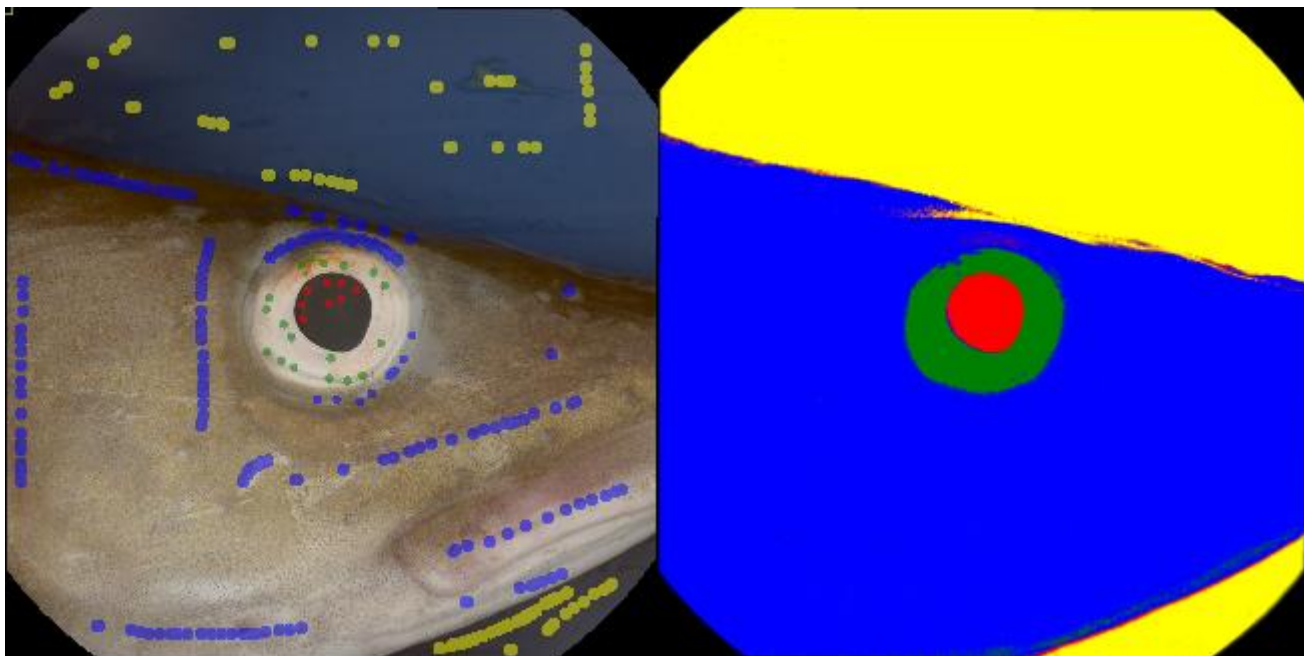


Figure 9 Pixel Classifier applied on salmon fillet

### 2.3.3 Segmentation Builder

A Segmentation is an algorithm classifying individual pixels or multiple pixels (larger objects) in an image, for example it can remove the background and/or discriminate between different objects in an image. In many situations it is advantageous to first remove the background of an image when analyzing it, and then separate objects from one another.

The Segmentation Builder (version 2) in the VideometerLab Software allows you to perform segmentations on your images with a step-by-step process. Below we illustrate the different steps:

#### 1. Load

It is possible to load and edit a previously saved Segmentation.

#### 2. Pre-Segmentation

Before segmenting out the objects (blobs) in the image, this pre-segmentation allows you to do a standard segmentation of a petri dish or a specific defined area in the image.

#### 3. Foreground/Background Segmentation

In this step, it is possible to segment the foreground (your objects) from the background. This is done by making a Transformation or Pixel Classifier file and setting a threshold value with respect to this selected Transformation.

#### 4. Fill Holes

After the segmentation of the objects, it is possible to fill faulty “holes”/spaces within the objects. This may especially be advantageous before separating the objects in the later step. If there are holes within the objects, this is very likely to cause wrong separations of the objects.

#### 5. Morphological Filtering

In this step the user can apply any type of Morphological Filtering to the segmentation before the separation of objects.

In the “Morphological Filtering” step it is possible to perform a long list of morphological operations to your segmented image. The basis morphological operations are:

- “Erode”: The erode function will shrink the segmented object around the entire object. It can, for example, be used if defects around the boarder of blobs should be excluded.
- “Dilate”: This function will add pixels around the boarder of the object(s), hence making it/them larger. It can, for example, be used if the boarder of blobs should be expanded further outwards.
- “Open”: The open function removes “lonely pixels” on the boarder of the object(s). It can be used, for example, if users encounter issues with faulty separating objects in the later stages of the segmentation.

- “Close”: This function will close potential “holes” in the segmented object(s). This can also help improve separating touching objects.

### 6. Separate Touching Objects

In the “Separating Touching Objects” step, it is possible to separate individual objects that are merged in the segmentation by use of the h-domes algorithm. The h-domes transformation finds “tops” and “holes” in an image based on their height h in pixel values irrespective of size and shape. Based on the tops and holes in the image, it is, then, possible to separate touching object. Hence, one pixel thick lines in between the objects are inserted. To apply the separation of the objects, there are 2 possibilities, either “Adaptive” or “Fixed”.

- “Adaptive”: The software will automatically estimate and apply the parameter values for the h-domes algorithm to separate the objects itself.
- “Fixed”: This option is available for the few cases where the users want to adjust the h-domes values by themselves.

### 7. Morphological Filtering

In this step, the user can apply additional Morphological Filtering to adjust for unwanted effects created by “Separating Touching Objects”.

### 8. Post-Segmentation

In the finishing step unwanted objects can be removed from the final segmentation by, for example, specifying a given object size threshold.

### 9. Apply and/or Save

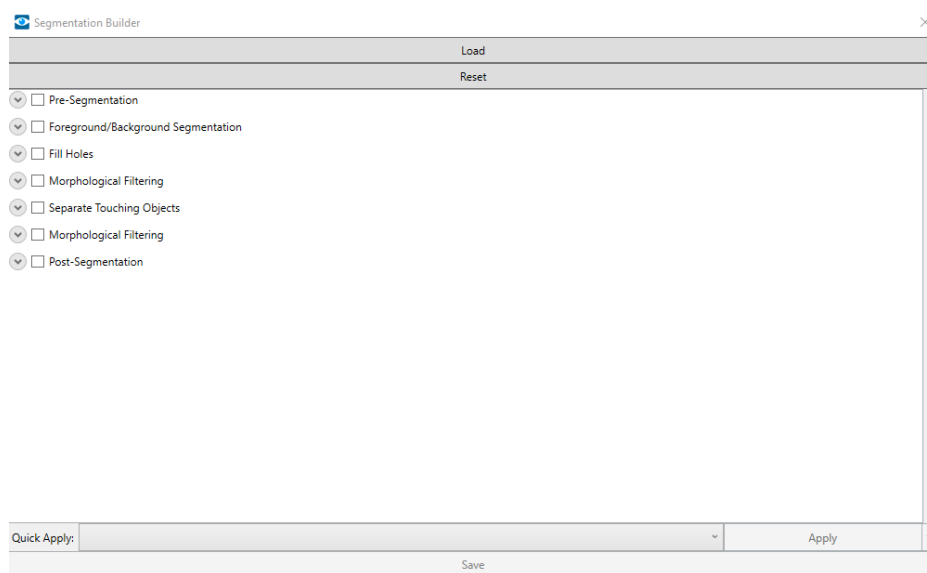


Figure 10 Segmentation Builder

Finally Apply the segmentation and then save the segmentation. The segmentation can both be applied as layer or as foreground.

### 2.3.4 Segmentation Sequence builder

The Segmentation Sequence Builder is a tool that allows you to combine multiple segmentations together, based on different logical methods:

- Intersection: The intersection function performs an AND operation between two segmentations. The outcome of the operation is true when both segmentations are true.
- Union: This function performs an OR operation between two segmentations. The outcome is true when either segmentation is true.
- Xor: This function performs an EXCLUSIVE OR operation between two segmentations. The outcome is true when either segmentation is true, but not when both are.
- Negate: This function performs a NOT operation on one or multiple segmentations. The outcome is true when the segmentation is not true.

### 2.3.5 Blob tool

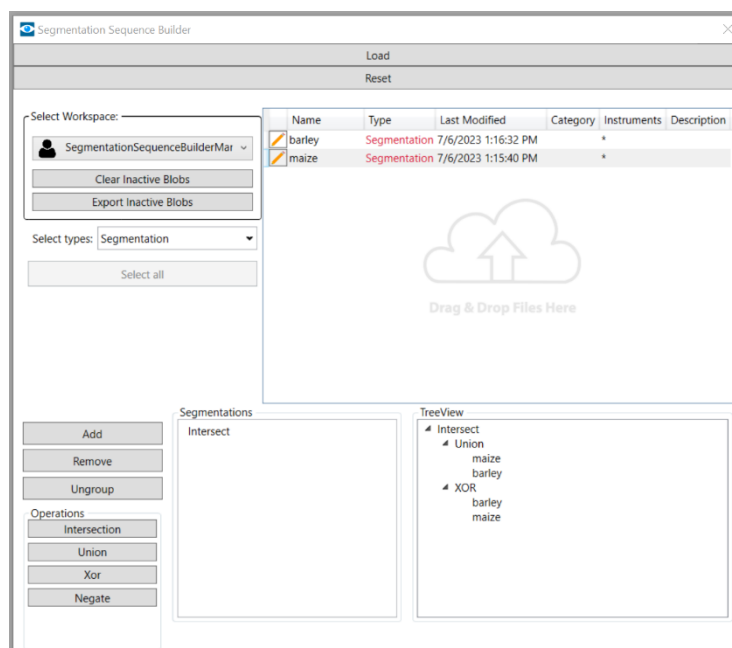


Figure 11 Segmentation Sequence Builder Tool

The Blob Toolbox can be used to extract and separate different objects from an image based on a segmentation. When the objects are separated and loaded into the toolbox, they become blobs (Binary Labeled Objects), and a classification model can be trained based on the categories (classes) which the user has defined and labelled the blobs with. The classification model will then be able to predict which Output class a blob belongs to when it is applied on new unseen samples consisting of the same type of objects.

The classification procedure is a machine learning method, where each class of objects are associated and defined by a set of common features which are able to separate blobs from each other. The features which the separation of classes is built upon varies from color (UV, visual and NIR) including fluorescence to size and shape characteristics. The set of features can be very specialized, making it possible to distinguish between visually almost identical objects.

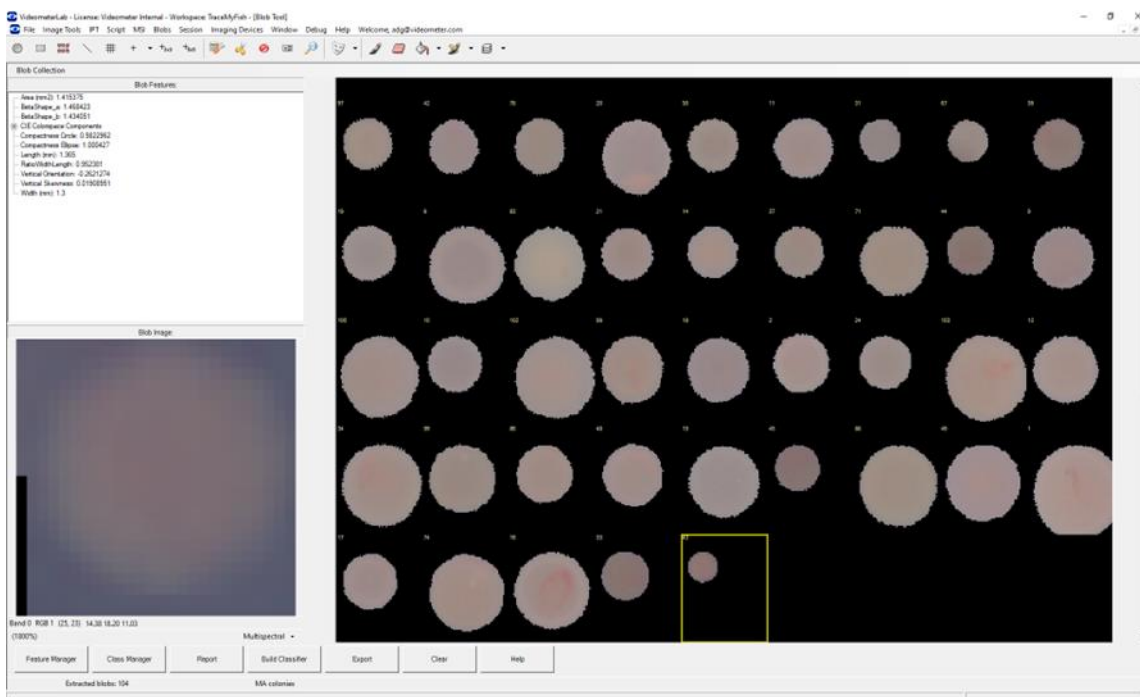


Figure 12 Colonies sorted in Blob Tool

## 2.4 DATA MANAGEMENT AND SHARING

In the VideometerLab software there are two types of cloud workspaces – private and public. The former solution allows organizations and teams to store data securely on the cloud to optimize data sharing between stakeholders where only authorized users have access to the storage and its retrieval. The Public Cloud Workspaces allow all logged-in users to utilize the available materials provided by Videometer, as a way to exchange solutions, analyses, and images. This solution makes communication between many stakeholders more accessible, transparent, and ordered.

The Private Cloud Workspace can, besides for personal use, be used across an organization that wants to share the models and session recipes for analyzing seafood. Furthermore, as the cloud is secure and requires authorization from the user, the workspace can store confidential information. This solution provides, for example, TraceMyFish parties to easily transfer and monitor shared data regarding the analyzed seafood

products. The users can choose if their data should be accessible to others by selecting the appropriate workspace.



## 3 APPLICATIONS OF THE VIDEOMETERLITE V.1 AND V.2

### 3.1 ATLANTIC SALMON USE CASE

#### 3.1.1 Imaging protocol of VideometerLite on Atlantic Salmon

In the experiment where the spectra and color of salmon fillets were measured with VideometerLite (V.1), the investigating parties were also interested in assessing the multispectral imaging prototype's ability to take images directly on whole salmon fillets. This was tested by placing the instrument directly on the fillets region of interest (ROI). The instrument's weight was supported by the salmon fillet surface and stabilized by the operator holding the instrument with one arm. This imaging protocol, named "direct protocol" was compared against another imaging protocol called "control protocol". The latter imaging protocol used the same ROI parts of the fillet, cut into 10x10 cm pieces, and placed in petri dishes on a white cutting board before imaging. Comparison of spectral reflection values between the two imaging protocols showed a significant difference where the control protocol had higher reflection values than the direct protocol, especially in the red to near infrared (NIR) spectrum. Analysis of the results led to the conclusion that the differences in spectral reflection between protocols could be caused by three factors. The first factor hypothesized to impact the spectral reflection values was lighting from external environment, reflecting on the sample surface and into the multispectral detector. The reasoning for this explanation is that there are gaps between the sample and the VideometerLite's integrating sphere, allowing external light to contaminate the reflectance values of the spectral bands. At the same time, light from the VideometerLite's LEDs escaping through the gaps would give lower reflections and create shadowed areas on the outer side of the image. An additional observed factor was the misalignment between each single wavelength band for the direct imaging protocol. Caused by movement during image capturing, this error may be corrected in future updates of the VideometerLab software. It was also suspected that the difference in materials present in the imaging area between protocols had an effect on the reflectance values. The control imaging protocol had a white cutting board as a background that could cause greater illumination of the sample during imaging than the direct protocol.

#### 3.1.2 Comparison of VideometerLite V.1 and V.2

To compare the VideometerLite V.1 and V.2, an experiment on Atlantic Salmon fillets was conducted. In this experiment both prototypes were used to capture images of the fillets throughout a period of 16 days. The reflection data was extracted after pre-processing in the VideometerLab software. Figure 13 shows images of the same fillet piece captured with V.1 (a) and V.2 (b). Since prototype V.2 covers a broader array of wavelengths in the red spectra (630, 660 and 690 nm) than V.1 prototype (621 and 690 nm), images of salmon fillets captured by V.2 could give more powerful data in this region and improved accuracy.

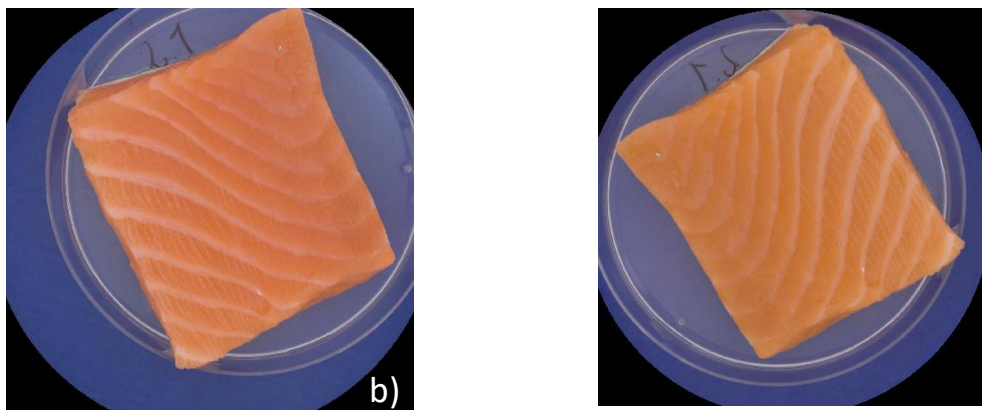


Figure 13 Images of salmon fillet captured with VideometerLite version V.1 (a) and V.2 (b)

The additional UV wavelength of 365 nm made white spots visible on salmon fillets, which indicate presence of bacteria, blood or pin bones that were not detected in visible light. The spots were most visible at bandpass 570-900 nm (Figure 14), which could be due to the stronger contrasts between the white spots and the red spectra having the highest intensity in the salmon fillet.

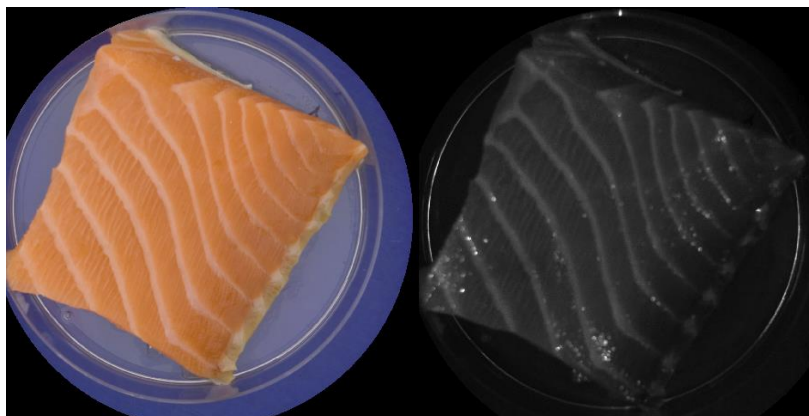


Figure 14 Image of salmon fillet shown in sRGB (left) and at UV 65 nm bandpass 570-900 nm (right)

The reflection data from images of the same sample taken with the two prototypes were compared at the different wavelengths and no significant differences ( $p > 0.336$ ) were found. Although there were no significant differences between the two versions for each of the wavelength, the sum of reflection values for all the bands, varied significantly ( $p < 0.001$ ) between the two prototypes. This can be observed as the reflection values from prototype V.2 are slightly lower than the reflection values for prototype V.1. (Table 2).

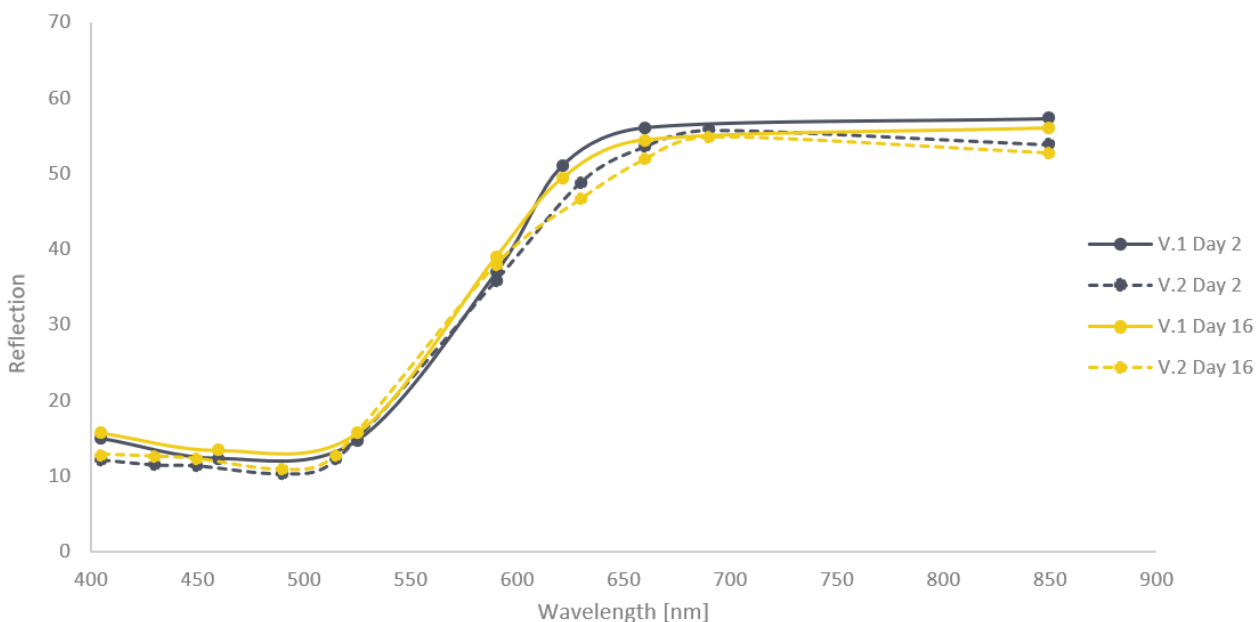


Table 2 Reflection data from images of salmon fillets captured by VideometerLite V.1 and V.2

### 3.2 WHITEFISH USE CASE

The whitefish pilot case evaluated the possibility of using the Videometer technologies for detecting parasites, such as ring worms at different depths in Atlantic cod fillets, along with predictions of microbiological, physicochemical and sensory quality changes.

Parasite detection was evaluated through imaging Atlantic cod fillets using the VideometerLab 4 and the VideometerLite V1. The equipment was brought to a processing facility for testing of the equipment in industrial living lab settings. Comparisons were made between the images captured using the Videometer equipment and a trained processing facility employee removing parasites through the traditional candling/light table method. Furthermore, the image performance differences of the equipment while using them open or closed dome settings were also assessed.

Texture analysis and microbiological and chemical spoilage were evaluated through imaging Atlantic cod fillet pieces throughout cold storage using the VideometerLab4 and the VideometerLite V1 and V2, along with analyses of Total Viable Count (TVC), total volatile base nitrogen (TVB-N) and texture.

The quality of whole fish was evaluated through imaging of eyes, gills, and skin of whole gutted Atlantic cod which was stored for different amount of times prior to it being analyzed by a trained sensory panel using the Quality Index Method (QIM), along with total viable counts (TVC) of skin and muscle, and TVB-N of muscle. Prediction models based on extracted image characteristics and spectral data will be built and tested and their efficiency in predicting microbial spoilage in whole Atlantic cod will be evaluated.

Data and image analysis is currently ongoing in all three trials mentioned above but are planned to be finished during the autumn months of 2023.

### **3.3 MEDITERRANEAN SEABREAM USE CASE**

The assessment of seabream's freshness using the two Videometer prototypes was attempted both by correlating data from imaging analysis with microbiological data and by analyzing images of fish heads/eyes. The detailed experimental design, procedures and results are presented in the framework of WP2 (D2.3, D2.4) and WP6 deliverables (D6.1, D6.2, D6.3).

#### **3.3.1 Estimation of the microbiological quality of seabream using the two different VideometerLite versions (1 and 2)**

The estimation of the microbial populations was performed by setting up experiments storing the samples at different temperature and packaging conditions for certain time period. MSI and microbiological analyses were performed every day throughout storage. Images of seabream fillets with the different VideometerLite versions, along with the relevant bands used in each one of them are shown in Figure 15. Experimental designs, as well as specific results are presented in WP2, WP4 and WP6 deliverables.

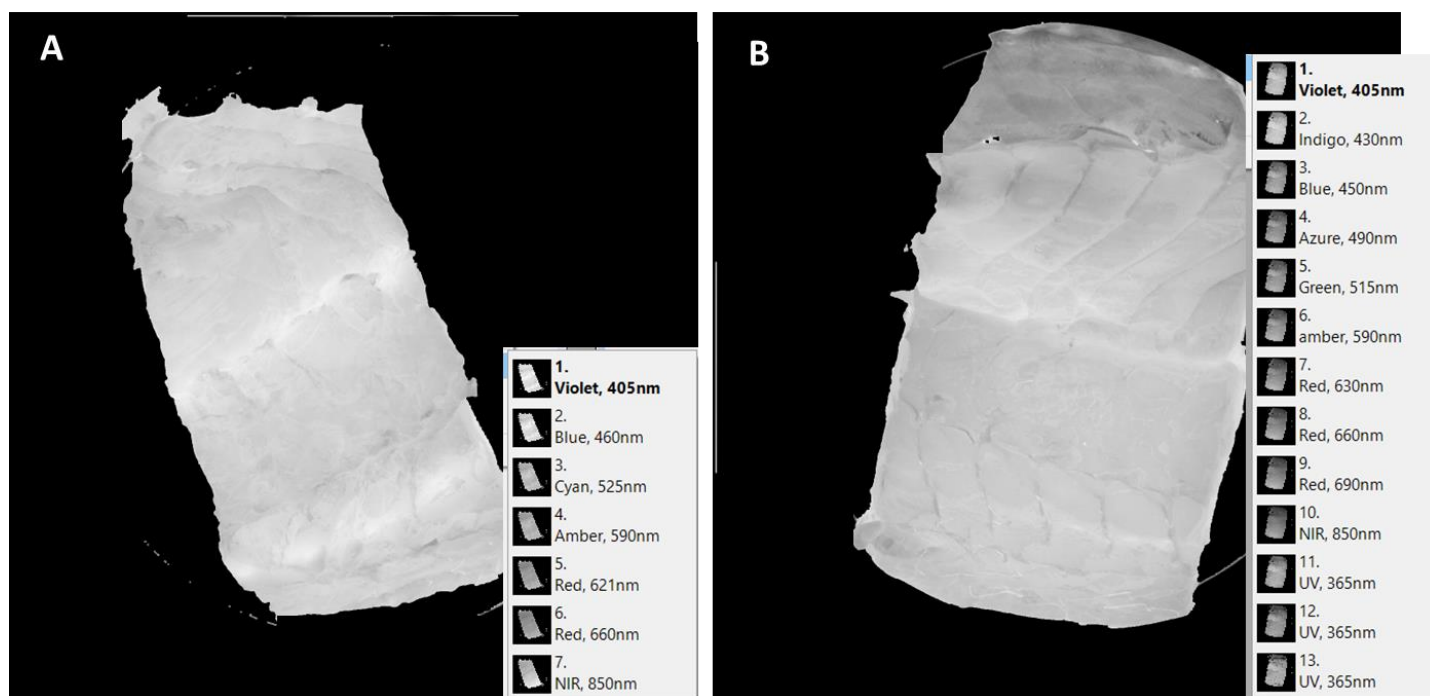


Figure 15 Images of seabream fillets using VideometerLite V.1 (a) and V.2 (b)

Comparisons of the performance of the two Videometer versions (1 and 2)

A PLS regression model was applied to estimate the efficiency of the different instruments in assessing the microbial counts and consequently the freshness of seabream fillets. The preliminary results show that the VideometerLite V.2 seems to be able to predict more accurately the microbial populations based on  $R^2$  and RMSE values. In regard to the features that were most informative for the prediction, there are slight differences among the most important ones. However, 460 nm, 590 nm, 621 nm, 630 nm and 405 nm are found to be among the most relevant ones for addressing this problem (Table 3).

Instruments	Packaging	$R^2$	RMSE	Important bands (nm)
VMLite 1	Air	0,534	1,305	405, 460, 590, 621
	Vacuum	0,456	0,733	460, 590, 621
VMLite 2	Air	0,638	1,008	405, 630, 660, 690, UV365(F2)
	Vacuum	0,679	0,496	590, 630, 660 UV365(F1)

Table 3 PLS regression performance indices for the prediction of microbial counts using the two versions of VMLite under different packaging conditions.

### 3.3.2 Estimation of the changes on fish heads/eyes throughout storage

As has been described in detail in D6.2 VideometerLite1 and VideometerLite2 images have been collected from fish heads. The aim was to segment the fisheye from the fish head, since the changes occurring during storage are considered as a quality index. Figure 16 presents VideometerLite1 and VideometerLite2 images of the same fish-head, the segmented eyes and the respective spectra of eyes and pupils.

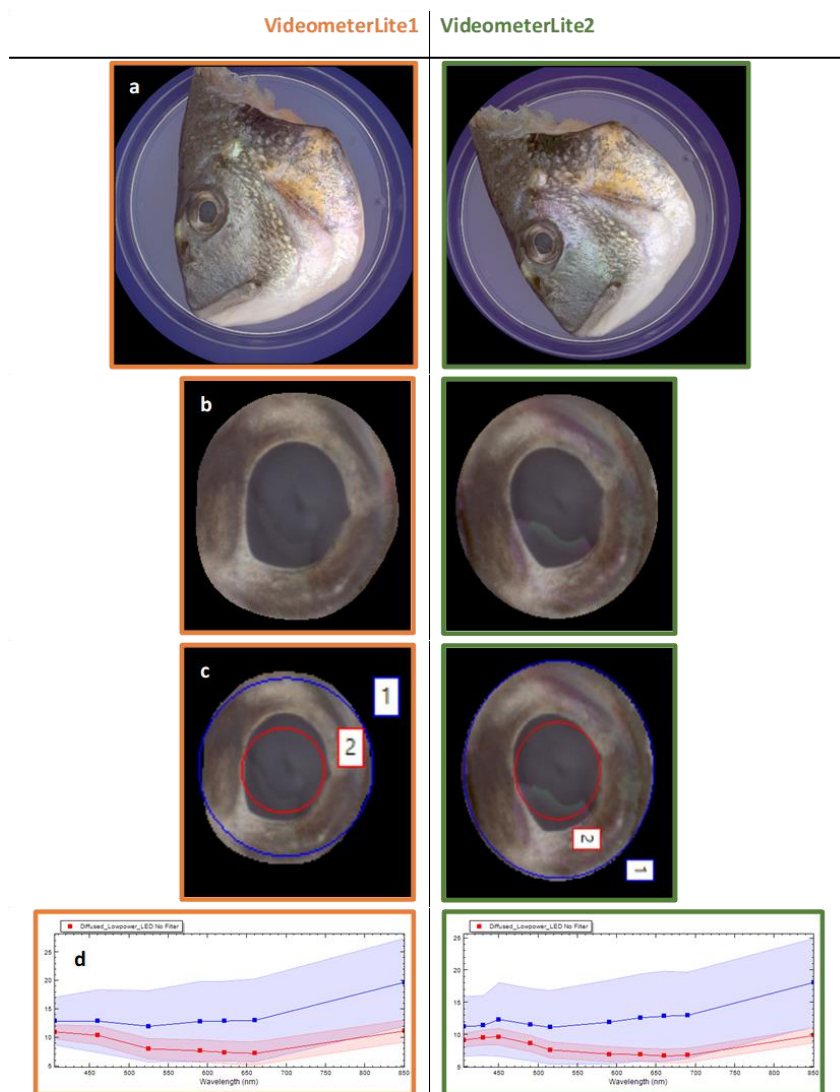


Figure 16 (a) Images from VideometerLite1 and VideometerLite2 of the same fish-head, (b) segmented fisheyes (c) ROIs of fisheyes and pupils, (d) the respective spectra of eyes and pupils.

## 4 ANALYSIS OF THE ACQUIRED IMAGES – DATA PREPROCESSING

### 4.1 ATLANTIC SALMON

Images of skin, gills and eyes from Atlantic salmon were pre-processed by either manual extraction from the region of interest (ROI) of each image, or by running a Session based on a pre-made transformation and segmentation recipe.

#### 4.1.1 Skin

To segment the skin, a nCDA transformation and foreground background segmentation that separated the white parts, that is mother of pearl, from the darker grey parts as well as red blood spots, shown in Figure 18 and 19, was created. Then, a session was run to perform the segmentation on all images.

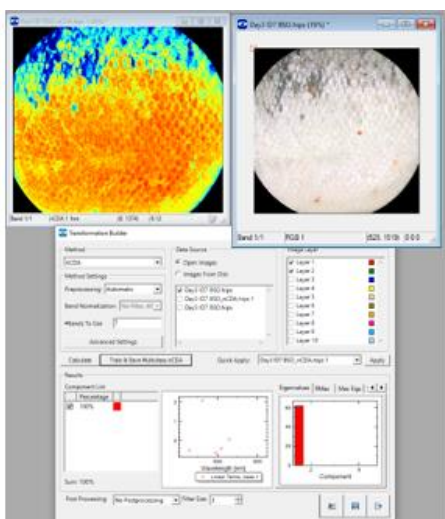


Figure 17 Transformation of salmon skin

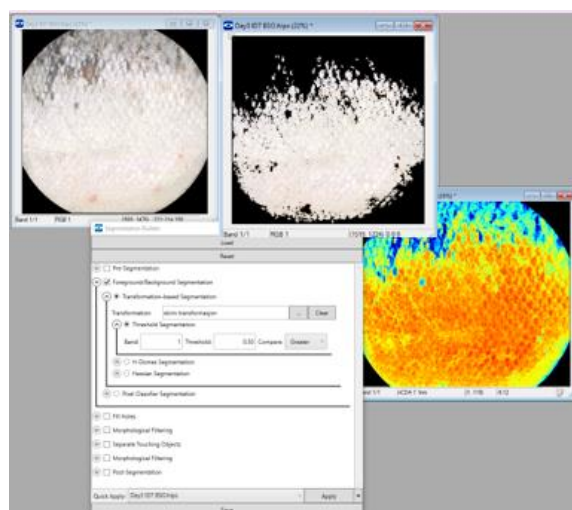


Figure 18 Segmentation of salmon skin

#### 4.1.2 Gills

For the gills, challenges were encountered when collecting MSI data by segmentation, because of shadows in between the gill threads, as well as slime that was present on some individuals. Consequently, the data was manually extracted by marking a ROI on one gill thread and copying the MSI data from Statistics, shown in Figure 20. The same size of ROI area was used for each gill.

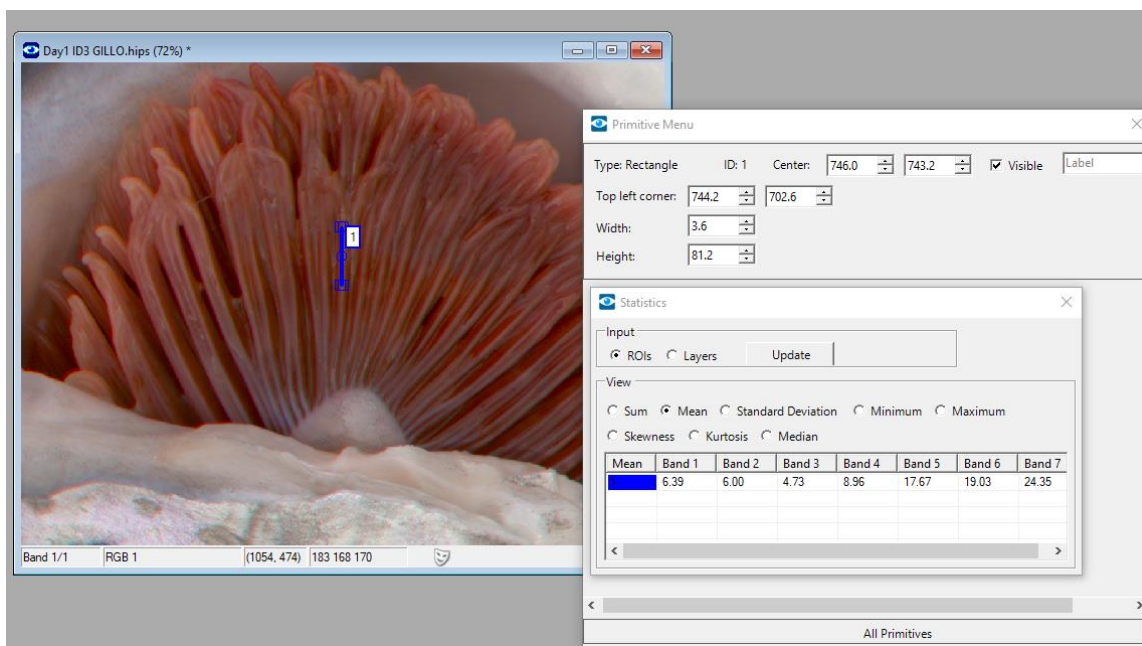


Figure 19 Example of data extraction from images of gills captured with VideometerLite 1.

### 4.1.3 Eyes

Imaging of salmon eyes was performed using VideometerLite and VideometerLab, followed by processing in the VideometerLab software. For quality measurement, it was desired to extract data from only the pupils. The following challenges were related to Segmenting of salmon pupils:

- The colors of the eyes are too similar to the surrounding imaging area of the salmon which leads to difficulties for the VM software to separate the different skin colors.
- The pupils have different sizes, making it challenging to pick a filter size that can be applied for all individuals.
- Light reflection from the pupils makes the spectral analysis inaccurate.

A segmentation recipe for salmon eye was developed by Videometer. Firstly, an nCDA transformation and a foreground/background segmentation of the eye were created using given parameters (Figure 20). Secondly, the pupil was segmented from the eyes (Figure 21). The two segmentations were grouped into an intersection (Figure 22) in the Segmentation Sequence Builder and lastly, a Session was run on the images. In blob toolbox all segmented parts were shown, and Class manager allowed to separate pupils and not-pupil fractions. The segmentation was considered as successful if only the pupil was covered by the segmentation, shown in Figure 23. From the storage experiments performed on whole salmon, the segmentation recipe was successful on 48 % and 69 % on images captured during the two storage experiments by VMLite, and 38 % on images captured with VMLAB. Further adjustments of the parameters in the segmentation recipe can improve the success rate.



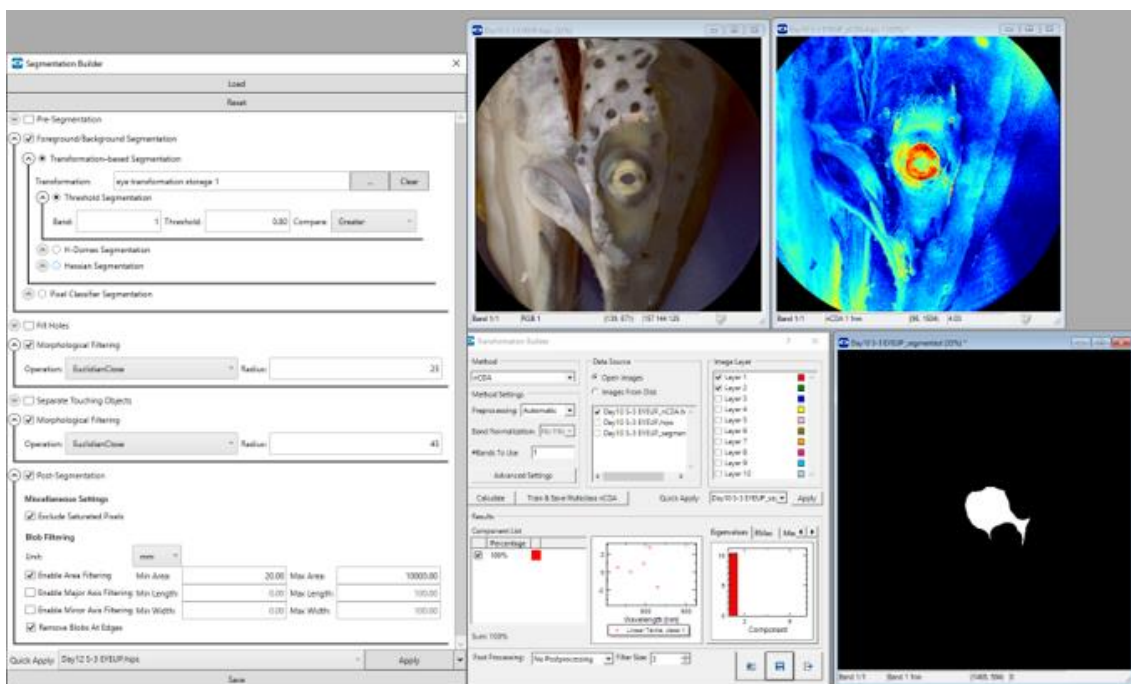


Figure 20 Eye transformation and segmentation vs surroundings.

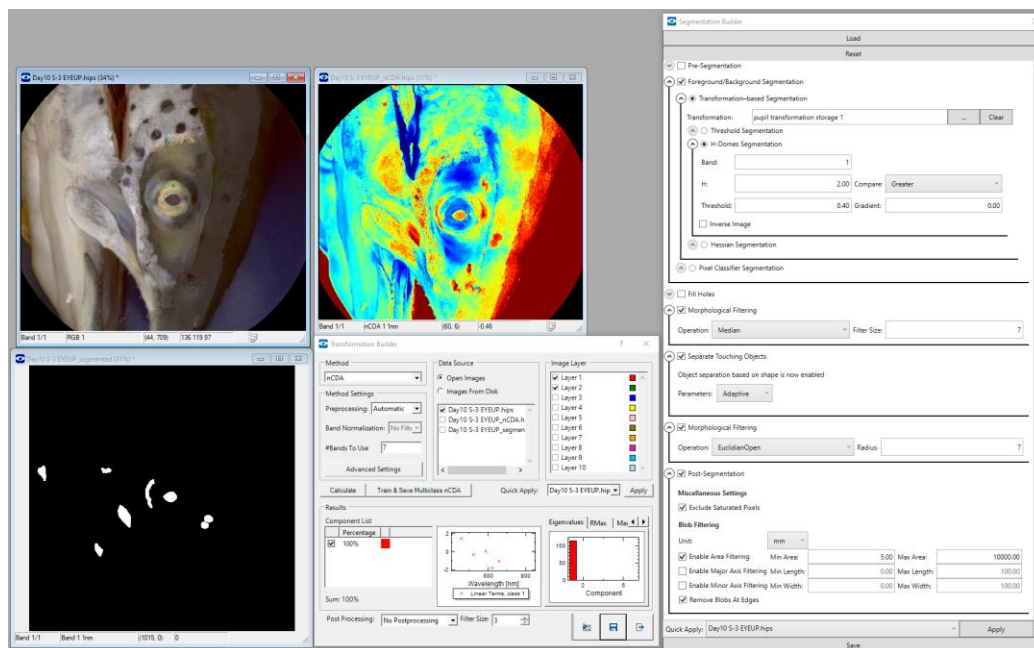


Figure 21 Pupil transformation and segmentation vs surroundings

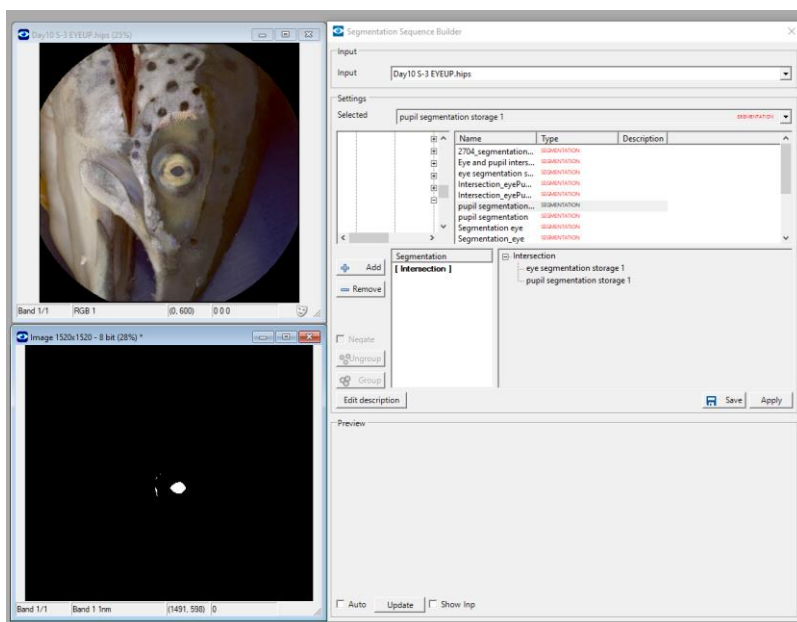


Figure 22 Eye and pupil segmentation are grouped into an intersection.

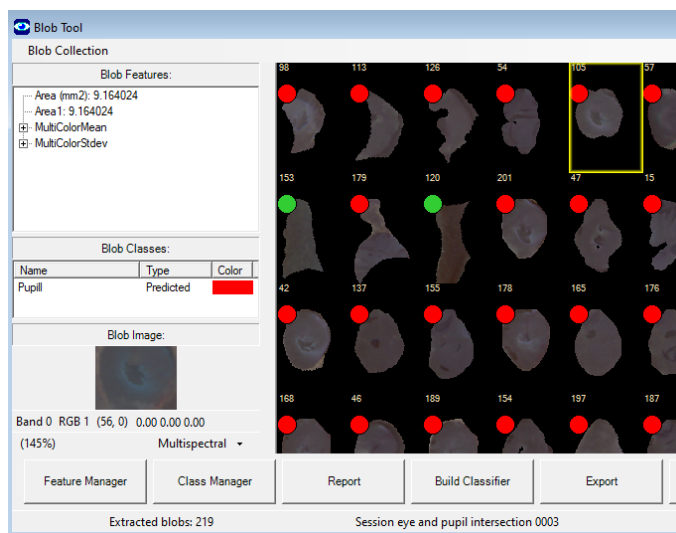


Figure 23 Segmented pupils and not-pupils are separated, and data imported into Blob Tool.

## 4.2 WHITEFISH

Data and image analysis is ongoing in the mentioned trials for the whitefish. A comparison between use of the CLIP image analysis method and segmentations is being performed to assess their precision and efficiency in finding and identifying parasites.

Furthermore, other data and image analysis of the eyes, skin, and gills of the Atlantic cod will be performed in a similar way as the Atlantic salmon trials performed by NTNU, with the aim of allowing interspecies comparison, and hopefully the development of more universal imaging and image processing solutions than

can be reached by analyzing quality changes within one species at a time. Some parameters will, though, only apply for the individual species, as mentioned earlier.

## 4.3 MEDITERRANEAN SEABREAM

### 4.3.1 Microbiological quality estimation

The different steps of data preprocessing are illustrated in Figure 24. At first, what is part of the sample or not is defined (Layers), then we apply the transformation function (Transformation Builder), which is followed by segmentation of the image (Segmentation Builder). Based on the parameters determined in previous steps we create the recipe and run the session (Session Manager). The output includes the segmented images and an .xls file with the relevant information. The output of each one of the aforementioned steps is illustrated in Figure 25. Each one of these steps is described below in detail.

#### 1. Layers

At first, the two different layers are defined, representing the sample (Layer 2) and what it is not considered as the sample (Layer 1). In this case, background surface and petri dish are excluded from further analysis.

#### 2. Transformation builder

The Transformation function is capable of “transforming” the information in the images. Among the transformation methods, the nCDA (Normalized Canonical Discriminant Analysis) method was used. It is appropriate for high dimensional data, while it is used to enhance differences between two defined classes (known vs known) visualizing them in a new transformed image. Apart from other applications, transformations are essential for the segmentation.

#### 3. Segmentation builder

The next step was the segmentation of the region of interest (ROI) on the sample from non-relevant areas. The foreground/background segmentation was selected with a transformation-based segmentation. Then the appropriate transformation file was selected, and the simple threshold Segmentation method was used. The unit for threshold comparison was set on less for all segmentations. Other parameters were also tested (i.e., Area Filtering) to remove small artifacts.

#### 4. Session Manager

The Session Manager tool was used to extract spectral data from a whole set of sample images. The effect of segmentations on each image was monitored during the session processing.

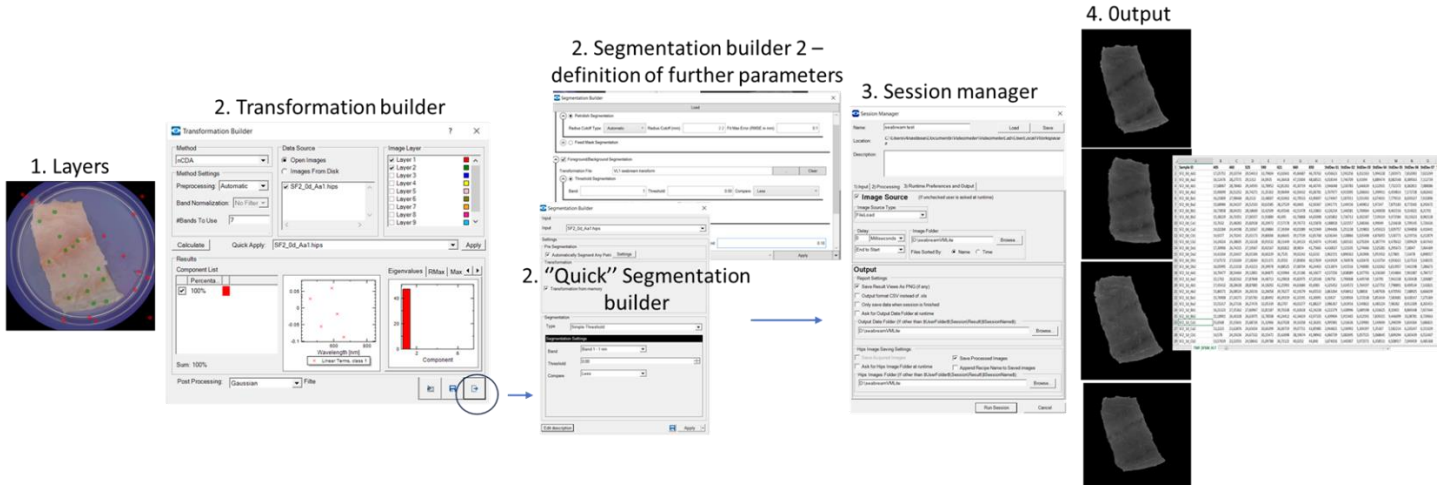


Figure 24 Steps for image processing using VideometerLab Software

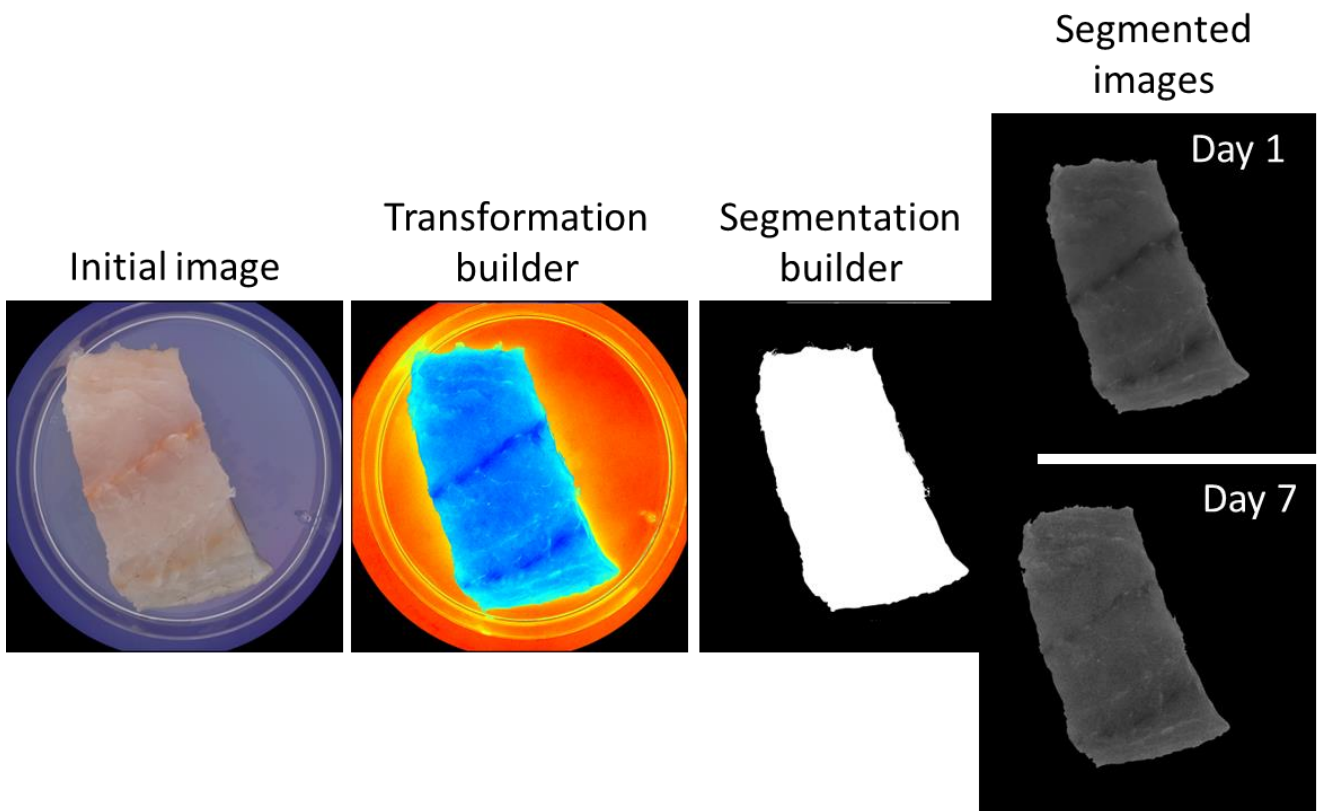


Figure 25 Visualization of the different image processing steps

### 4.3.2 Determination of changes on fish heads/eyes

The segmentation was applied as an image-processing step to remove image background and the petri dish from the actual region of interest, where the fisheye was present. The respective routines from the VideometerLab software were used. Figure 26 shows the workflow followed, which is consisted of:

- Building three independent segmentations (seabass vs. forehead, seabass pupil and seabass vs. background) for the detection of the fisheye pupil only. For that purpose, canonical discriminant analysis (CDA) was employed as a supervised transformation building method to divide the images into regions of interest. A simple threshold was used to separate between pixels of the pupil (foreground) and other (background) pixels. Subsequently, morphological filtering, separation of objects and blob filtering were applied on the images and the saturated pixels were excluded to segment fisheye pupils only.
- The built segmentations were applied on images as an intercept in the Segmentation Sequence Builder 2, which helps narrow down the analysis for the specific relevant areas, enabling to isolate pupils within the acquired images.
- The workflow described above was applied on all VideometerLite1 images and pupil eyes were acquired. Afterwards, a script was prepared in the VideometerLab software's Script Editor to dilate the masked fisheye pupils and capture the entire fisheye (iris and pupil).

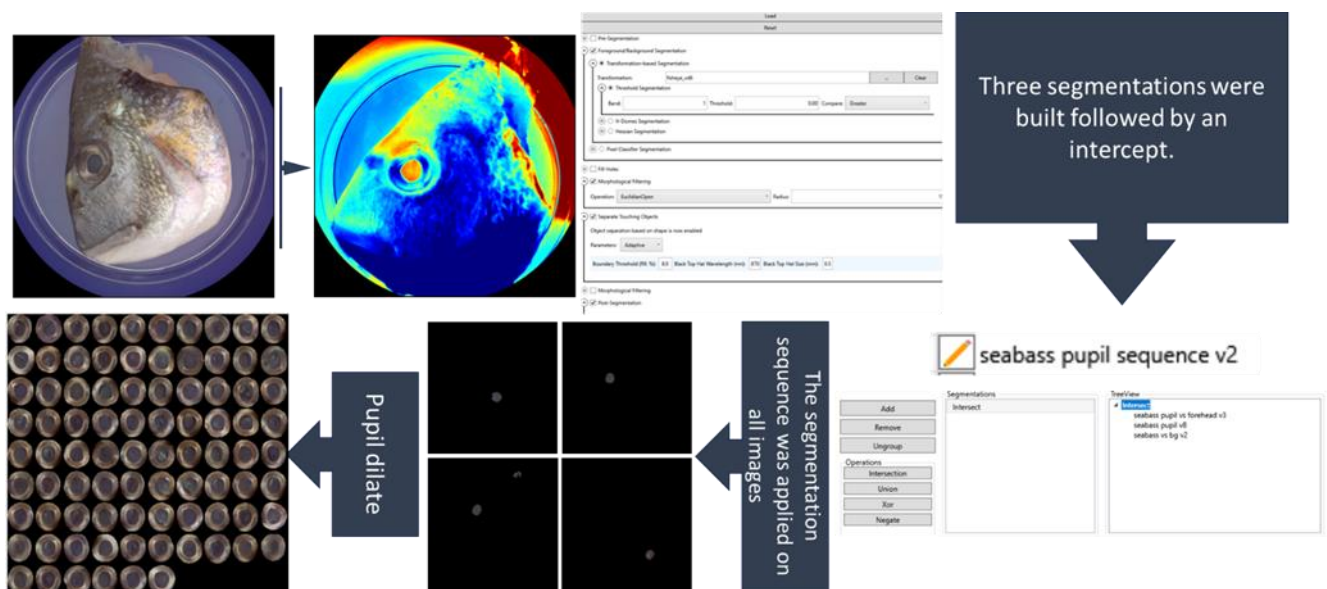


Figure 26 Segmentation of the fisheye region in VideometerLite1 images, where the eye is separated from the surrounding background

The different ‘characteristics’ of pupil and iris (please see Figure 27) lead us to the decision to, additionally, segment the iris from the fisheye separately.

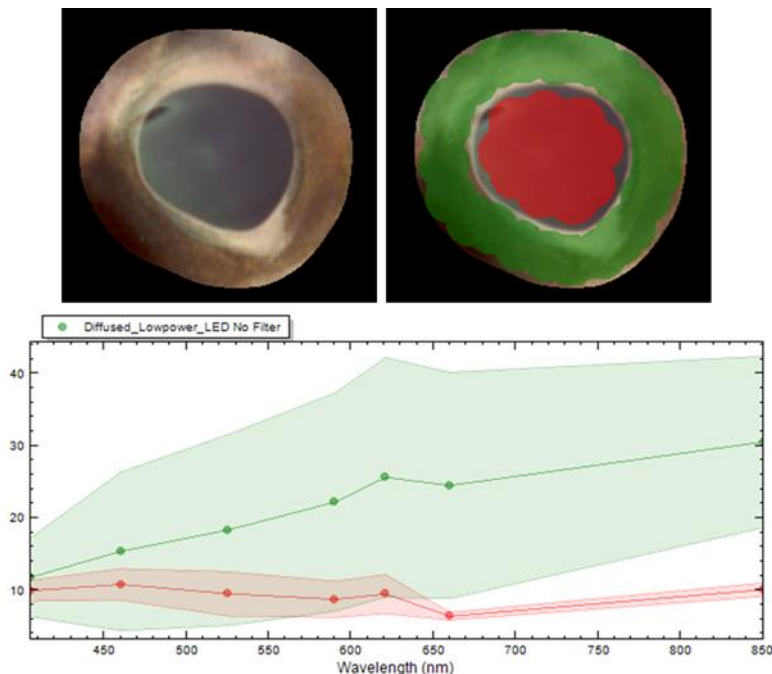


Figure 27 Fisheye pupil and iris spectra

In brief, the fisheyes (segmented images acquired from the workflow described above) were used. CDA was employed to separate between pixels of irises (foreground) and pupils (background) using a simple threshold. Morphological filtering (erode, Euclidean close) was applied for acquiring only the region of interest. In Figure 28 shows the segmented images of pupil, iris and fisheye.

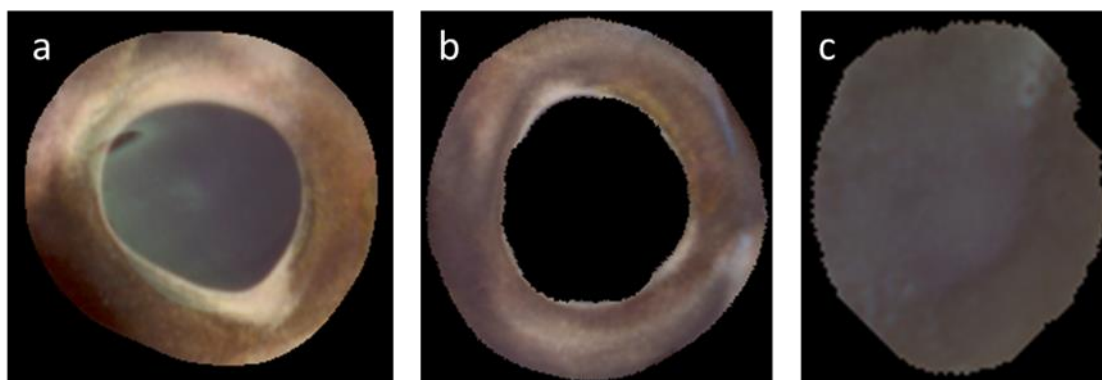


Figure 28 Segmented images of (a) fisheye, (b) eye iris and (c) eye pupil

Figure 29 shows the fish eyes’ images acquired from four different fish stored for a period of 10 days. The images were acquired at eight time points. The different colours correspond to a different day and the respective

microbial population of the fish (log CFU/g) is available, which ranged from 3.00 to 10.00 log CFU/g. Comparing images acquired at 0d (green labelled eyes) to 10d (red labelled eyes) shows some differences. The first are clearer and brighter, while the second are dull and milky. The upload of images as a Blob Collection allows the calculation of features related to colour, texture, shape and other. These will be used for the data analysis aiming for the prediction of quality through fish eyes.

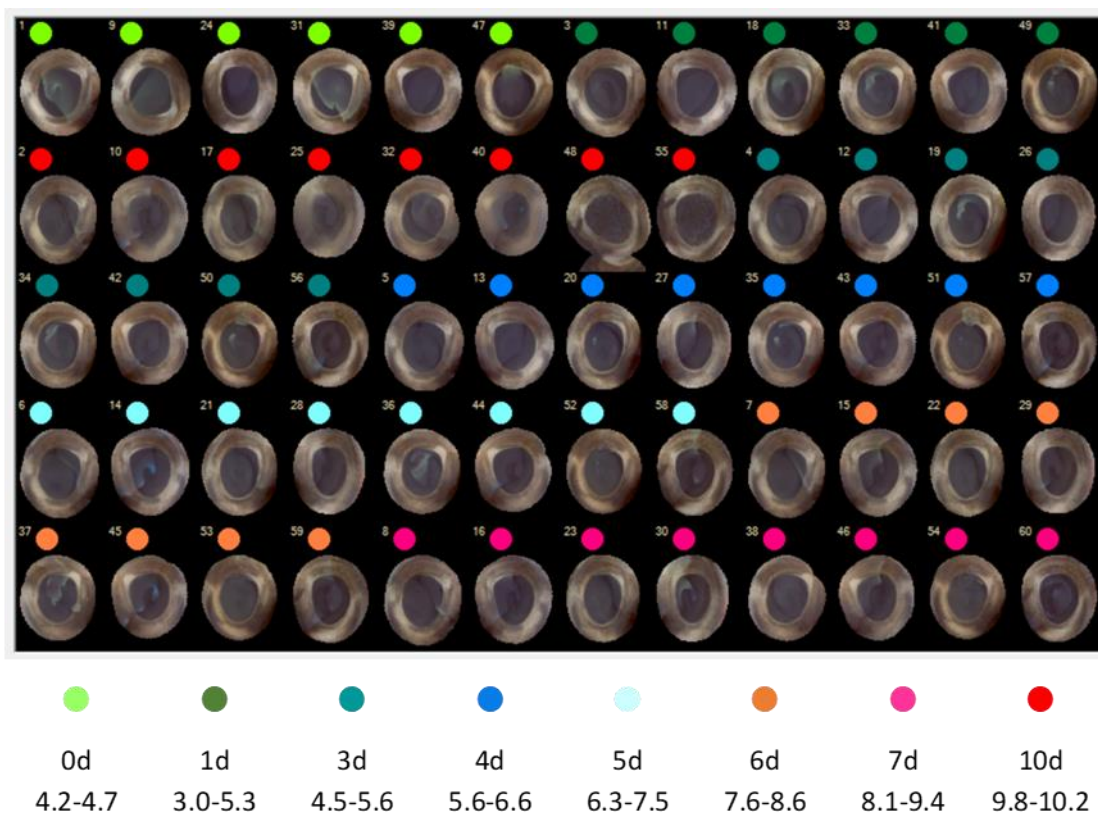


Figure 29 The image was acquired from Blob Collection, where the fisheyes were uploaded and labelled according to storage day.

The workflow described above was conducted in close collaboration between the Videometer and AUA partners during the visit of AUA staff at the Videometer premises. The Segmentation Sequence Builder, and the respective session for the segmentation of fisheyes are available in the TraceMyFish workspace. Additionally, training on the software took place for using the full capabilities of the software.

## 5 CONCLUSIONS

This Deliverable focused on the developed spectral imaging technology and data analysis methodology framework, exemplified by indicative applications of VideometerLite TMF prototype 1 and 2 from an analytical perspective, including the comparison of the two different prototype versions for the same application. The last part of this deliverable focused on data pre-processing based on the specific task/problem.

In the second VideometerLite TMF prototype, UV wavelengths were extended to 365 nm, and fluorescence filters added, differentiating to some extent the instrument's performance. Experiments that were conducted on fisheyes and gills explored the use of the VideometerLite's spectral imaging and cloud-based technology for estimating seafood freshness. Although important progress has been made on this field, there are still limitations for the application of this device in the Aquaculture Sector, especially the need for acquisition of more data is necessary to obtain robust results.



## 6 REFERENCES

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