STERapp User Guide

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1 What is STERapp?

STERapp is a free software tool to do stereological analysis. The present distribution includes a plugin to estimate fish reproductive parameters like fecundity, based on digital analysis of histological samples of fish ovaries. STERapp was developed by the following four Spanish research groups: Instituto de Investigaciones Mariñas (IIM)[1] part of the CSIC (Centro Superior de Investigaciones Científicas), Oceanographic Center of Vigo from the Spanish Institute of Oceanography (IEO)[2], Laboratorio de Informática Aplicada[3] of the University of Vigo and Centro de Investigación en Tecnoloxías Intelixentes (CiTIUS)[4] of University of Santiago de Compostela. STERapp is a multi-platform software written in the programming language C/C++. The current implemented plugin analyses automatically ovary histological images, using advanced computer vision and machine learning techniques. Due to the different histological processes and fish species analyzed in each laboratory, precision of the results obtained during the automatic processing may differ, because of this, STERapp includes a friendly GUI (Graphical User Interface) to visualise, review and interact with the classification of the fish gonad histological manually.

STERapp works with three types of files: image files, XML files (eXtensible Markup Language) and CSV files (Comma-Separated Values). The image formats supported are the most frequently used like GIF, TIF, PNG, BMP, PPM, JPG, etc. Every processed image generates a XML file which contains the contour and classification of each oocyte detected. This XML file is stored and its graphical content can be superimposed to the image in any moment. The statistical information of the quantitative analysis of every image is exported in a CSV file, such as number of objects, cell area and diameter, type of oocyte, etc. Calculated data can also be saved by STERapp in order to be reviewed by experts any time later.

The present user guide is organized as follows: Sections 2 and 3 describe the installation steps for the operating systems Windows and Linux; Section 4 describes how to run the Graphical User Interface (GUI) of STERapp. Section 5 describes the configuration of the preferences. Sections 6, 7 and 8 describe the File, Edit and View menus, respectively. In the Sections 9 and 10 users can find information about the analysis of the results with STERapp and the classification process. Finally, Section 11 refers to Help menu.

2 Windows installation

Once the user has downloaded the installation file `setupSTERapp.exe`, a double click on the file will open a dialog window which asks for permission to install a foreign program on your computer, click on "Yes" and a window will show you the Setup Wizard. Clicking on "Next" will start the installation.

The next window allows the user to choose the installation folder, click on "Next" and continue with the installation. A dialog will ask if you want to create a desktop icon. Click on "Next" to continue and confirm if you want to install STERapp, clicking on "Install", or cancelling the installation.

[1] http://www.iim.csic.es/

[2] http://www.ieo.es/es/web/vigo/

[3] http://lia.esei.uvigo.es/projects/gonadas/index.php

[4] http://citius.usc.es/

Once the installation process has started, a pop up window will show the progress; this overall process may take a few seconds. If you click on "Cancel", the installation process will be aborted. When the installation has finished the system will inform you and ask for launching the program. Click on "Finish" and check the box *Launch STERapp* if you want to run STERapp immediately.

3 Linux installation

In Linux, there are several types of packages, and every distribution has its own preferred package format. Ubuntu distributions used the Debian packages (format DEB). The .deb/Debian files containing STERapp is the file `sterapp_1.0_all.deb`. It is provided the linux package for Ubuntu 20.04 version.

To install STERapp, go to terminal, change to the folder where the file `sterapp_1.0_all.deb` is and type the following command: `sudo dpkg -i sterapp_1.0_all.deb` and the system asks you by the administrator password.

If the installation gives errors due to the lack of some packages required by STERapp, the user must uninstall STERapp with the command `sudo apt --fix-broken install`, install the required packages with the command `sudo apt install package-name`, and install STERapp again with the command `sudo dpkg -i sterapp_1.0_all.deb`. Then, you can run STERapp using the following command: `sterapp`.

STERapp can be removed from the computer using the command: `sudo apt remove sterapp`.

4 Run STERapp

After installing, click on the desktop icon in Windows. If it is the first time you run STERapp, a pop-up window (Figure S1) will inform you that there are no species defined in the program, click **ok** and the main program window (Figure S2) will be opened. This window encloses a menu bar with several sections, a toolbar containing the main functionality commands of STERapp, and a window panel where the histological images are visualized.

Figure S1: Pop-up message to inform that there are no species defined in STERapp.

The menu bar lists all **STERapp** commands and it is organized in six menus:

1. **File**: basic file operations, opening images and XML files, saving the overlays (as xml) and the statistical results (as csv); set and load the working preferences; and exit STERapp.

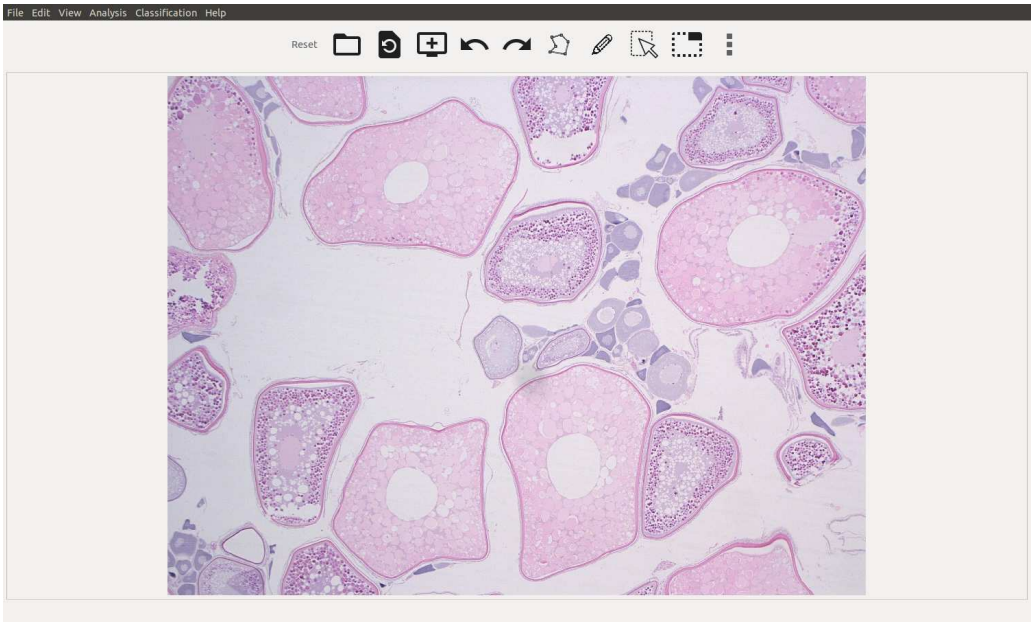

Figure S2: Main window of STERapp.

2. **Edit**: redo and undo operations, fitting image size and set the image to its original size.
3. **View**: show or hide the processing (lateral) panel.
4. **Analysis**: provides functionalities to calculate the results from several images of the same sample.
5. **Classification**: provides functionalities to train the classifier (machine learning model) with different species, and to classify the detected cells by developing stage and presence or absence of visible nucleous in the oocyte.
6. **Help**: provides information about the development team.

The toolbar provides a faster access to the main functionalities of STERapp, and contains the following icons (a pop up label shows a short description when the mouse is placed over the icon):

1. **Reset**: clears all the objects drawn on the image.
2. **Open**: opens a dialog to select the histological image (see Section 6).
3. **Zoom Fit**: fits the zoom of the image window.
4. **Original Zoom**: resets to the original image size.
5. **Undo**: undoes the last drawing command.
6. **Redo**: performs again the last drawing command.

Figure S3: Main window of STERapp with the lateral panel being open, which shows the plugin GONAD to process histological images of fish gonads.

7. **Draw by points**: marks the oocyte contour by points; use the left button of the mouse to draw them. The contour will be closed by clicking the middle button of the mouse.

8. **Draw freehand**: marks the oocyte contour freehand. To draw a contour press the left button of mouse and keep it pressed while drawing the region. The contour will be closed when the button is released.

9. **Select**: selects a drawn contour on the image by clicking into the object with the left button of the mouse. To select more than one object, keep the key **Ctrl** or key "Control" pressed while selecting objects. You can also select various objects with the following option **Select with rectangle** or select all the objects with the button *Select all cells* in the lateral panel.

10. **Select with rectangle**: to select several drawn objects on the image, click the left button of the mouse and keep it pressed, drag the mouse to draw a rectangle and release the left button. The objects under the rectangle area will be selected.

11. **Lateral panel**: open the lateral panel that shows the commands to process and analyse the image (see the section 8 and Figure S3).

In the following sections the functionalities of the Graphical User Interface (GUI) of STERapp will be described. In the File menu there are several commands to work with the required

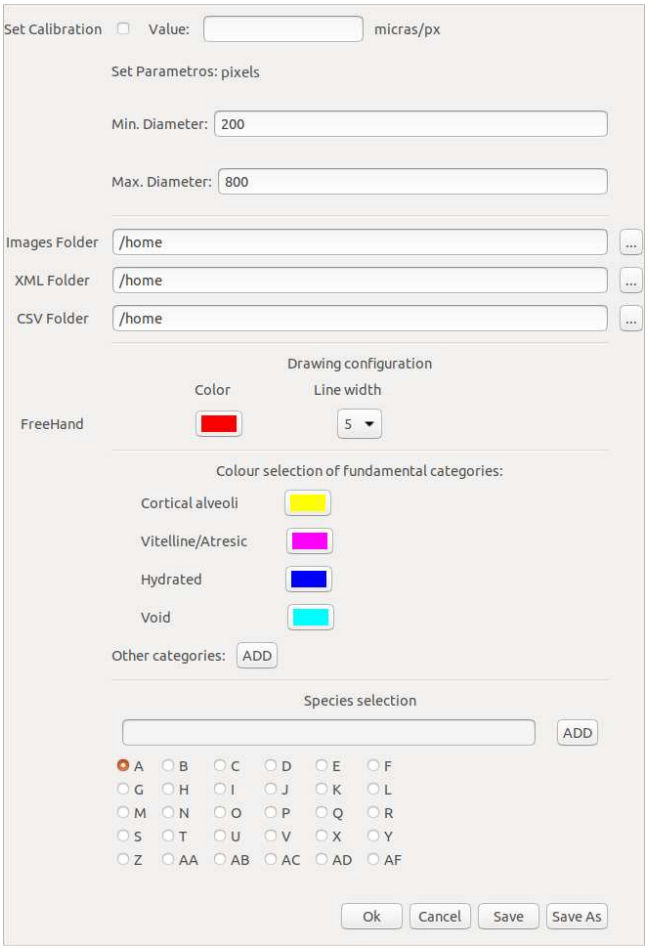

Figure S4: Preferences panel: default configuration.

files:

1. **Open image**: opens a dialog window to load a new image.
2. **Open image and XML**: opens a dialog window to select the image to load the XML file with the contours of the cells superimposed, when the XML file for that image already exists in the XML directory and the option is set in the preferences.
3. **Open XML**: opens a dialog window to select the XML file and loads it on a preloaded image. The user should check that the XML file matches with the image.
4. **Save XML**: opens a dialog window to enter the name of the XML file and chooses the path to save it. By default, STERapp uses the name of the image with extension xml, and saves the contours in the XML directory as set in the preferences.
5. **Export CSV**: opens a dialog window to enter the name of a CSV file in which you want to save the statistical analysis of the image. By default, STERapp uses the name of the image with extension CSV, and saves the statistical data in the CSV directory as set in the preferences.

6. **Preferences**: opens a panel to set the working preferences (see Figure S4), such as working directories, colour and width of lines, calibration of the images, fish species, maximum and minimum diameters of the oocytes for the selected species, and developing stage of the oocytes. The working preferences can be saved to be available in future sessions, or exported to a XML file.

7. **Reset preferences**: resets the preferences as previously set in the program.

8. **Load preferences**: allows to load the working preferences from a XML file given by the user.

9. **Exit**: exits the program.

In the on-going, Section 5 describes the configuration of the working preferences in STER-app.

5 Set preferences

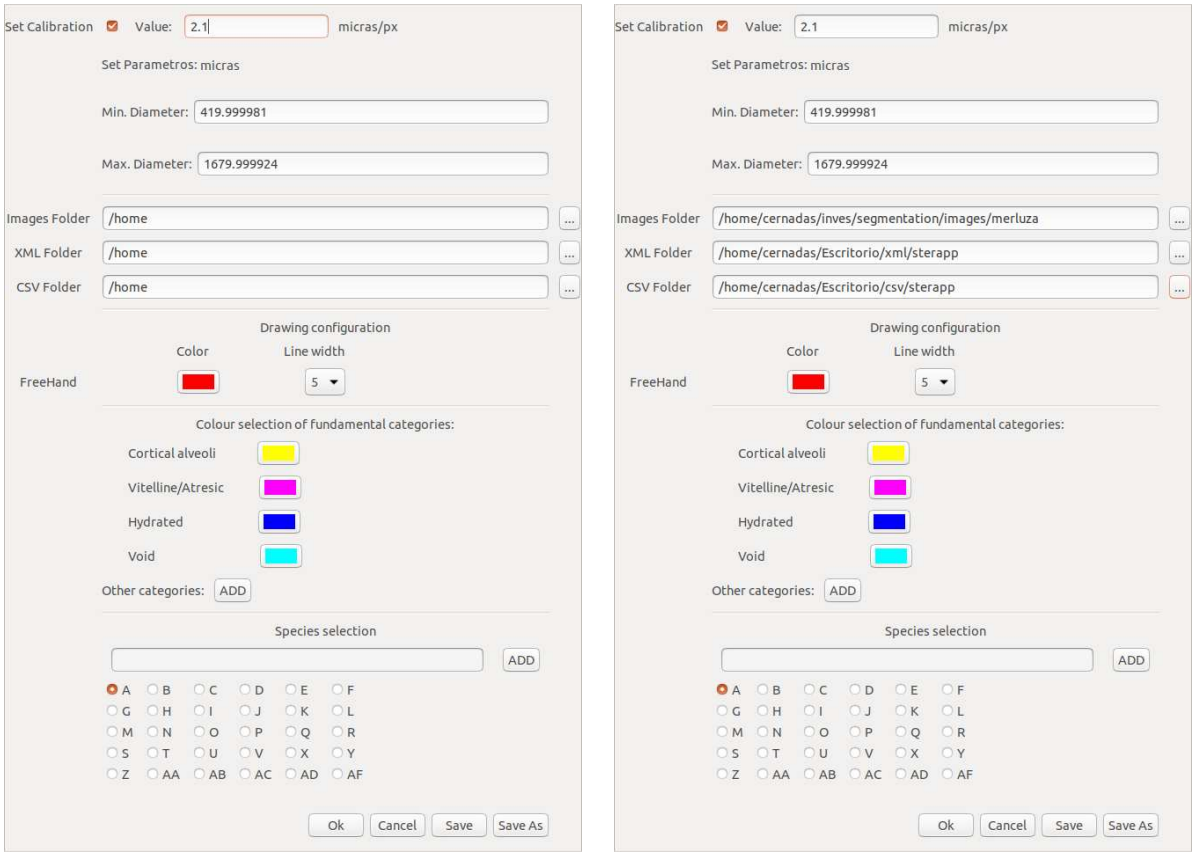

Figure S5: Setting preferences: (left panel) after setting the calibration, i.e., the relation between micrometers and pixels; (right panel) after setting the working directories.

Selecting "Preferences" in the **File menu**, the window shown in Figure S4 (left panel) will be opened. There, you can configure the following items:

1. **Calibration**: It is the size (in microns) of a pixel in the image. The user must set the value of every image to get the measuring of the objects in their real units. Otherwise, values will be given in pixels.

2. **Diameters**: sets the minimum and maximum size of the oocytes in the images. These parameters can be set in pixels or microns depending of the Calibration setting.

3. **Working directories**: sets the default directories for images, xml and CSV files.

4. **Drawing configuration**: sets the colour and width of the line to draw the contours over the oocytes.

5. **Colour selection of fundamental categories**: As default, STERapp only can automatically classify the following three oocytes development stages: cortical alveoli, vitelline / atretic and hydrated. The user can select a colour to represent every category and also the category "void" that corresponds to these parts of the image that correponds to space out of the ovary tissue, i.e. it will excluded from the tissue area estimates. New categories can be added manually using the GUI of STERapp.

6. **Species Selection**: defines new fish species.

7. **Setting or changing configuration**: use buttons **Ok**, **Cancel**, **Save** and **Save As** at the bottom (Figure S4) to set the preferences to this working session, cancel, save the preferences in the system or save the preferences in a xml user file, respectively.

5.1 Calibration

The calibration of an image is fixed in the digitalization process, and depends on the magnification used in the microscope and the spatial resolution of the digital camera connected to it. If the user knows the actual calibration of the image, or set of images, he/she should introduce the *Value* in microns per pixel. Therefore press "Enter" and check the box after the label *Set Calibration* to confirm that the real calibration value has been added. If it is non-checked, the calibration will be considered in pixels. When this value is fixed, the minimum and maximum diameters will be converted from pixels to microns according to the calibration, as you can see in the left panel of Figure S5.

5.2 Diameters

The range of variation of oocyte diameters can be a species specific parameter. Keeping this in mind, STERapp provides more versatility allowing the user to choose the minimum and maximum diameters of the oocytes according to the species. These parameters can be set in the boxes *Min. Diameter* and *Max. Diameter*, respectively, by inserting the values in microns or in pixels depending if the calibration is active or not. After setting the values, press the "Intro" or "Enter" key to update. This parameter can also be set on the lateral panel.

5.3 Working directories

In this panel the user can introduce the path to set the default directories to store the working files, *Images Folder*, *XML Folder* and *CSV Folder*, as in the right panel of Figure S5. Clicking the button next to the box, users can choose the working directory in an open dialog window (see Figure S6).

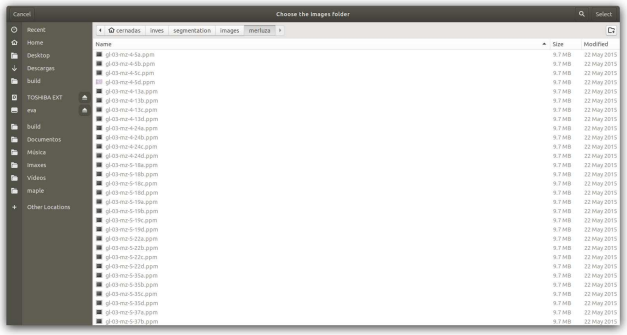

Figure S6: Open dialog box to choose the working directory.

5.4 Drawing configuration

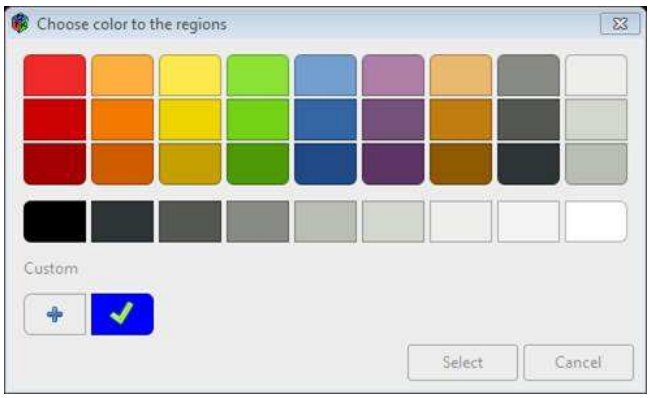

Figure S7: Chart colour for contour lines.

The colour and width of the line to draw the oocytes over the image can be changed in this panel. Clicking the coloured button after the label *FreeHand*, opens the colour chart dialog shown in Figure S7, choose the colour and press "Select". Clicking on the button "Line width" (Figure S5) the width of the line can be chosen in a drop-down menu. The colour of every oocyte category can be changed in their own coloured button, below the section *Colour selection of fundamental categories*, with the same process as the *FreeHand* colour.

5.5 Adding categories

With the button **ADD** after the label "Other categories", in section *Colour selection of fundamental categories* of Figure S8, you can add new categories of oocytes to STERapp. As mentioned

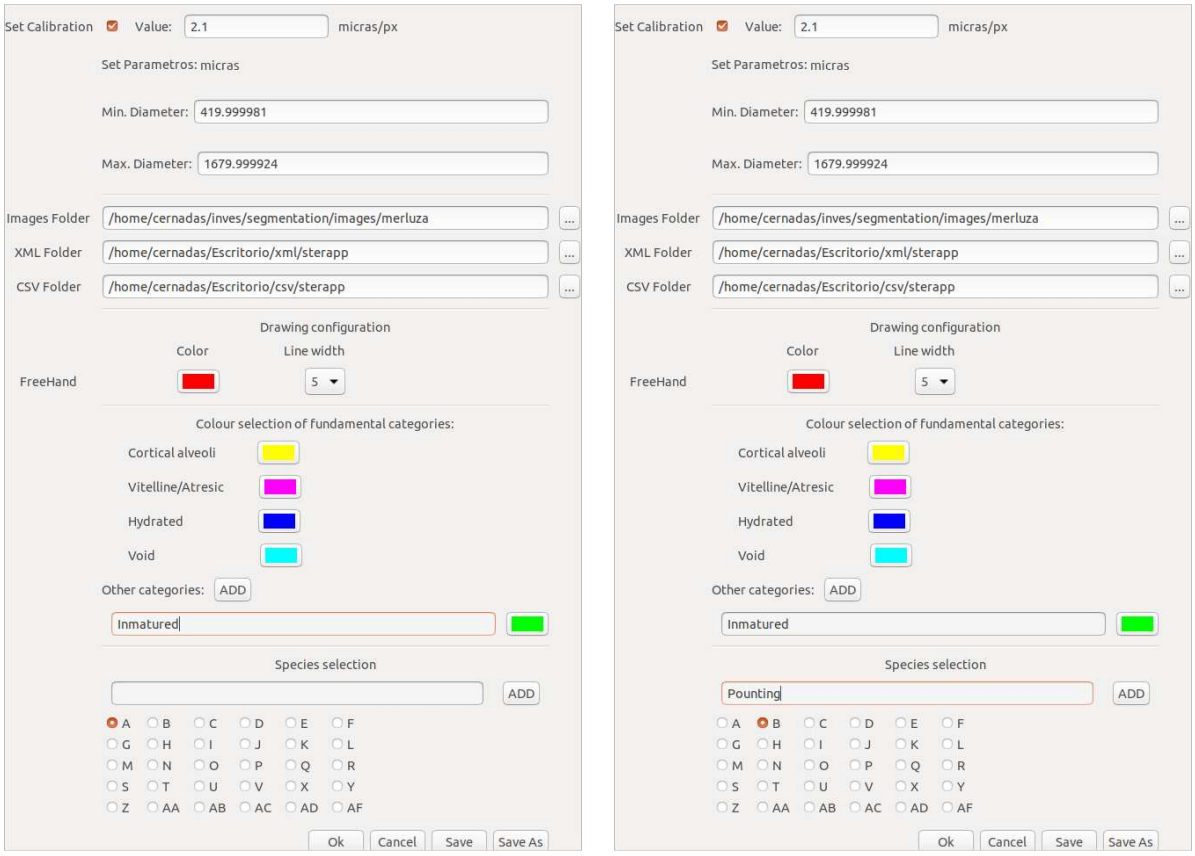

Figure S8: Setting preferences: (left panel) after adding new cell categories; (right panel) after setting some fish species.

before, these new categories will not be considered in the automatic classification, but they can be manually managed in the program. You can manually assign these categories to oocytes contours on the images and STERapp will use them for the statistics. The left panel of Figure S8 shows, as an example, where a new category "Inmatured" is added with an associated green colour.

5.6 Species selection

STERapp manages a different classifier (or machine learning model) to each fish specie, so is necessary to train the classifier with each new species. In the current version, STERapp can manage 30 different fish species. By default there is no species defined in the system. To add a new fish species, select a radio button with one of the characters (A, B, C, D, E, ...), write the name of the species in the entry widget below the label "Species selection" and press "Intro" or "Enter" to update the value or click the button **ADD** after the text box. This process creates an internal link between the label used for the software (A, B, C, ...) and the name of the species, which allows to preserve data consistency. We defined in the radio button A the specie European hake (*Merluccius merluccius*) and in the radio button B the specie Pounting (*Trispterus luscus*) (see the right panel of Figure S8). After that, the radio button A was selected and the lateral panel only shows the fish selected species, but not the character associated (see

Figure S11 after the label "Species analysed").

5.7 Setting the configuration

1. Button **Ok**, sets these preferences to the present working session.

2. Button **Cancel**, cancels the operation of setting the preferences. Changes will not be saved.

3. Button **Save**, saves the preferences for present and future working sessions. The first time the user runs STERapp, the pop-up message of Figure S9 informs you that "There is no trained classifiers", the user must complete this process in order to use the automatic classification (see Section 10). Once the classifier is trained, the program will classify the oocytes in an image and will show the development stage of each oocyte using di
erent colours (set in the preferences). The presence or absence of visible nucleous in each oocyte will be indicated using a continuous line to show oocytes with visible nucleous and dashed line to oocytes with non-visible nucleous. This information is relevant as STERapp only uses those cells with visible nucleous to perform the diameter calculations, since they are the only one from which the measurement of the real diameter can be assured. It is important to emphasize that the user must check that the loaded image belongs to the working specie, shown in the lateral panel after the label "Species analysed" (otherwise, the user must go to the preferences and change the species in the radio buttons at the botton of the preferences window in Figure S8).

4. Button **Save As**, saves the preferences of the present working session and saves them in an XML file selected by the user, which can be loaded using the submenu "Load preferences" of menu "File". This option allows to share the preferences files between users.

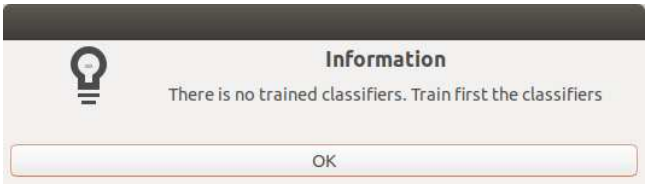

Figure S9: Pop-up message to inform that the classifier must be trained in the future.

6 File menu

The commands available in the **File** menu are: **Open Image**, **Open Image and XML**, **Open XML**, **Save XML**, **Export CSV**, **Preferences**, **Reset preferences**, **Load Preferences** and **Exit**. STERapp works with three types of files: images, xml (eXtensible Markup Language) and csv (Comma-Separated Values) files. The supported image formats are the most frequently used ones, such as .gif, .tif, .png, .bmp, .ppm, .jpg, etc. Every image file will have its associated XML file, w hich w ill i nclude t he c ontours a nd c ategories o f t he o ocytes, a nd c an b e loaded

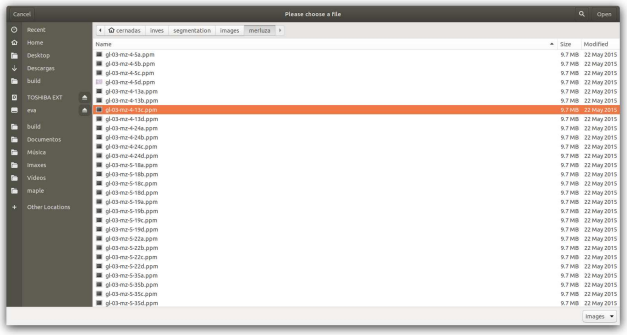

Figure S10: File chooser dialog to choose the image to be loaded in STERapp.

into the software at any time. The CSV file will include the statistical information of the quantitative analysis of every image.

Open Image opens a dialog (Figure S10) where you can choose an image file from the directory set in Preferences (see Section 5) or where you can select another path. Choose the file and click **Open** to load the image in STERapp. Be aware that the path must not be larger than 256 characters and must not contain rare symbols ("/", "%", " ,", "*", etc).

Figure S11: The image selected in Figure S10 is loaded in STERapp.

Figure S11 shows the main window of the program with an image loaded and with the oocytes contours overlapped. When an image is loaded, the lateral panel opens and the state bar at the left-bottom corner of the main window shows the name of image file and its resolution. If the

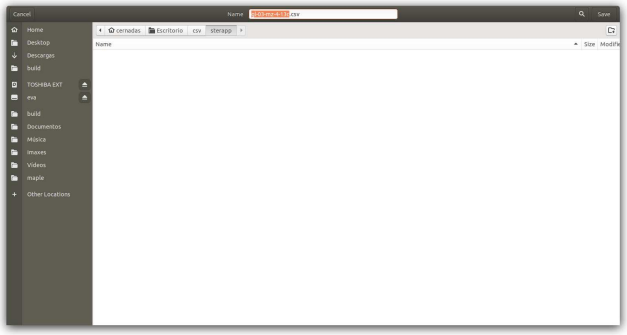

Figure S12: Dialog to choose the CSV file to store the statistical analysis of the image.

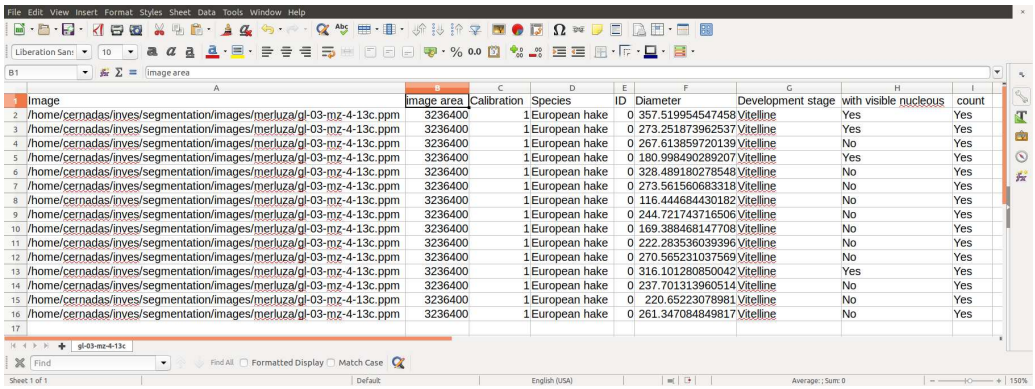

Image	image area	Calibration	Species	ID	Diameter	Development stage	with visible nucleous	count
/home/cernadas/inves/segmentation/images/merluza/gl-03-mz-4-13c.ppm	3236400	1	European hake	0	357.519954547458	Vitelline	Yes	Yes
/home/cernadas/inves/segmentation/images/merluza/gl-03-mz-4-13c.ppm	3236400	1	European hake	0	273.251873962537	Vitelline	Yes	Yes
/home/cernadas/inves/segmentation/images/merluza/gl-03-mz-4-13c.ppm	3236400	1	European hake	0	267.613859720139	Vitelline	No	Yes
/home/cernadas/inves/segmentation/images/merluza/gl-03-mz-4-13c.ppm	3236400	1	European hake	0	180.998490289207	Vitelline	Yes	Yes
/home/cernadas/inves/segmentation/images/merluza/gl-03-mz-4-13c.ppm	3236400	1	European hake	0	328.489180278548	Vitelline	No	Yes
/home/cernadas/inves/segmentation/images/merluza/gl-03-mz-4-13c.ppm	3236400	1	European hake	0	273.561560683318	Vitelline	No	Yes
/home/cernadas/inves/segmentation/images/merluza/gl-03-mz-4-13c.ppm	3236400	1	European hake	0	116.444684430182	Vitelline	No	Yes
/home/cernadas/inves/segmentation/images/merluza/gl-03-mz-4-13c.ppm	3236400	1	European hake	0	244.721743716506	Vitelline	No	Yes
/home/cernadas/inves/segmentation/images/merluza/gl-03-mz-4-13c.ppm	3236400	1	European hake	0	169.388468147708	Vitelline	No	Yes
/home/cernadas/inves/segmentation/images/merluza/gl-03-mz-4-13c.ppm	3236400	1	European hake	0	222.283536039396	Vitelline	No	Yes
/home/cernadas/inves/segmentation/images/merluza/gl-03-mz-4-13c.ppm	3236400	1	European hake	0	270.565231037569	Vitelline	No	Yes
/home/cernadas/inves/segmentation/images/merluza/gl-03-mz-4-13c.ppm	3236400	1	European hake	0	316.101280850042	Vitelline	Yes	Yes
/home/cernadas/inves/segmentation/images/merluza/gl-03-mz-4-13c.ppm	3236400	1	European hake	0	237.701313960514	Vitelline	No	Yes
/home/cernadas/inves/segmentation/images/merluza/gl-03-mz-4-13c.ppm	3236400	1	European hake	0	220.65223078981	Vitelline	No	Yes
/home/cernadas/inves/segmentation/images/merluza/gl-03-mz-4-13c.ppm	3236400	1	European hake	0	261.347084849817	Vitelline	No	Yes

Figure S13: An example of CSV file of the image of Figure S11 imported in LibreOffice Calc.

image was already analysed and the XML file was stored, you can load it selecting the submenu **Open XML** and the contours will be shown overlapped to the image, as i t can be seen in Figure S11. The user must reassure that the selected XML file is the corresponding one for each image, STERapp does not check this automatically. Using the submenu **Open Image and XML** (open the window of Figure S10 to select the image), STERapp will check automatically in the xml directory set in Preferences, if there is a file with the same name as the image. If this is the case, STERapp will open the image file and overlap its xml in a single process (see Figure S11).

Once the contours of the oocytes have been drawn on the image, the user should open the submenu **Export CSV** to open the window of Figure S12 and set the file name (by default the name of the image with extension csv is used). The CSV file will store the statistical results of the image in the directory selected in "Preferences". This CSV file contains the directory path, the selected area, the calibration, the fish species, the development stage of every oocyte, the presence/absence of a visible nucleous, and if the oocyte will be used in the statistical analysis or not. Following the rules of the stereological method, when you use more than one image of a sample and they are adjacent, the probability of counting the same object twice is high. Thus, in order to minimize this probability, the method suggests to count only the cells in touch with the bottom and right border of the image and discard the oocytes touching upper and left side of the image. The information of the CSV file can be loaded in a spreadsheet such

as LibreOffice Calc[5], as it can be seen in Figure S13, or in Excel.

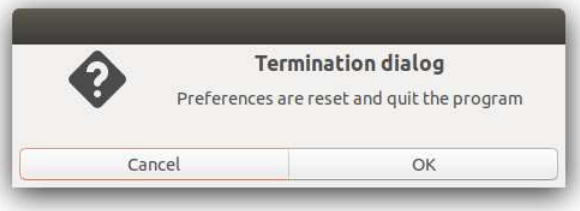

Figure S14: Pop-up message shown when the user resets the preferences.

The command **Reset preferences** removes all preferences previously set and shows the message of Figure S14. The program closes if you click on the **OK** button and does nothing if you click on the **Cancel** button. The user can also remove the set preferences manually. The preferences file (a XML file named `preferences.xml`) and the configuration of classifiers to each fish specie are located in the directory `.govocitos` of the home path. In Windows, the home path ususally is the directory `C:\users\myUserName` or `C:\usuarios \myUserName` where `myUserName` is the session's name where the program was installed in the computer. Deleting the directory `C:\users\myUserName\.govocitos`, all the preferences are removed. In Linux, the home path is the directory `/home/myUserName`.

The **Load preferences** command opens a file chooser dialog to select the XML file preferences (`preferences.xml` file to be loaded. Preferences do not include the configuration files of the classifier which cannot be loaded. All the preferences (file `preferences.xml`) and the configuration files of the classifiers for each fish specie can be transferred manually to another computer copying the `.govocitos` directory from one computer to another in the same path, e.g., `C:\users\myUserName` or `C:\usuarios \myUserName` for a Windows system.

7 Edit menu

All the items available within this menu are also available in the toolbar (see Section 4). They are: **Undo**, **Redo**, **Fit Image** and **Original Size**.

Fit Image fits the zoom of the image panel and **Original Size** sets the original size of the image. Image zoom can be changed rolling up and down the mouse wheel to increase or decrease the zoom. The portion of the area which is visible in the image window can be seen in the iconized image located in the bottom of the lateral panel (see Figure S15). Keeping the zoom, you can move to another part of the image by two methods: 1) pressing simultaneously the left and right buttons of the mouse over the image panel and displacing the visible area; or 2) pressing the left button of the mouse on the blue square in the iconized image of the lateral panel and displacing the square. Both movements, the visible area in the window image and the iconized image of the lateral panel are synchronized. The overlays of the image are zoomed with the image as well.

[5] `https://www.libreoffice.org/discover/calc/`

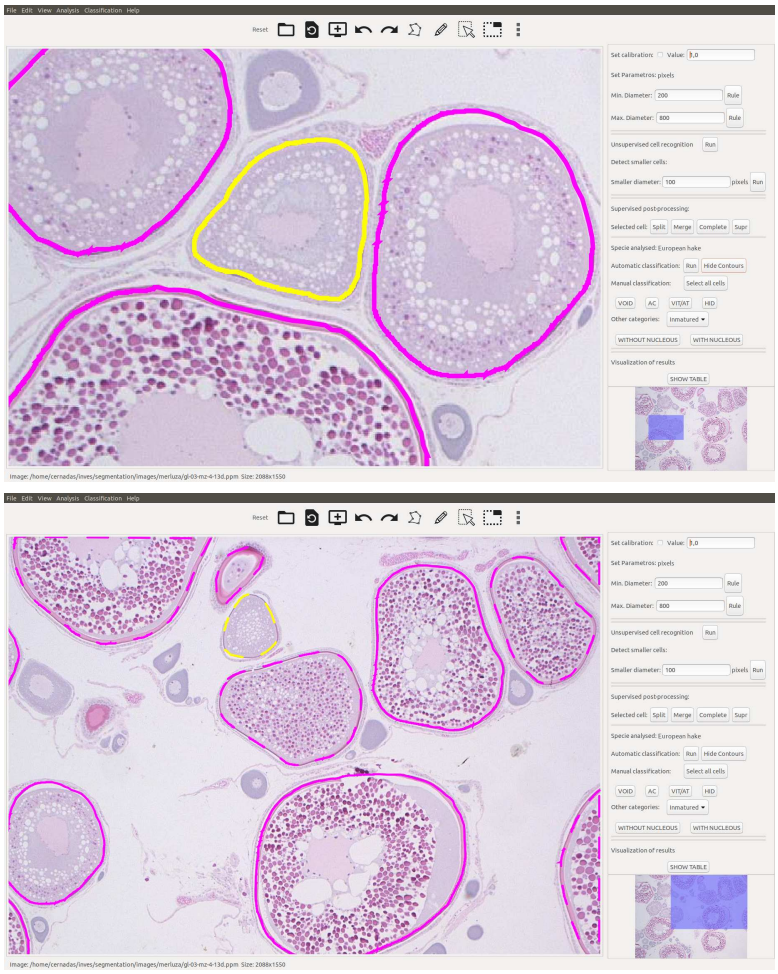

Figure S15: An example of the zoom functionality in STERapp.

8 View menu or lateral panel

The lateral panel is a simple way of visualizing and managing the commands needed in the processing of an image; it contains the same functionalities already seen in other sections as **Calibration** and **Diameters**. **Diameters** in the lateral panel has also a tool to introduce the values both graphically and manually: the button **Rule** allows to measure one object of the loaded image and will transfer the result to the diameters box when the left button of the mouse is released. The value will be referred in pixels or microns depending on the current calibration. When the calibration or diameters are modified, all the overlays of the image are deleted. The value will not be updated if there is an inconsistency between maximum and minimum values. Other utilities of the lateral panel are:

1. **Unsupervised cell Recognition** automatically processes the image and shows, overlapped on the image window, the recognized oocytes. Clicking on the **Run** button, the image panel will show, in a few seconds, the results of the operation (see the upper panel of Figure S16).

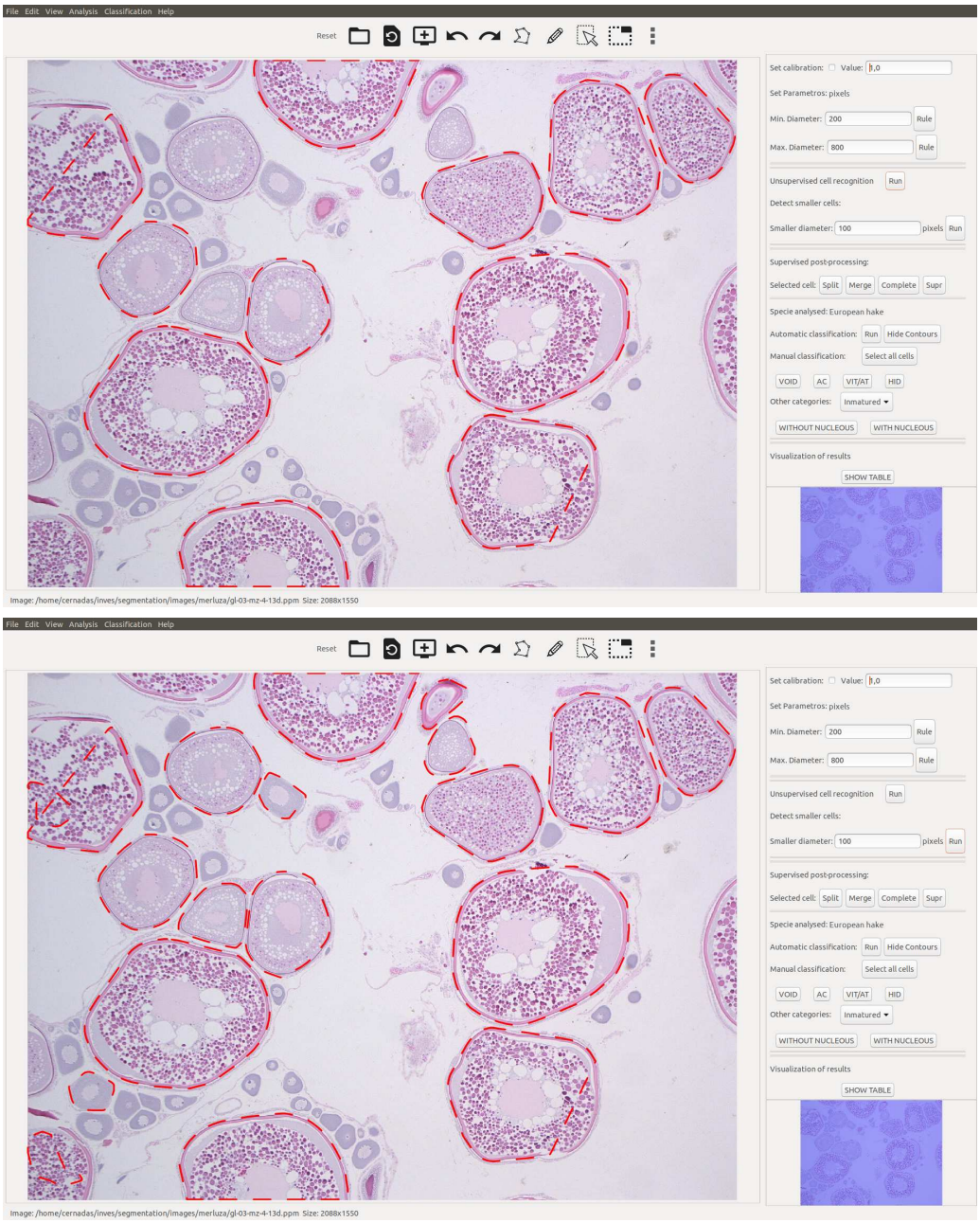

Figure S16: Output after automatic processing of the histological image (upper panel) and output after automatic processing using the option "detect smaller cells" (bottom panel).

2. **Detect smaller cells** can be used to detect oocytes smaller than the minimum diameter set in the program after the run of the **Unsupervised cell Recognition**. In the entry widget after the label "smaller diameter", you can set a value lower than the minimum diameter $d_{smaller}$. Pressing **Run** after this entry widget, STERapp automatically recognizes the oocytes, whose diameter is between $d_{smaller}$ and the minimum diameter set in the preferences, and ensures that the newly recognized oocytes do not overlap the previously recognized ones. As well, this process can provide less false positives, because of its

two phases of recognition and revision. The lower panel of Figure S16 shows an example of oocyte detections added when the automatic algorithm to recognize oocytes is used with $d_{smaller} = 100$ and the minimum diameter is 200 pixels.

3. **Supervised post-processing**, in this block there are some tools to help the user to supervise the recognition results of the automatic algorithms. These tools are detailed in Section 8.1.

4. **Classification utilities**, there are a set of functionalities to manage the issues related to the assignment of categories to the oocytes being automatically or manually recognized (see Section 10 for a futher description).

5. **Visualization of results**, pressing the **Show Table** button, a table containing analysis information will be opened at the bottom of the main window. The table will show the diameter, the area, the development stage, the visible/not visible nucleous and if the cell is counted or not. A detailed description of these functionalities is provided in Section 8.2.

6. **Iconized image for visualize position**, at the bottom of the lateral panel, there is a miniature image of the original image loaded in STERapp. Over this miniature there is a blue shaded square representing the area of the original image currently shown in the image window. The position and size of this area depends on the zoom used, as it can be seen in Figure S15.

8.1 Supervised post-processing

After the automatic processing to recognize the oocytes (see Figure S16), the user can review and edit the results using the tools to modify, add and/or remove misclassified oocytes. To add an oocyte you must select the drawing tool on the toolbar and draw the contour using the mouse. To remove one or more oocytes you must first select them and then press the **Supr** button of the keyboard or the **Supr** button on the lateral panel. To select oocytes, press the **Select cells** icon on the toolbar and click inside the oocyte with the left button of the mouse. To select more than one object repeat the process keeping pressed "Ctrl" in the keyboard. Buttons **Split**, **Merge**, **Complete** and **Supr** must be applied for a selection of oocytes.

1. **Split**: divides one selected object into two oocytes when the automatic processing fails in the recognition of more than one particle. To do the split operation, the user must first select one object, press then the button **Split** (this button will be activated), and draw a line or an arc on the selection which follows the contour of the desired oocyte contour. The **Split** button will be deactivated and the selected object will be divided into two. Figure S17 shows two images representing the state before and after the split operation. If one of the oocytes as a result of the splitting process is smaller than allowed by the minimum diameter, this oocyte or object is automatically removed.

2. **Merge**: fuses the selected contours, which do not correspond to single oocytes, into one. To do the merge operation, you must select the set of objects you want to merge (i.e.,

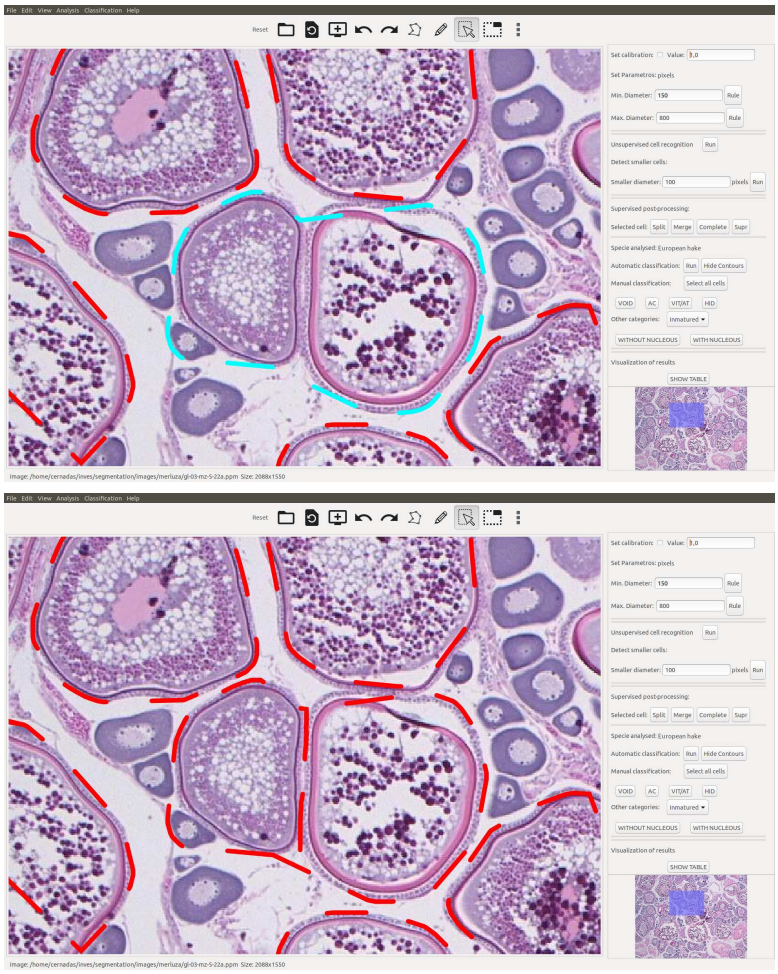

Figure S17: Split operation (button **Split** in the lateral panel) to divide one selected object (magenta in the upper panel) into two regions.

using "Ctrl") and click **Merge** in the lateral panel. After that, the separated contours will not be visible any more and they will appear as a single oocyte. Figure 18 shows two images that represent the state before and after the merge operation.

3. **Complete**: adds an outline to a selected oocyte when the automatic processing has not recognized the entire contour. To do such a completing operation, the user must select the incomplete oocyte and click **Complete** in the lateral panel. This button will be activated as long as the user draws on the image to complete the actual outline of the selected oocyte. When the user releases the left button of the mouse, the selected oocyte will be completed and the **Complete** button will be deactivated, as it is shown in the Figure S19.

4. **Supr**: selects an oocyte or a set of oocytes. You can click the button **Supr** to remove them. The selected objects can also be removed pressing "Supr" on the keyboard.

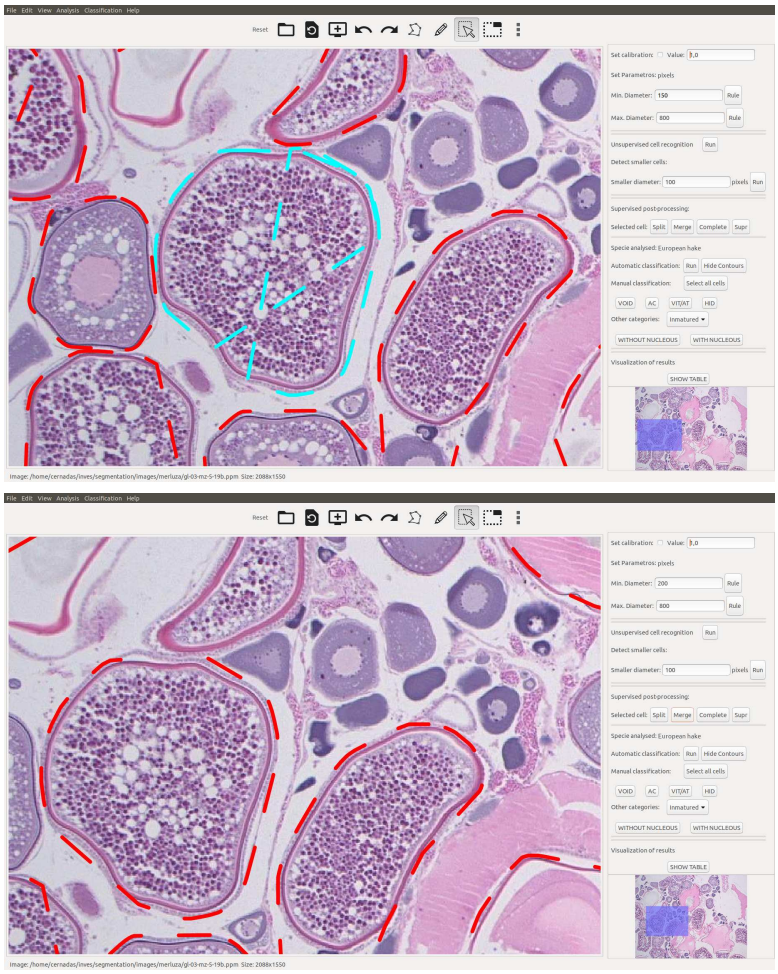

Figure S18: Merge operation (button **Merge** in the lateral panel) to merge various selected cells into one cell. The selected oocytes to merge in the upper panel are in magenta colour.

8.2 Visualization of results

Press the button **Show Table** of the lateral panel to open a table at the bottom of the main window (see Figure S20) that shows the information of the detected oocytes in the image. The results table will show diameter, area, development stage, visible/not visible nucleous and whether the oocyte is counted or not. The user can select an oocyte outline on the image (cyan contour) and the row of the table containing the information of that cell will be activated, as it can be seen in the upper panel of Figure S20. Inversely, if you select a row in the table, its corresponding oocyte in the image window will be appear as selected (cyan colour), as it can be seen in the lower panel of Figure S20. The information of this table can be exported to a CSV file, as it has been described in Section 6.

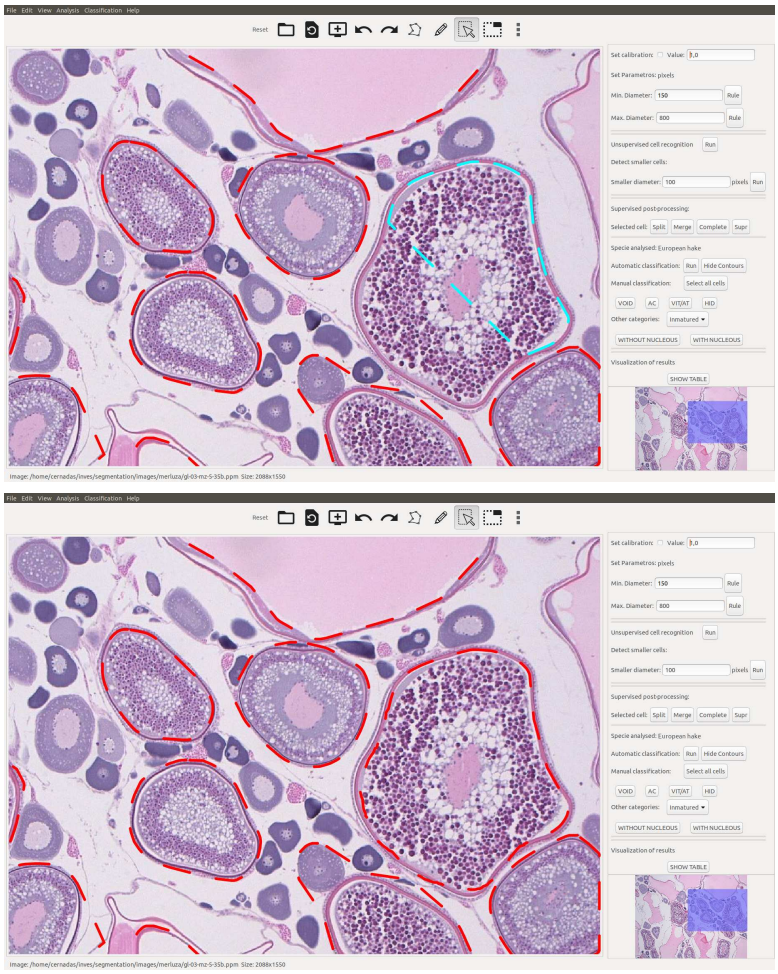

Figure S19: Result of the operation **Complete** of the lateral panel. Complete the contour of a selected oocyte that was inaccurately recognized (magenta colour).

9 Analysis menu

The only item available in this menu is **XML files**, which opens a pop-up window (Figure 21). There, the user can choose the XML files of every processed image in order to gather the results in one single CSV file, as they can correspond to a single individual, samples, etc. For this purpose, the user must first process individually each image and save the recognition results (outline of the oocytes of each image) in an XML file; once finished, run **Analysis**, select the **XML files**, and get one single results sheet.

In Figure S21, by default the selected xml directory is the folder of the preferences for XML files, but it can be changed by clicking on the three points button. After that, click in the button **Click to select files** to select the XML files to be included in the anlysis, opening the window of Figure S22. To select contiguous files, click on the file name with the left button mouse and keeping the key **Alt** pressed, choose the last file. To select non-contiguous files, click with the left button mouse on the file and, keeeping the **Ctrl** key pressed, click on other files. Once the selection is made, click **Select** and the files selected appear in the selection XML

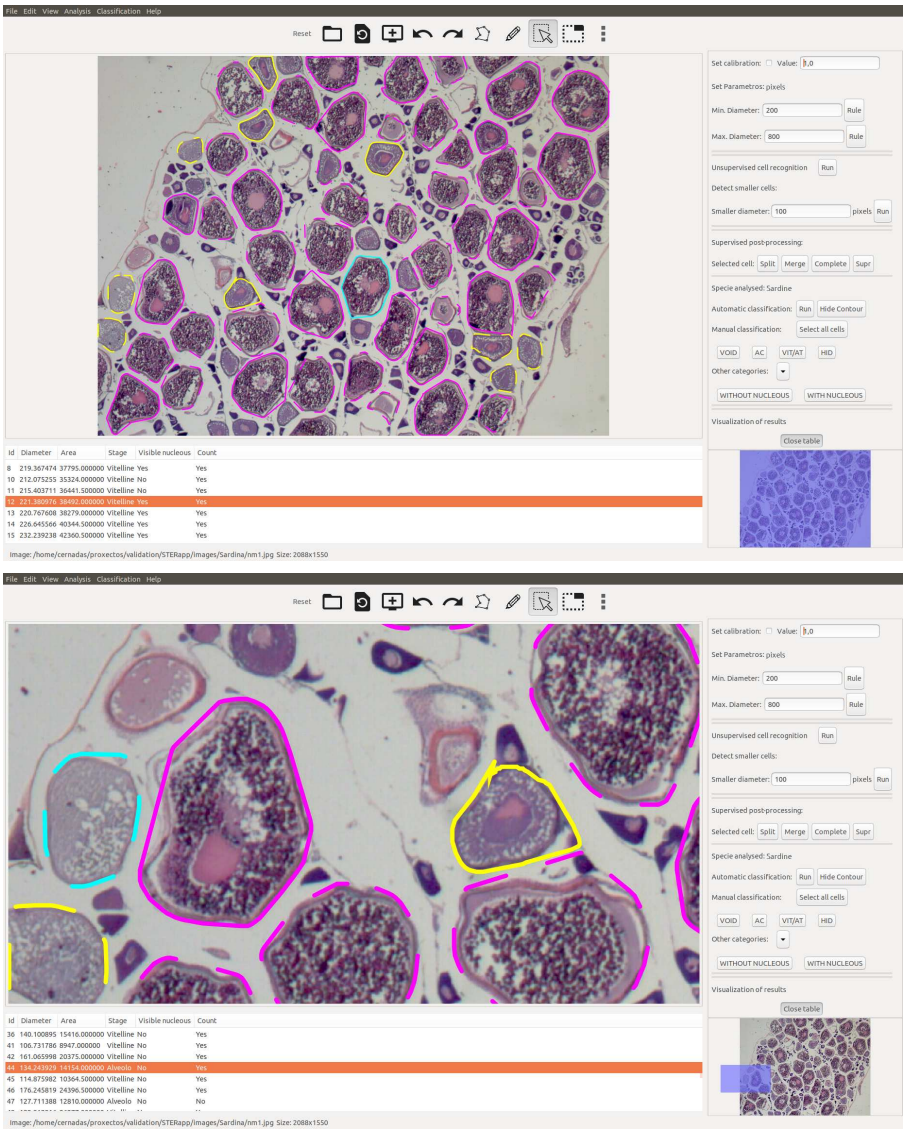

Figure S20: Visualization of information about the oocytes overlapped to the image in the bottom table (button **Show Table** in the lateral panel). The cyan contour is the selected cell.

files box (Figure S23). Clicking on button **Export CSV** (see Figure S23) to export the statistical results to a CSV file (Figure S24 shows the CSV files generated); or press the **Close** button to cancel the operation. Following, the user will be asked for the name of the results CSV file. The operation could require some seconds depending of the amount of files to analyse. After finishing the process, the user must close the window in the **Close** button (in Figure S23) or perform another procedure.

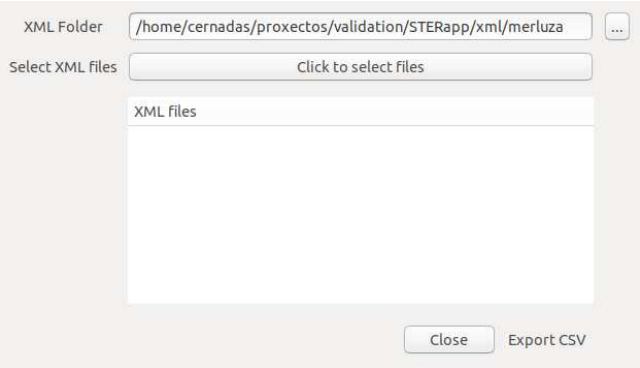

Figure S21: Window open when the submenu **XML File** of the menu **Analysis** is chosen.

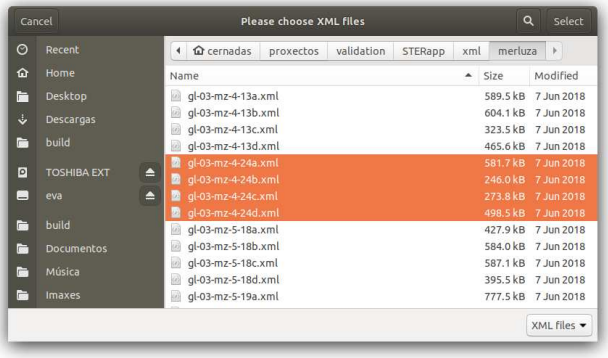

Figure S22: File chooser dialog to choose the XML files included in the joint analysis.

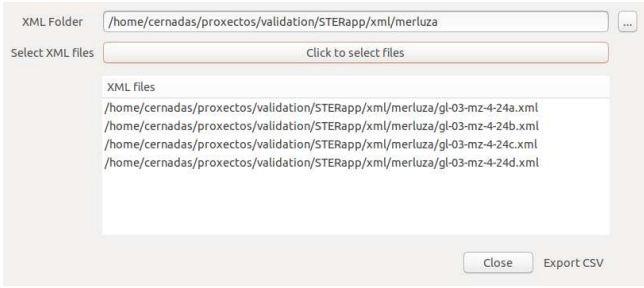

Figure S23: Window open when the submenu **XML File** of the menu **Analysis** is chosen and after selecting the XML files used in the analysis.

10 Classification menu

The classification menu has two submenus: **Classify** and **Train classifier**, whose use will be described below.

The oocytes as recognized in the histological image can be classified following two criteria, "Classes" and "Stage".

1. **Classes**: describes the way in which the oocyte has been sectioned, i.e., showing or not showing nucleous. It deeply affects the diameter estimates. An oocyte without visible

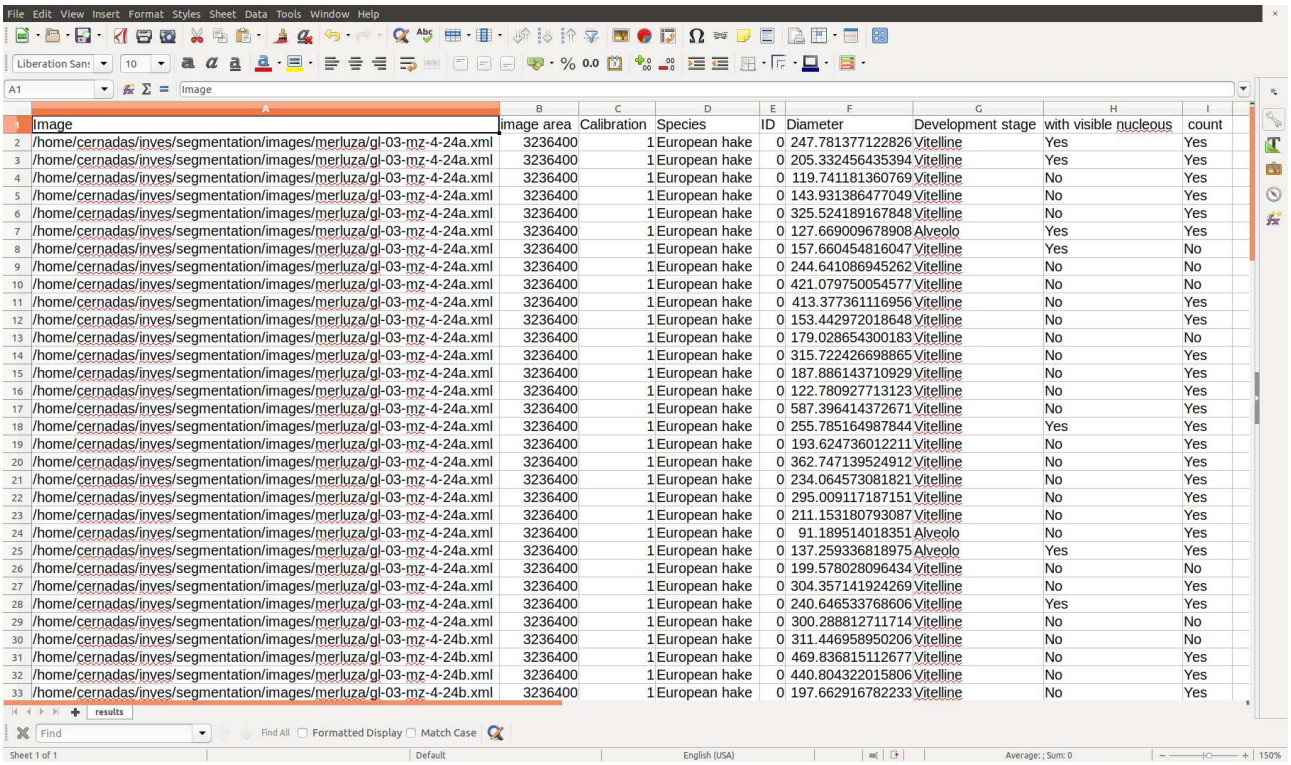

Image	image area	Calibration	Species	ID	Diameter	Development stage	with visible nucleous	count
/home/cernadas/inves/segmentation/images/merluza/gl-03-mz-4-24a.xml	3236400	1	European hake	0	247.781377122826	Vitelline	Yes	Yes
/home/cernadas/inves/segmentation/images/merluza/gl-03-mz-4-24a.xml	3236400	1	European hake	0	205.332456435394	Vitelline	Yes	Yes
/home/cernadas/inves/segmentation/images/merluza/gl-03-mz-4-24a.xml	3236400	1	European hake	0	119.741181360769	Vitelline	No	Yes
/home/cernadas/inves/segmentation/images/merluza/gl-03-mz-4-24a.xml	3236400	1	European hake	0	143.931386477049	Vitelline	No	Yes
/home/cernadas/inves/segmentation/images/merluza/gl-03-mz-4-24a.xml	3236400	1	European hake	0	325.524189167848	Vitelline	No	Yes
/home/cernadas/inves/segmentation/images/merluza/gl-03-mz-4-24a.xml	3236400	1	European hake	0	127.669009678908	Alveolo	Yes	Yes
/home/cernadas/inves/segmentation/images/merluza/gl-03-mz-4-24a.xml	3236400	1	European hake	0	157.660454816047	Vitelline	Yes	No
/home/cernadas/inves/segmentation/images/merluza/gl-03-mz-4-24a.xml	3236400	1	European hake	0	244.641086945262	Vitelline	No	No
/home/cernadas/inves/segmentation/images/merluza/gl-03-mz-4-24a.xml	3236400	1	European hake	0	421.079750054577	Vitelline	No	No
/home/cernadas/inves/segmentation/images/merluza/gl-03-mz-4-24a.xml	3236400	1	European hake	0	413.377361116956	Vitelline	No	Yes
/home/cernadas/inves/segmentation/images/merluza/gl-03-mz-4-24a.xml	3236400	1	European hake	0	153.442972018648	Vitelline	No	Yes
/home/cernadas/inves/segmentation/images/merluza/gl-03-mz-4-24a.xml	3236400	1	European hake	0	179.028654300183	Vitelline	No	No
/home/cernadas/inves/segmentation/images/merluza/gl-03-mz-4-24a.xml	3236400	1	European hake	0	315.722426698865	Vitelline	No	Yes
/home/cernadas/inves/segmentation/images/merluza/gl-03-mz-4-24a.xml	3236400	1	European hake	0	187.886143710929	Vitelline	No	Yes
/home/cernadas/inves/segmentation/images/merluza/gl-03-mz-4-24a.xml	3236400	1	European hake	0	122.780927713123	Vitelline	No	Yes
/home/cernadas/inves/segmentation/images/merluza/gl-03-mz-4-24a.xml	3236400	1	European hake	0	587.396414372671	Vitelline	No	Yes
/home/cernadas/inves/segmentation/images/merluza/gl-03-mz-4-24a.xml	3236400	1	European hake	0	255.785164987844	Vitelline	Yes	Yes
/home/cernadas/inves/segmentation/images/merluza/gl-03-mz-4-24a.xml	3236400	1	European hake	0	193.624736012211	Vitelline	No	Yes
/home/cernadas/inves/segmentation/images/merluza/gl-03-mz-4-24a.xml	3236400	1	European hake	0	362.747139524912	Vitelline	No	Yes
/home/cernadas/inves/segmentation/images/merluza/gl-03-mz-4-24a.xml	3236400	1	European hake	0	234.064573081821	Vitelline	No	Yes
/home/cernadas/inves/segmentation/images/merluza/gl-03-mz-4-24a.xml	3236400	1	European hake	0	295.009117187151	Vitelline	No	Yes
/home/cernadas/inves/segmentation/images/merluza/gl-03-mz-4-24a.xml	3236400	1	European hake	0	211.153180793087	Vitelline	No	Yes
/home/cernadas/inves/segmentation/images/merluza/gl-03-mz-4-24a.xml	3236400	1	European hake	0	91.189514018351	Alveolo	No	Yes
/home/cernadas/inves/segmentation/images/merluza/gl-03-mz-4-24a.xml	3236400	1	European hake	0	137.259336818975	Alveolo	Yes	Yes
/home/cernadas/inves/segmentation/images/merluza/gl-03-mz-4-24a.xml	3236400	1	European hake	0	199.578028096434	Vitelline	No	No
/home/cernadas/inves/segmentation/images/merluza/gl-03-mz-4-24a.xml	3236400	1	European hake	0	304.357141924269	Vitelline	No	Yes
/home/cernadas/inves/segmentation/images/merluza/gl-03-mz-4-24a.xml	3236400	1	European hake	0	240.646533768606	Vitelline	Yes	Yes
/home/cernadas/inves/segmentation/images/merluza/gl-03-mz-4-24a.xml	3236400	1	European hake	0	300.288812711714	Vitelline	No	No
/home/cernadas/inves/segmentation/images/merluza/gl-03-mz-4-24b.xml	3236400	1	European hake	0	311.446958950206	Vitelline	No	No
/home/cernadas/inves/segmentation/images/merluza/gl-03-mz-4-24b.xml	3236400	1	European hake	0	469.836815112677	Vitelline	No	Yes
/home/cernadas/inves/segmentation/images/merluza/gl-03-mz-4-24b.xml	3236400	1	European hake	0	440.804322015806	Vitelline	No	Yes
/home/cernadas/inves/segmentation/images/merluza/gl-03-mz-4-24b.xml	3236400	1	European hake	0	197.662916782233	Vitelline	No	Yes

Figure S24: An example of a CSV file of the analysis of various XML files imported in LibreOffice Calc.

Figure S25: Pop-up message informing that the classifier is not trained for the fish specie con-sidered.

nucleous in the image indicates that the oocyte has not been cut by its middle section so, its diameter does not correspond to the real diameter of the spherical cell. Once an oocyte or set of oocytes are selected on the image, the class of the selected oocytes can be manually set clicking the buttons **WITHOUT NUCLEOUS** or **WITH NUCLEOUS** in the lateral panel. The oocytes with visible nucleous are shown with a continuous line and the oocytes with no visible nucleous are shown with a dashed line.

2. **Stages**: the oocytes can be at different developmental stage within the same ovary section depending on the specie. These maturity stages can be cortical alveoli, vitelogenic, atretic, hydrated, primary growth, etc [1]. For automatic classification, STERapp allows, by default, only three of this categories: cortical alveoli, vitelogenic/atretic and hydrated, plus the category "Void", that represents the area in the image that does not correspond to gonadal tissue and therefore shouldn't be evaluated. As it was described in Section 5,

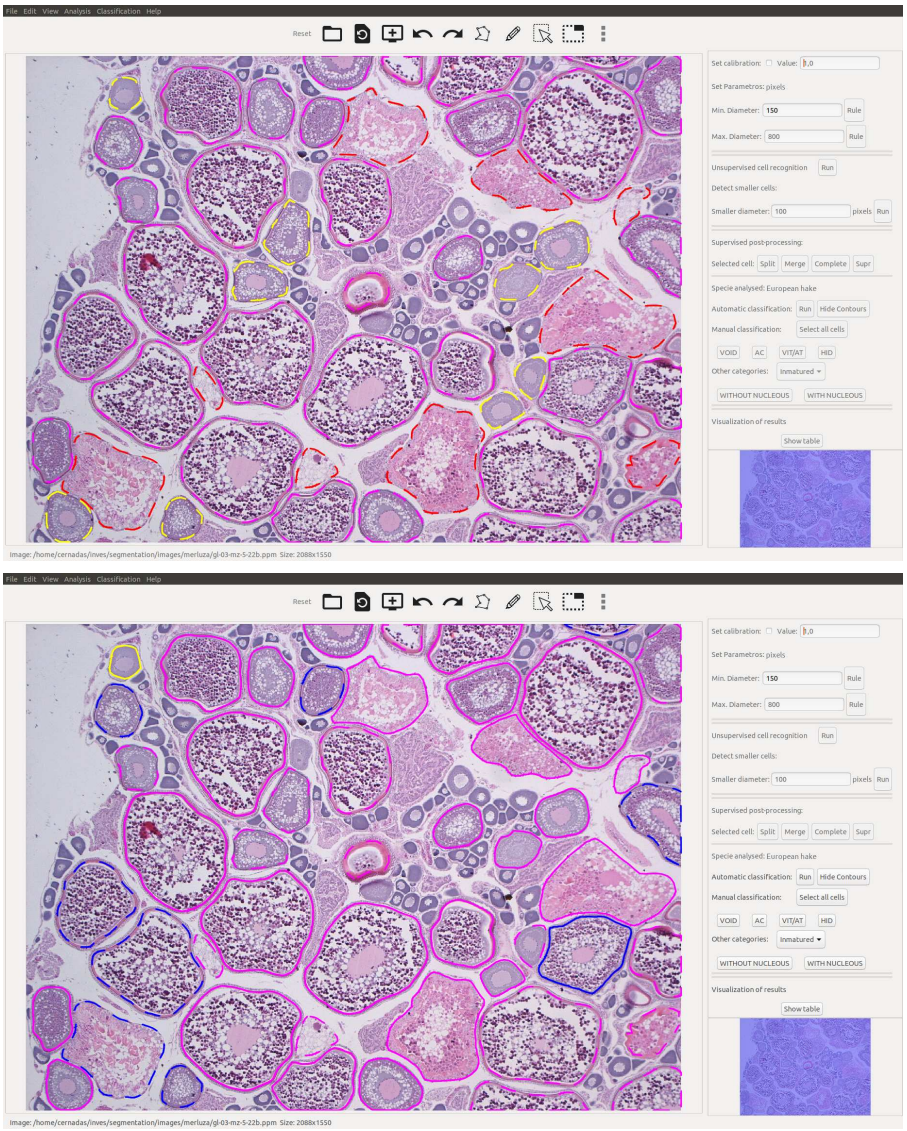

Figure S26: An example of oocytes classified manually by expert (upper panel) and after applying the classifier (lower panel).

STERapp allows to define or register new categories or stages and the user can associate a colour to show them on the image window. If the user wants to assign a non predefined category, she must select an item in the drop-down list after the label *Other categories* (see Section 5 to define new categories in the working preferences). Once an oocyte or set of them are selected, the category or stage can be manually set by clicking the corresponding buttons **VOID**, **AC**, **VIT/AT** and **HID** of the lateral panel to assign the stage "void", "cortical alveoli", "vitelogenic/atretic" and "hydrated", respectively.

The user can only apply the classification process over recognized oocytes in an image loaded in the image window. To classify automatically the oocytes, go to the submenu **Classify** or click the button **Run** in the lateral panel after the label "Automatic classification". Before running

the classification, the user must make sure that the selected fish species in the preferences is correct (shown in the lateral panel after the label "Specie Analysed"). If the user tries to do the classification before the training, the program will show a pop-up message (Figure 25), warning that the classifier for the mentioned species was not trained. If there already exists a trained classifier for that species, STERapp runs it and shows the results (the upper panel of Figure S26 shows the cells manually classified by the expert, and the lower panel shows the output provided by the automatic classifier).

The stage or class of the oocytes can be manually assigned using the GUI. STERapp also implements the automatic classification of oocytes for the predefined stages. This procedure operates over the recognized oocytes, but again, the classifier needs to be trained. The classifier is a supervised machine learning technique, which needs to learn the function to predict, in this case, the stage and class of the oocytes which have not been seen before. To learn this function it is necessary to provide the classifier a set of images which contains oocytes of every predefined stage and class. So first, the user must manually analyse a limited number of images for a specific fish species, save the annotated oocytes overlapped to the image in their corresponding XML files, and train the classifier for that species. After the training, the classification operation can be applied with new images or individuals, using the submenu **Classify** or clicking the button **Run** in the lateral panel after"Automatic classification".

10.1 Training the classifier

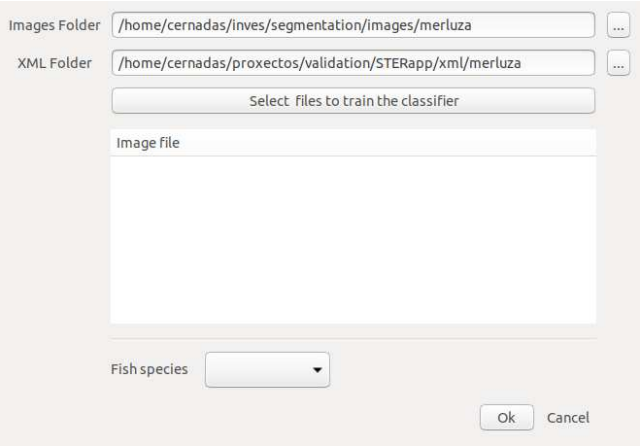

Figure S27: Dialog window to train the classifier.

To train the classifier, please select the submenu **Train classifier** in the "Classification" menu, which will open the dialog window of Figure S27. Select the image and XML files used in the training process, and click on the button **Select files to train the classifier** which will open a window similar to Figure S22 to select the files used. STERapp will train the classifier. At the bottom of the panel of Figure S27, there is a drop-up list "Fish species" where the user has to select the fish species for which he wants to train (among the fish species defined in STERapp when setting the preferences in Section 5). Clicking **Ok** starts the training, and clicking on **Cancel** closes the training process (Figure S28). This process can take several

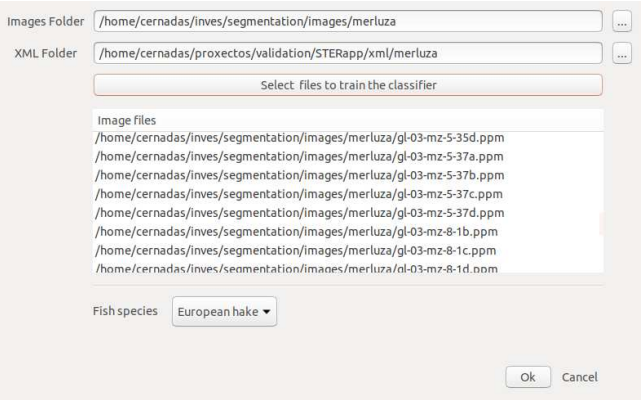

Figure S28: Figure S27 after the configuration of the parameters to train the classifier.

minutes, depending on the number of images used. During the training, the program cannot be used.

11 Help menu

The **Help** menu is loaded within the menu bar with the submenus **User Manual** and **About Us**. The submenu **About Us** pop-ups a window with a short description of STERapp and information about its developers and the license (see Figure S29).

Figure S29: Pop-up dialog to inform about help.

References

[1] H. J. Grier, M. C. Uribe-Aranzábal, and R. Patiño. The ovary, folliculogenesis, and oogenesis in teleosts. In B. G. M. Jamieson, editor, *Reproductive biology and phylogeny of fishes (agnathans and bony fishes)*, pages 25–84. Enfield, New Hampshire: Science Publishers, 2009.