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Mode d'emploi

User Manual

Histolab

Analysis of histological tissue

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1. Overview of Histolab

Histolab has been devised in order to take several kinds of measurements on various types of histological tissue. It thus provides rich functionalities for processing images, printing images and numerous interactive and powerful measurement tools. We have taken particular care with regards to simplicity and comfort of use and we wanted the documents produced to be usable straight away: annotation of the images, print-out of the images and the results, storage in files that can be manipulated with other applications.

Histolab provides for the accumulation of measurements in an unlimited number of fields. It allows the simultaneous analysis of several types of structures. Statistics concerning each type of structure are provided at all times.

The measurements provided are as follows: X-axis and Y-axis of the centre, length, width, perimeter, surface and light intensity. The results are all expressed in real units, either in a comprehensive table or summed up with statistical values.

Histolab provides the user with a whole range of measurement tools, thus adapting to many kinds of histological sections. Certain tools are automatic, i.e. once they have been set, they measure a structure on all (or part) of the field displayed in the video window. Other tools are pure plotting tools, particularly for counting, measuring thicknesses or even for when the colorations of the section are not sufficiently homogeneous to be detected with shade criteria.

Finally, everything is done so that the user can use the results provided by Histolab as smoothly as possible: a work sheet can be saved and resumed later. The results, statistics and counts can be exported to common calculation applications, either in compatible files or simply by using Copy / Paste. Finally, images, lists of values and statistics can be edited on a printer.

Starting up Histolab

A Histolab shortcut is normally accessible in the "Microvision" program group, situated in the "Programs" menu within the Windows "Start" menu.

The Histolab work sheet

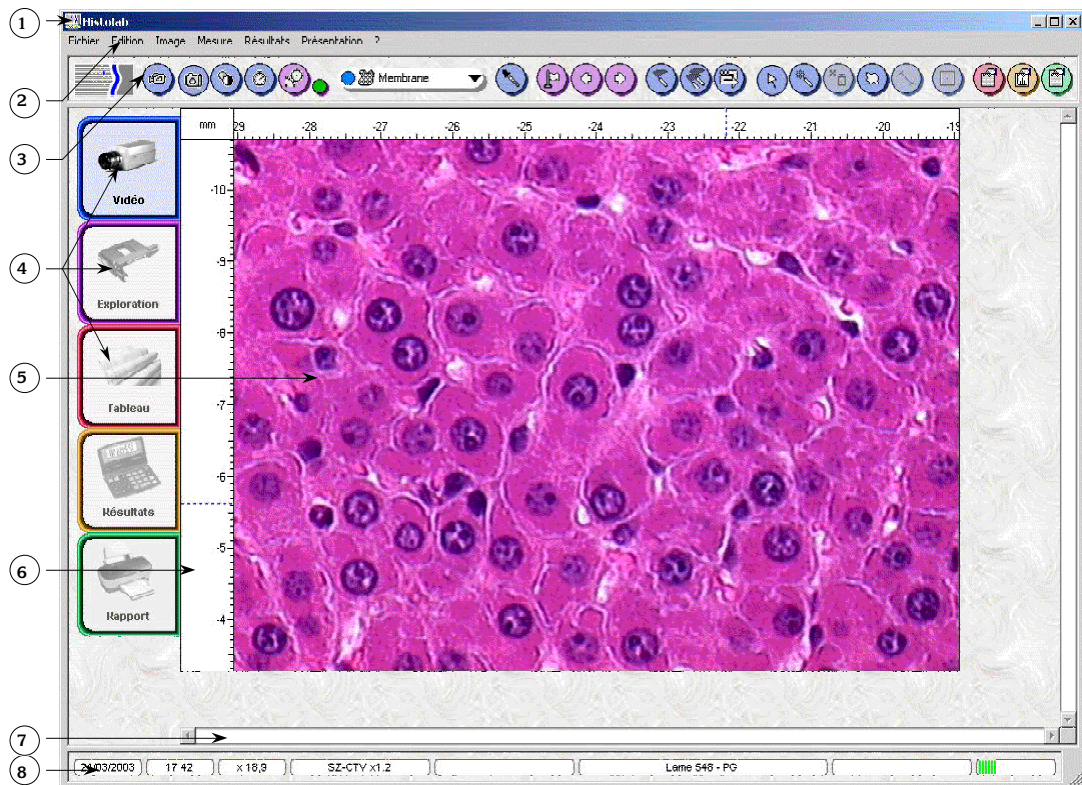
The work sheet is the window displayed when Histolab starts up. It contains the standard elements of the Windows environment: the title bar (1) which includes the system menu box (on the left), and the window enlargement / reduction boxed (on the right), the menu bar (2) provides access to all of the software's functions. When the sheet does not occupy the entire screen, it can be moved (by clicking on the title bar) or deformed (by clicking within the frame).

The toolbar (3) contains some buttons for quickly accessing the software's most used functions.

On the left of the sheet, some tabs (4) allow you to display either the video image or various documents that are representative of the work in hand: a mapping or a histogram of the fields measured, the measurements table, a graph and statistical tables, the printed report.

The video image or the document occupies most of the sheet (5). They are bordered by rules (6), graduated in real units, and by scroll bars (7) that are active when the document or image is larger than the window itself.

In the lower part of the work sheet, the status bar (8) contains information relating to the work in progress.



The Histolab work sheet

The menus

They provide access to all Histolab functions. Here is a quick overview of them:

- the "File" menu is dedicated to the studies: start of a study, and definition of its main properties; saving the study, exporting and printing the results. It also enables you to archive, load and print images.
- the "Edit" menu provides classic "Copy/Paste" functions that allow you to exchange images or documents between several different software applications. You can also cancel an action and access the image annotation module.
- the "Image" menu is dedicated to video management: acquisition, storage and improvement of images.
- the "Measure" menu contains the functions used during a study: system calibration, properties of the study, choice of structures and measurement tools.
- the "Results" menu concerns the documents presented on the "Table", "Results" and "Report" pages. It also provides access to dialog boxes for defining the contents of these

documents and items allowing you to display certain of them in windows that remain visible at all times.

- the "Layout" menu contains various more general options: personalising the environment and options for plotting measurements.
- the "?" menu: information about the Histolab software and secure operating modes.

The functionalities provided in these menus are described in detail in the "Reference Manual".

The toolbar

The toolbar provides quick access to certain frequently used functions and information:



The Histolab toolbar

1. Commonly used video functions (from left to right): "Acquisition", "Freeze image", "Automatic contrast", "Image integration".
2. Activation / Deactivation of position tracking, when the system is equipped with an indexed or motorized displacement stage.
3. Name of the structure being measured. This is a local menu: click on it to display the list of the defined structures and it allows you to change the structure.
4. Setting the detection of the selected structure.
5. Switching from field to field:

With a motorized stage, all of the buttons are active. The displacement buttons pilot the stage; the button on the left allows you to define an initial position.

With an indexed displacement stage, the buttons are inactive: moving the stage changes the field.

With a manual system, the field displacement buttons are active and allow you to display the measurements already taken.
6. Measurement of a structure / Measurement of all of the structures / Measurement of the sample (motorized systems).
7. Interactive measurement tools (from left to right): selection of objects, measurement in a manually plotted area, counting, plotting a contour, plotting a segment, measurement of the reference light intensity. The tools are validated according to the type of structure selected.

8. Setting the properties for the displayed documents: measurements displayed in the tables, definition of the graph, report page layout.

The tabs, pages and documents

The tabs located on the left of the window allow you to display five pages that are representative of the current study:

- The real time video image is of course the most important page as it allows you to observe the sample and to take the measurements.

The video image and the possibly superimposed profiles can be saved or printed using the corresponding options in the "File" menu. A part of the image can be boxed and then transferred to another software, using the "Copy" option in the "Edit" menu.

- The "Overview" page presents a background history of the fields measured: all of the objects plotted are shown, superimposed on the image that is under the camera while the measurement is being taken.

When the system is equipped with an indexed or motorized displacement stage, the background history is replaced by a mapping of the sample, in which the images and measurements are plotted in their real positions.

The overview (background history or mapping) can also be displayed in a separate window and included in the printed report. A part of the overview can be boxed, copied, and then transferred to another software application.

- The "Table" shows all of the objects plotted during the study (one object per line) and a certain number of measurements chosen by the operator for each one.

The measurements table can be inserted into the report. A part of the table can be selected, copied, and transferred to a spreadsheet program.

- The "Results" page allows you to display four separate documents: the statistical values calculated for each structure, the counting table, a distribution histogram as a graph and as a table.

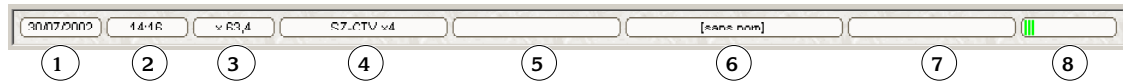
These four documents can be displayed individually in separate windows, included in the report, or copied to an external software application.

- The "Report" is a true copy of the document that is likely to be printed. It always follows the progress of the work in hand.

The report page layout can be personalised: size and contents of the header and footer, title and order of the printed documents.

The status bar

The status bar is situated at the very bottom of the sheet. It contains information relating to the work in hand:



The Histolab status bar

1. System date.
2. System time.
3. Scale in which the video image or overview is displayed.
4. Name of the currently applied calibration.
5. Name of the image file, if loaded.
6. Reference of the sample being analysed (or name of the "study" file in which the measurements are recorded)
7. Position of the crosshairs when you click on one of the graduated rules. When you use the framing tool, the size of the selected area is displayed here.
8. Indicator of memory occupied on the microcomputer.

The structure of a work session

Preparing the equipment

During this stage, you essentially set:

- the acquisition and framing of the video image: using the options in the "Image" menu, you try to display the image on the screen with a sufficient contrast.
- the calibration of the system, operation that consist in establishing the equivalence between a real distance and the length that is visible on the screen.

The preparation of the equipment is presented in Chapter 2. The reference manual deals with many aspects of this in detail (video acquisition in Chapter 4, calibration in Chapter 5).

Preparing the study

The preparation of the study consists in providing the software with the number and the names of the structures to be measured as well as some of the properties of the sample to be analysed

and, if the system is equipped with a motorized stage, the form and the size of the area to be scanned.

Then, the "detection" of each structure is set, i.e. all of the image processes that will lead the equipment to detect the structure in the video image. The programmed processes are summarised in a palette that can be displayed via the "Measure" menu.

These operations are described in Chapter 3.

The slide analysis

The analysis can be completely automatic if the system is equipped with a motorized stage and if the slide preparation is sufficiently homogeneous so that the settings made may apply to the entire sample, from one end to the other. Otherwise, the analysis is semi-automatic: after having positioned the microscope stage so that the area of interest is displayed in the video image, the operator must select the structure then he wants to analyse and then a measurement tool.

Chapter 4 is dedicated to taking measurements with both manual plotting tools and automatic measurement tools.

Chapter 5 presents the "Overview" page which displays the mapping of the section or the background history of the measurements taken.

Using the results

This final stage consists in defining the presentation for the tables and graphs provided as well as the page layout for the measurement report.

These functions are described in Chapters 6 to 8.

Protecting the settings

The settings made during the first two stages are fundamental and determine the validity of the measurement results. It is possible to restrict access to these settings by setting the software to a secure operating mode.

The secure operating modes are described in Chapter 9.

2. Preparing the equipment

Image acquisition

The first stage consists in acquiring the image of the field to be measured. It is stored in the image memory, and a part of it is displayed on the work sheet.



Real-time acquisition is activated using this button or the "New image" option.



This button automatically improves the image contrast.



It may be useful to freeze the image using this button or the "Freeze image" option.



Acquisition by integration is used when the light intensity is very weak: the image is acquired with a special camera, called "an integration camera". The image integration is set via the corresponding item in the "Image" menu.

During a study, image acquisition is repeated for each analysed field. The techniques mentioned here are described in detail in Chapter 4 of the Reference Manual.

Access to the stage controller

If an indexed or motorized displacement stage is installed, it is controlled by an independent software application. The necessary settings are set at the factory or during commissioning of the equipment.

This software can be displayed via the "Show stage driver" item in the "?" menu: the streaked button on the bottom right of the display provides access to the menu and to the material settings; the red button, on the bottom left, closes the display, as it is of no use during normal use of Histolab.

System calibration

This stage is fundamental as it conditions the validity of the measurements that will be taken. It is used more or less frequently depending on the optical system used:

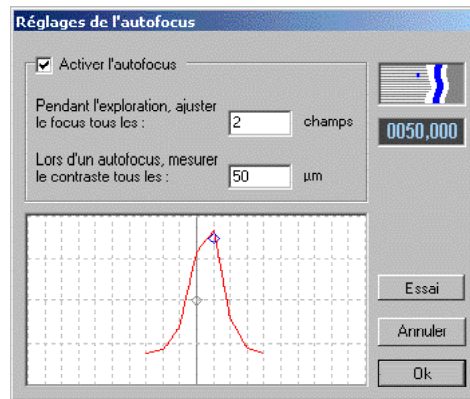
- If you often modify the optical system (magnification of an objective, modification of the position of the camera on the bracket), you have to recalibrate the system after each modification using the "Define..." option in the "Calibration" sub-menu of the "Measure" menu.
- If you have an optical system with a few pre-defined positions (objectives of the microscope, for example), you just have to activate the correct distance scale from those provided by the "Calibration" sub-menu in the "Measurement" menu.
- Finally, if you use a fixed optical system, you could make do with a routine inspection: place a ruler or a micrometer under the camera and check that the graduations match those displayed by Histolab.

For more precisions, refer to Chapter 5 of the Reference Manual.

Setting the autofocus

If the observations are made with a microscope equipped with a motorized focusing axis (three-axis motorized system), it is possible to carry out automatic focusing during the analyses.

This operation requires a setting that depends on the objective chosen. The "set autofocus..." item is available in the "Measure" menu. It displays the following dialog box:



Setting the autofocus

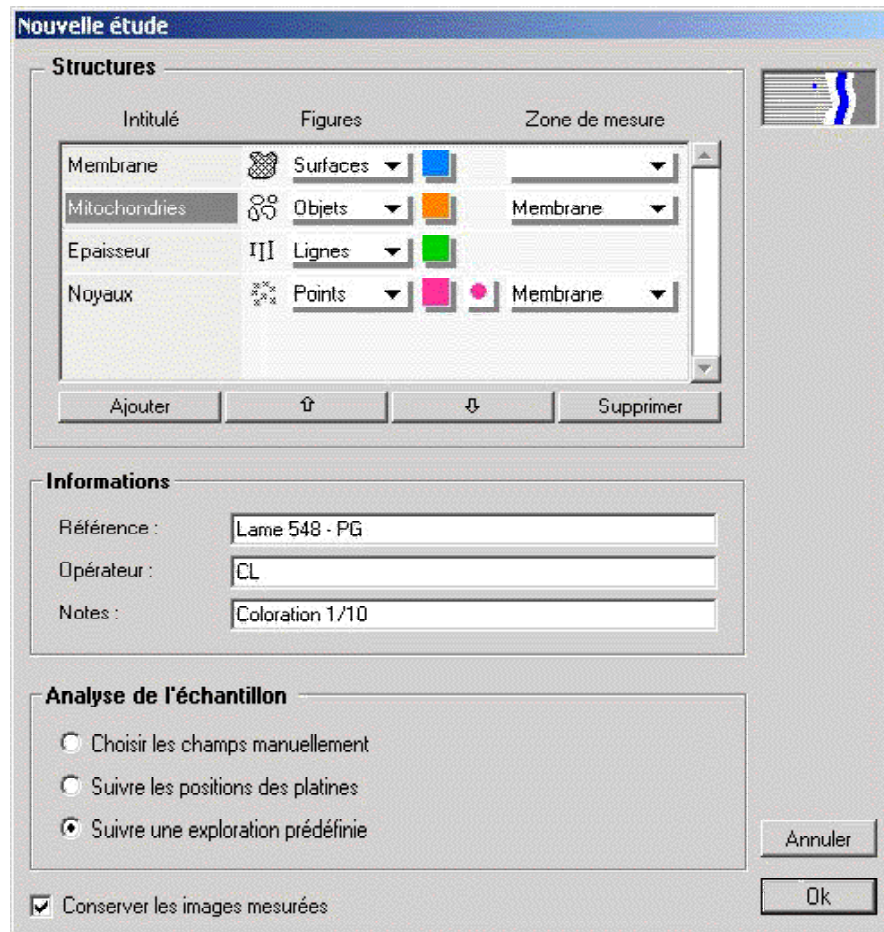
In order to set the autofocus:

- enter in the second text box a distance with the same order of magnitude as the field depth of the objective used: it defines the analysis pitch.
- click on the "Test" button: the system moves the focusing axis and takes a measurement at each stage. A curve is plotted (in red), and the system moves to the position that corresponds to the maximum level of this curve.
- if the curve is large and flattened, the correct position is likely to be outwith the analysed zone: increase the analysis pitch.
- if the curve is too narrow, the system is likely to miss the correct position: reduce the analysis pitch.

3. Preparing the study

Defining the study

During this first stage, the user defines the structures that he wishes to analyse and documents the study that is starting (reference of the section, name of the operator, comment(s)). This is done using the "New study..." item in the "File" menu.



The structures are used for coherently grouping together the measurements, especially for the statistical calculations.

The name of each structure is indicated in the left column. It can be modified once it has been partially or totally selected.

Each structure will contain a certain number of figures whose type determines the measurements taken. This is selected in the local menu situated in the centre of the list, which features:

- "points" intended for carrying out counts,
- "lines" intended for measuring lengths and thicknesses,
- "objects" intended for carrying out individual geometric measurements on closed contours: length, width, equivalent diameter, perimeter, surface and light intensity.
- "areas" intended for taking global measurements.

All of the figures can be plotted manually. All of them, except lines, can be acquired automatically.

The structures taken can be restricted inside a "reference area" by using the local menu situated to the right to the list: unless otherwise stated, measurements are taken for the entire video field. The local menu features the structures that can be considered as the reference area (they are defined by a closed contour).

Setting the detection of structures



Setting the detection of structures with automatic tools is presented in a palette that is displayed by pressing this button or via the "Detection settings..." item in the "Measure" menu.

You first choose whether to apply automatic detection or not (depending on whether the structure to be measured is in a homogeneous shade or not).



The detection setting palette

Structures in an image are detected in four stages:

- Thresholding, which extracts the phase to be measured from the image. There are two methods:

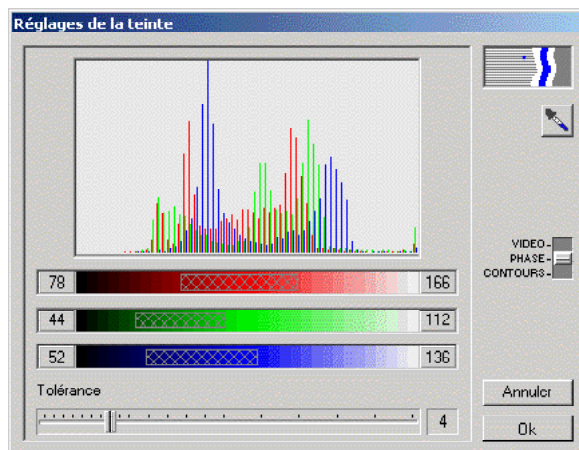
"Fixed" thresholding consists in manually setting the values of shades characterising the objects. This method allows greater precision of detection but requires stable lighting and uniform preparation of the samples. There are two versions that take account of either the grey levels in the image or the levels of each of the red, green and blue components.

"Adaptative" thresholding allows you to detect the objects in fields where lighting is not homogeneous. It is always carried out on an image converted into grey levels. The detection takes a few seconds.

- Filtering, which corrects imperfect thresholding that leaves artefacts or does not fully detect objects. Filtering can be either a simple "smoothing" of the contours of the detected objects or a sequence of personalised processes.
- possibly, a separation of the detected objects (often useful for "points", sometimes for "objects"),
- the application of a criteria to the measured values (this stage is also optional, useful for "objects").

Setting fixed thresholding

It is set in the following dialog box:



Setting threshold levels for a colour image

This box shows the shades present in the image (depending on the type of image, in shades of grey or three red, green and blue channels), the lower and upper detection thresholds currently defined for each of the red, green and blue channels (or, if need be, only in black and white) under the histogram.

During automatic analysis of the video field (<F5>), the points considered in the image are those with each of their red, green and blue components inside the defined range,



click on this button and box the shade to be extracted in the video image. Histolab then calculates the mean value and the standard deviation for each of the components of the image points in the designated area and the lower and upper detection thresholds modulated by the defined tolerance.

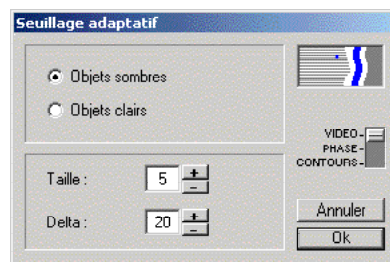
The result of the thresholding operation appears if you click on the "Phase" button (superimposition of a mask on the thresholded parts of the image) or on the "Outlines" button (the thresholded parts are bordered).

If the detection carried out is not satisfactory, there are several ways of adjusting it:

- If the field measurement has "missed" elements in the structure being analysed: increase the tolerance if elements have been unduly allocated to the structure: reduce the tolerance.
- If the setting is still not satisfactory, it is possible to individually modify the upper and lower levels for each of the red, green and blue channels by clicking on one of the coloured bars until the threshold reaches the desired value.

Setting adaptive thresholding

This method is set with the following dialog box:



Setting adaptive thresholding

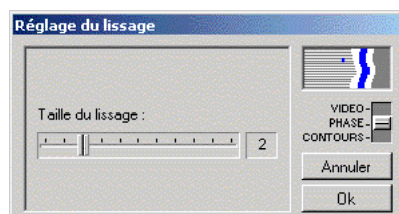
This dialog box indicates:

- the general shade of the objects,
- a size parameter that corresponds to the radius (in pixels) of the largest objects to be detected,
- the minimum shade variation to be taken into account between the objects and the image background.

As in the previous case, the result of the thresholding operation appears if you click on the "Phase" button (superimposition of a mask on the thresholded parts of the image) or on the "Outlines" button (the thresholded parts are bordered).

Setting smoothing

The smoothing dialog box is presented as follows:



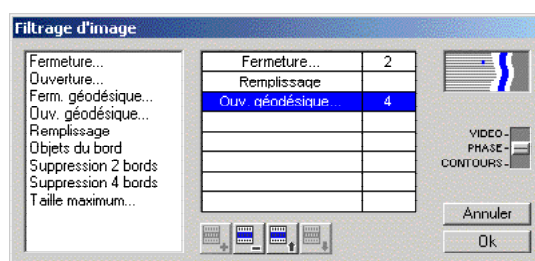
Setting smoothing

If small insignificant objects interfere with the measurement: increase the smoothing; if various elements of a structure are assimilated, reduce the smoothing.

The result of the thresholding operation appears if you click on the "Phase" button (superimposition of a mask on the thresholded parts of the image) or on the "Contours" button (the thresholded parts are bordered).

Defining a macro

A "macro" is a series of cleverly arranged image transformations: it is defined in the following window:



Definition of a sequence

The dialog box that appears shows on the left a list of the transformations that can be applied to the image. Here is a brief description of the possible transformations:

- closing: gap filling with contour smoothing.
- opening: removal of the minor artefacts with contour smoothing.
- geodesic closing: gap filling while preserving the contours.
- geodesic opening: removal of the minor artefacts while preserving the contours.
- filling: filling all of the holes, whatever their size, while preserving the contours.
- objects on the edge: removal of the objects entirely contained in the field.
- removal of 2 edges: removal of the objects intersecting the upper edge or the left edge of the field.
- removal of 4 edges: removal of all of the objects intersecting one of the field edges.
- maximum size: removal of the objects that are larger than the indicated parameter while preserving the contours.

The list on the right is the sequence formed by some of these transformations. Proceed in the following way to include a transformation:

- click in the left-hand area on the desired transformation and then click on the "+" button (or simply double-click in the left-hand area on the desired transformation): the transformation is

then placed at the end of the list.

- click on the button marked with an arrow in order to move the transformation to the desired location.
- if required by the transformation, indicate the size then validate with the <Enter> key.

The effects of the sequence on the image can be visualized by placing the switch on the right in the "Phase" or "Outlines" position.

Choice of separation

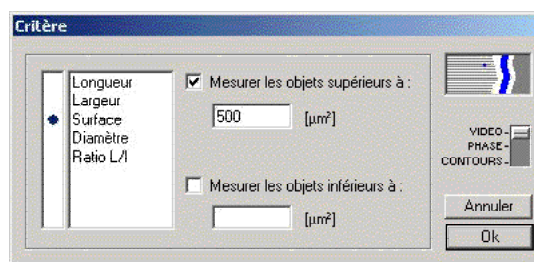
Separation is useful for structures for which an object count has been carried out (object count or measurement). It allows you to consider to a certain extent objects that appear to be agglomerated as separate objects.

Three automatic separation methods are available:

- rapid for only separating large objects;
- standard for separating medium-sized objects;
- fine for separating small objects.

Setting the criteria

It is possible to terminate detection with classification according to one of the measurements taken, using the following dialog box:



You should indicate a min. and/or max. size for the detected objects.

The effects of the sequence on the image can be visualized by placing the switch on the right in the "Phase" or "Outlines" position.

Definition of scanning

When the system is equipped with a motorized stage, the sample analysis is completely automatic. The form and the size of the sample must then be defined.

The upper part of the dialog box shown above ("Set scanning..." item in the "Measure" menu) is used for this:

Définition de l'exploration

Echantillon

Rectangulaire Longueur : 67.4 mm
Largeur : 44.9 mm

Circulaire Diamètre : 67.40 mm

42 champs

Exploration

Horizontale Verticale En spirale

Inclure les champs périphériques

Parcours aléatoire

Choisir [] champs parmi 42

Disposition des champs

Champs jointifs

Pas horizontal : 10.61 mm

Pas vertical : 7.933 mm

Annuler
Ok

The zone thus defined can be scanned in several different ways with the options in the two other parts. You can for example define exhaustive scanning of the sample (check the "Include the peripheral fields" and "Joining fields" boxes) or prefer a more rapid analysis (by defining the disjoint fields or by letting the system chose fields at random).

The zone to be analysed is represented in the "Overview" page. The size and position of the zone can be adjusted with the mouse.

Saving a study

While the measurements are being taken, it is recommended to save the measured values on a regular basis. The "Save study" item in the "File" menu is used for that purpose.

The first time this item displays the standard Windows dialog box in which you should indicate a directory and a file name to identify the study (the suffix ".h1b" is automatically added). This

name then appears in the Histolab toolbar.

The same file is used for the following recordings unless it is changed explicitly with the "Save study as..." item.

Saved templates and files

It may be useful to define study templates that conserve in particular the definition of the structures and the settings for the detection thereof. In order to do this, the information prepared in a file is saved using the "Save study" item.

In order to make use of this template:

- Open the file with the "Open study..." item ("File" menu): this restores the defined structures.
- Enter the characteristics specific to the new study with the corresponding item in the "Measure" menu.
- Define a new file with the "Save study as..." item in the "File" menu.

Note: the template files can be protected from any accidental changes: from within the Windows Explorer, display the properties of the file, and check the "Read only" box.

4. The slide analysis

The characteristics of the study have been defined, the system calibration has been checked, the image of the section(s) is correct on the screen: everything is ready for the measurements. They are taken field by field, and then, in principle, structure by structure.

Pre-viewing the slide with a motorized stage

If the system is equipped with a motorized displacement stage, it is possible to pre-view the slide before the actual analysis in order to locate the areas of interest in the overview.

In order to do this,

- Choose an objective with weak magnification on the microscope and select the corresponding calibration in the list of calibrations given in the "Measurement" menu, and then
- Position the scanning area around the tissue section to be analysed (by moving and redimensioning its representation in the "Overview" page), and
- Select the "Acquire preview images" item in the "Measure" menu. The system then produces an image of the entire scanning area.
- Return to the desired objective for the analysis and select the corresponding calibration in the list.

Plotting a reference area

If, when defining the study, a structure has been defined as the reference area, it is generally

plotted before any other measurement. Select this structure in the local menu in the toolbar (or in the list of "Structures" in the "Measure" menu).

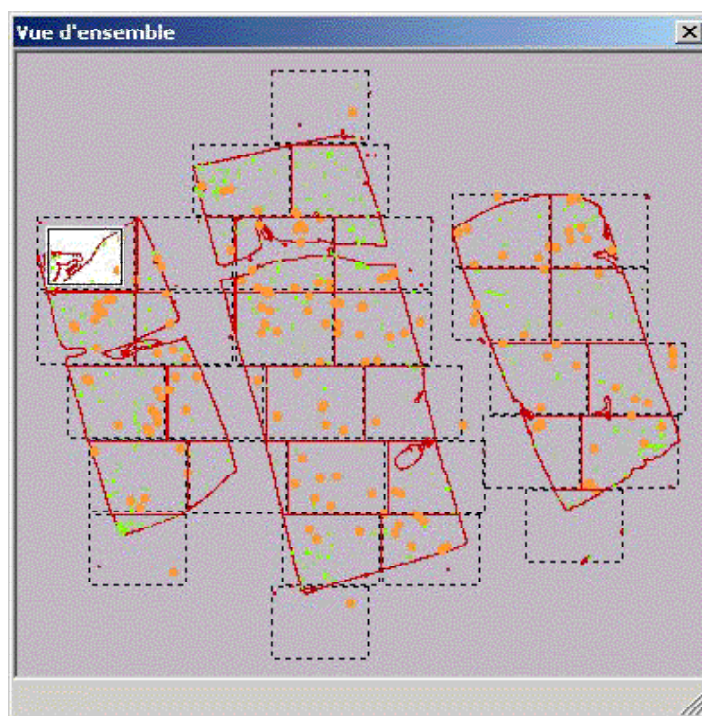


Once this is done, select the free plotting tool by pressing the corresponding button in the toolbar (or by selecting the "Outlines" item in the list of "Tools" in the "Measure" menu).

Then scan the sample and point at as many points as necessary in order to describe the section edge with the desired precision. However, the greater the number of points in the plotted profile, the longer the analysis of each field will take, as an inclusion test for each measurement within the profile must be carried out.

Repositioning a sample

In order to replace a sample in a reference area, display the overview (select the "Overview" page or display the palette by pressing the <F12> key or by using the "Results" menu).



Overview

Locate a detail that is characteristic of the profile, move the section until the detail is situated in the video field (i.e. until the white rectangle representing the video field in the overview and the detail in question are superimposed), then close the overview (<F12>) again, display the "Video"

page and



press this button or select the "Disengage stage" item in the "Measure" menu) in order to stop controlling the profiles at the position of the microscope stage; then move the stage until the located detail is superimposed on the corresponding tissue. Press this button again to return to automatic position control.

Selecting the structure

There are three methods for selecting the structure:

- In the "Measure" menu, the "Structures" item opens a sub-menu containing the names of the defined structures: you just have to select the required structure which is then checked.
- The name of the structure currently selected is displayed in the toolbar. This is a pull-down menu: clicking on it displays the other structures.
- During the measurements, there is a quick means of switching from one structure to the next: the "Next structure" item in the "Measure" menu, and in particular the corresponding keyboard shortcut, the <F4> key.

A colour, used for plotting the readings, is associated to each structure. The colour appears in particular in the toolbar and in the pull-down menu. In order to change the colour of a structure, you should use the "Drawings colors" item in the "Layout" menu.

Using the measurement tools

Manual or automatic measurement tools are provided depending on the type of structures chosen. The manual tools can be used directly whereas the automatic tools must be at least set at the start of a section analysis:

- The "Tools" item in the "Measure" menu opens a sub-menu that contains the tools list. The selected tool is checked.
- If the toolbar is displayed, each tool is represented by a button. The button corresponding to the selected tool appears depressed (sometimes, none of the buttons may be depressed at the start of a study). Most of the tools are used with the mouse by carried out a sequence of actions of the type <press>, <move>, <release>; each action is used for reading a particular point either in the selected area in the video image or in the plotted figure. The mouse buttons can be used indifferently.

No special keys on the keyboard are used, with one exception: the <Shift> + <F5> keys for automatic analysis of the entire video field and the <F5> key for automatically detecting a structure in the entire video field.

The uses of each tool are presented below. There is also a reminder of the measurements taken by each tool.



Double butterfly net: automatic measurement of the video field

- Measurements taken: coordinates, length, width, equivalent diameter, perimeter, surface.
- Principle: successively applies the defining settings for each of the automatically detected structures to the video field.



Butterfly net (shortcut: <F5>), automatic measurement of the selected structure in the video field

- Measurements taken: coordinates, length, equivalent diameter, perimeter, surface.
- Principle: applies the settings defined in the "Detection settings..." box for the selected structure to the video field.



Wizard: automatic measurement of an area in the video field

- Measurements taken: coordinates, length, width, equivalent diameter, perimeter, surface, light intensity.
- Operation: click on both extremities of an area of interest. This area is then automatically analysed according to the defined settings.



Free outlines

- Measurements taken: coordinates, length, width, equivalent diameter, perimeter, surface.
- Profile: click then move the mouse as many times as necessary in order to describe the form to be measured. Double-click to close the contour again and thus indicate the end of the measurement. Histolab smooths the outline.



Counter

- Measurements taken: coordinates.
- Profile: click on the location of the point to be marked.



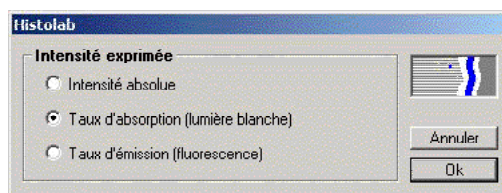
Thickness


- Measurements taken: coordinates, length.


- Profile: click on both extremities of the segment to be plotted.

Measuring the intensity

An additional measurement is taken for "object" structures: the light intensity of each of the detected or plotted objects. It is expressed in different ways depending on the selection made in the box that is displayed via the "Displayed intensity expressed..." item in the "Results" menu:



- the absolute intensity is a value between 0 and 255 corresponding to the mean value of the grey levels in an object.
- For an image from a sample highlighted in white light (transmission lighting), it can be more interesting to express the light absorption rate for each of the detected objects (for example for analysing the intensity BRDU marking on a histological section). Before taking the measurements, in this case, you must provide the system with a light reference corresponding to 100% of transmitted light (i.e. an absorption rate of 0%). In order to do that,
 -  press this button (or select the "Measure background intensity" item in the "Measure" menu) and click in an area of the image where all the light is transmitted.
- In fluorescence, the light source is used for stimulating fluorescent markers in specific wavelengths. It is then the emission rate of each of the fluorescent objects in comparison with the (dark) background intensity that is characteristic of the marking intensity. Before taking the measurements, in this case, you must provide the system with a light reference corresponding to 0% of emitted light. In order to do that,

-  press this button (or select the "Measure background intensity" item in the "Measure" menu) and click in an area of the image where nothing fluoresces.

Checking the measurements

The last values measured can be displayed in a small window, superimposed onto the work sheet ("Measurement results..." item in the "Results" menu).

| Champ 10 | Noyau | |
|-----------|--------|-----------------|
| Abscisse | 5,76 | mm |
| Ordonnée | -1,79 | mm |
| Longueur | 0,118 | mm |
| Largeur | 0,091 | mm |
| Diamètre | 0,094 | mm |
| Périmètre | 0,48 | mm |
| Surface | 0,0069 | mm ² |
| Emission | 86 | % |

Results expressed

Correcting the errors

In the case of an error during plotting or automatic measuring, there are several solutions:

- The last profile (or the last measurement) can be cancelled with the corresponding function in the "Edit" menu.



This tool allows you to select a profile... The "Delete" item in the "Edit" menu deletes the selected profile. If the profile contains handles, it is possible to correct it by clicking on one of the handles and, with the mouse button depressed, bringing it to the desired location.

- A measurement of the section that is being analysed can be deleted by removing the corresponding line from the results table.

It is recommended to save the current study on a regular basis. In the event of any wrong manipulations that are not seen at once and that can not be cancelled afterwards, re-opening the file with the corresponding item in the "File" menu still remains possible.

Validating a measured field



Press this button (or select the "Next field" item in the "Measure" menu) to validate the measurements already taken and to analyse a new field. Then move the section on its base until the field to be analysed is fully displayed in the video image.



This button allows you to return to a previously measured field.

Note: these buttons are invalidated when the microscope is equipped with an indexed displacement stage connected to the computer: you just have to move the stage to measure a new field or to return to a field already measured. When it is equipped with a motorized displacement stage, these buttons allow you to move from one field to another.

Automatic sample measuring with a motorized stage



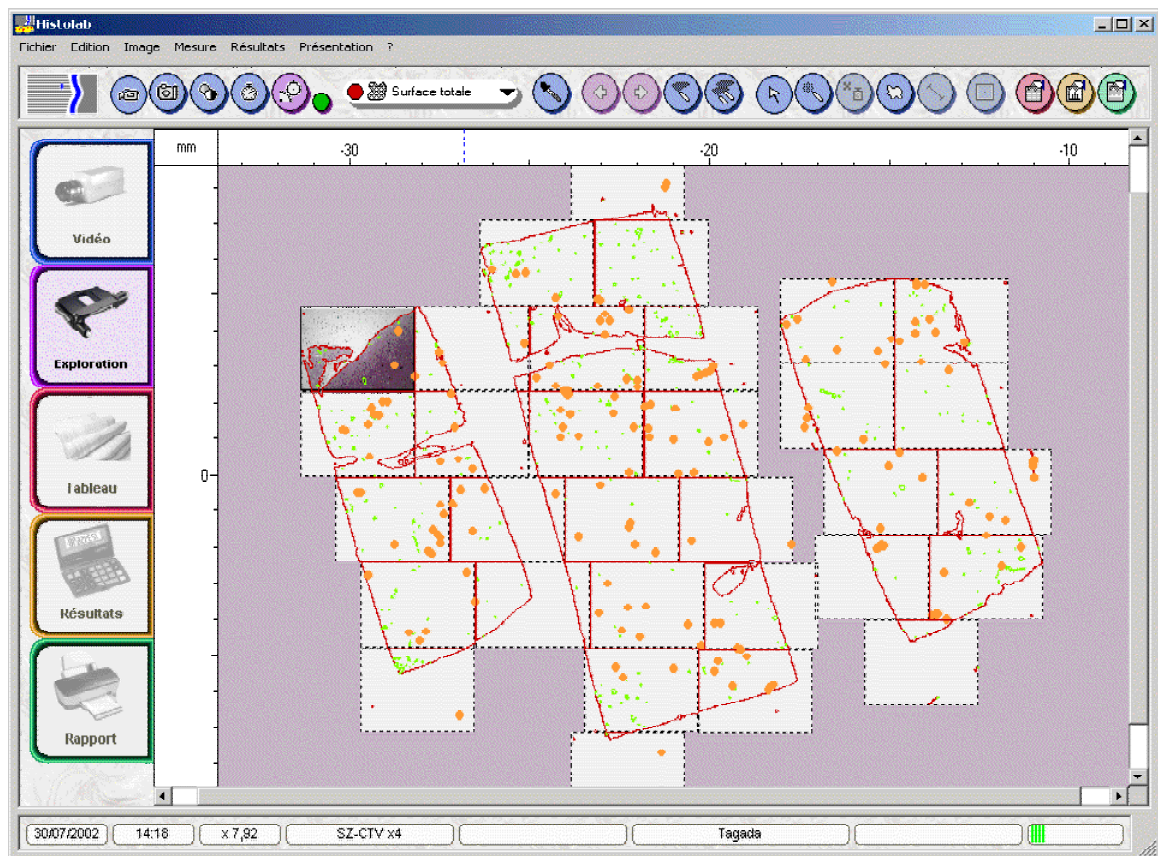
Move the sample to the upper left corner of the rectangle to be analysed (or the summit of the circle) and press this button: it defines the position of the first scanning field.



Click on this button: automatic scanning will begin.

5. Overview of the section

The overview is displayed when you select the "Overview" tab.



The overview presents a background history of the analysed fields.

The profiles corresponding to the measurements taken in each field for all of the defined structures are represented in a scaled-down view (the overview magnification is displayed in the status bar at the bottom of the window).

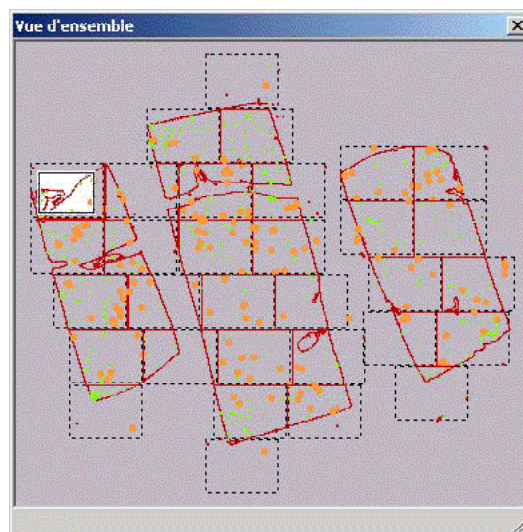
The images of the fields are also displayed if the "Keep measured images" option was activated when the study was started.

When the system is equipped with a stage that transmits its position to the microcomputer (indexed or motorized stage), the background history is replaced by a mapping of the section.

If the stage is motorized, the preview, if acquired, is displayed here with the scanning area. The latter can be moved and deformed with the mouse so that it can be adjusted to the area of interest on the tissue section.

The bordered field is the "active" field, the data of which is displayed in the "Video" page. In order to change the active field, use the "Previous field" and "Next field" options in the "Measure" menu, or click on the desired field while pressing simultaneously on the <Shift> and <Ctrl> keys on the keyboard.

The overview can be displayed in a separate window and thus remain visible even when you are working on the other pages of Histolab: for that, you use the "Display in window" option in the "Results" menu or the corresponding keyboard shortcut, the <F12> key.



Display of the overview in a palette

Exportation

- Transformation of the overview into a single image: activate the "Frame" tool in the "Edit" menu, and then frame the required zone. The "Copy" option allows you to transfer the framed part to another software application.
- Saving the measured images individually: in the "File" menu, use the "Export results" option then the "Measured images" sub-menu.

6. Measurements table

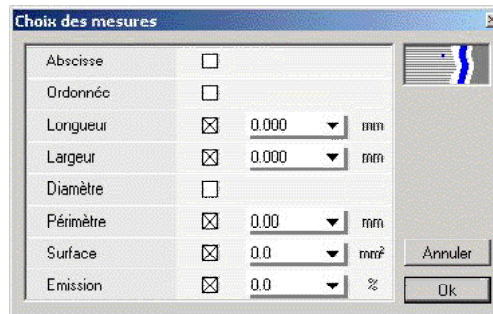
While the fields are being analysed, Histolab creates a table that groups together the values measured. Each object occupies a line and is described by the analysis field number, the name of the structure to which it belongs and by the result of the measurements taken.

The table is displayed by selecting the corresponding tab.

| Champ | Structure | Objet | Longueur (µm) | Largeur (µm) | Surface (µm ²) | Emission (%) |
|-------|-----------|-------|---------------|--------------|----------------------------|--------------|
| 20 | Objets | 410 | 59,3 | 47,2 | 1900,1 | 01,01 |
| 20 | Objets | 410 | 21,4 | 19,7 | 313,0 | 00,00 |
| 20 | Objets | 417 | 24,0 | 20,0 | 300,1 | 79,00 |
| 20 | Objets | 410 | 11,4 | 11,4 | 07,2 | 04,00 |
| 20 | Objets | 410 | 47,3 | 43,0 | 1909,0 | 00,00 |
| 20 | Objets | 420 | 43,3 | 24,3 | 013,4 | 04,10 |
| 20 | Objets | 421 | 27,3 | 20,4 | 410,4 | 01,70 |
| 20 | Objets | 422 | 17,0 | 17,0 | 220,0 | 00,40 |
| 20 | Objets | 420 | 24,0 | 20,0 | 300,1 | 70,32 |
| 20 | Objets | 424 | 41,0 | 22,3 | 079,9 | 73,10 |
| 20 | Objets | 420 | 40,4 | 35,0 | 1272,7 | 00,11 |
| 20 | Objets | 420 | 17,0 | 17,0 | 220,0 | 04,10 |
| 20 | Objets | 427 | 24,0 | 17,7 | 313,0 | 02,31 |
| 20 | Objets | 420 | 109,3 | 103,7 | 13249,0 | 01,32 |
| 20 | Objets | 420 | 37,0 | 27,7 | 704,0 | 70,42 |
| 20 | Objets | 430 | 30,0 | 23,0 | 992,7 | 01,35 |
| 20 | Objets | 431 | 20,3 | 19,0 | 300,0 | 00,42 |
| 20 | Objets | 432 | 30,4 | 23,2 | 902,0 | 01,40 |
| 27 | Objets | 430 | 70,0 | 30,0 | 1901,0 | 00,39 |
| 27 | Objets | 434 | 72,1 | 35,2 | 1429,0 | 00,00 |
| 27 | Objets | 430 | 70,0 | 01,2 | 2009,1 | 01,09 |
| 27 | Objets | 436 | 33,8 | 28,6 | 732,2 | 83,70 |
| 27 | Objets | 437 | 33,5 | 26,8 | 679,9 | 81,56 |
| 27 | Objets | 438 | 58,8 | 32,3 | 1429,6 | 79,30 |
| 28 | Objets | 439 | 49,4 | 31,7 | 1202,9 | 82,55 |
| 28 | Objets | 440 | 12,5 | 4,2 | 34,9 | 86,49 |
| 28 | Objets | 441 | 154,6 | 66,2 | 7479,0 | 81,17 |
| 28 | Objets | 442 | 27,5 | 23,3 | 470,7 | 76,41 |
| 28 | Objets | 443 | 4,2 | 4,2 | 17,4 | 84,31 |
| 28 | Objets | 444 | 84,5 | 27,3 | 1481,9 | 82,47 |
| 28 | Objets | 445 | 17,8 | 17,8 | 226,6 | 81,27 |
| 28 | Objets | 446 | 142,5 | 142,4 | 17700,1 | 93,72 |



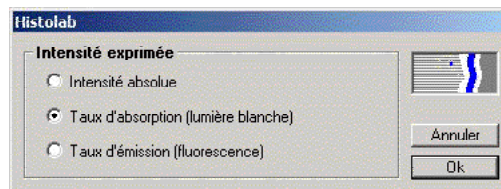
The displayed measurements can be selected by pressing this button or by using the corresponding item in the "Results "menu.



Selection of the displayed measurements

It is also in this window that you can choose the precision of a measurement by clicking on the corresponding pull-down menu.

In order to choose the intensity expressed, display the following window via the "Measure" menu:



Choose:

- "Absolute intensity" to display the mean value of the grey levels in an object.
- "Absorption rate" to analyse the intensity of marking on a histological section with transmission lighting (white light).
- "Emission rate" to analyse the intensity of marking on a fluorescent histological section.

Exportation

Exporting the results consists in formatting the results obtained by Histolab so that they can be used by external software applications (in general, spreadsheet programs). Two techniques are available:

- via the clipboard: select the block of cells. When it is highlighted, select the "Copy" option in the "Edit" menu. Activate the destination software application and paste in the desired position.

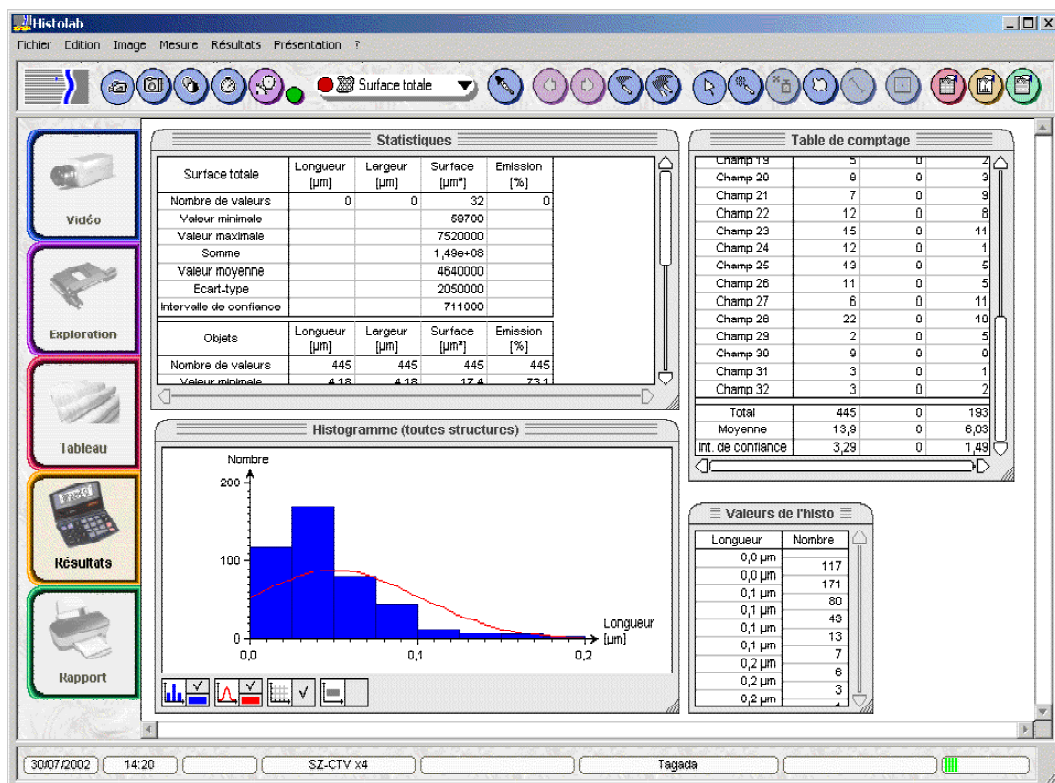
To select entire lines, click and drag in the legend columns. To select entire columns, click and move in the heading lines. To select the entire table, click in the top left corner of the table.

- via a "text" format file: in the "File" menu, use the "Export results" sub-menu then the "Measurements..." item.

IMPORTANT NOTE: Exportation must not be confused with saving a study. A study file contains the measurements and their environment but can only be read by Histolab. An exported file only contains the value but can be read by other software applications. It is always preferable to save the study, as you can then extract the values in order to export them, whereas the reverse is not possible.

7. Statistics and Graphs

Besides the measurements table presented above, Histolab also provides four other documents in the "Results" page: the statistics table, the counting table, a "bar" histogram and the numerical values associated with the graph.



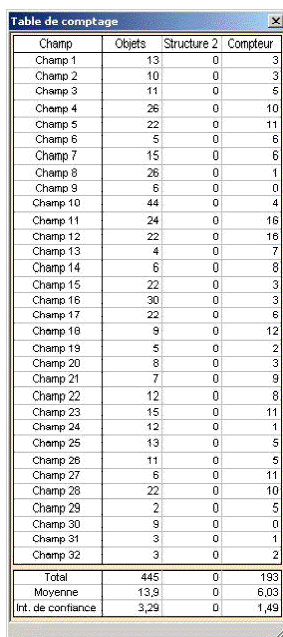
The statistics

The statistics table contains the measurements taken for each structure. It may remain visible at all times if the "Statistics" item of the "Display in window" sub-menu in the "Results" menu is checked.

The counting table

The counting table displays field by field the results of the counts for each structure during a study. Statistics for these counts are displayed at the end of the table. This table is updated during the analysis of a tissue section.

It may also be displayed at all times if the "Statistics" item of the "Display in window" sub-menu in the "Results" menu is checked:

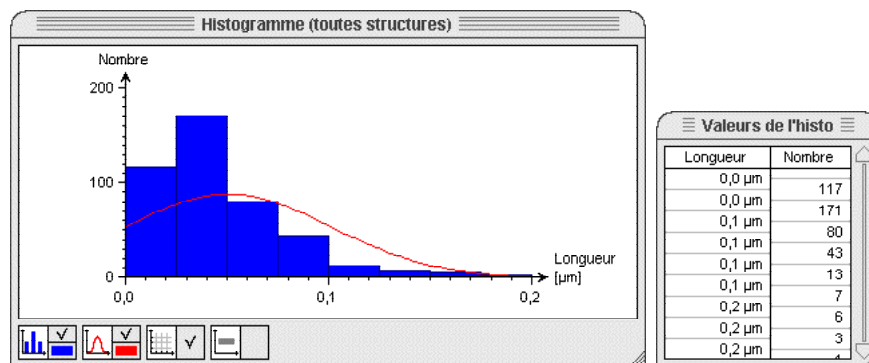


| Champ | Objets | Structure 2 | Compteur |
|-------------------|--------|-------------|----------|
| Champ 1 | 13 | 0 | 3 |
| Champ 2 | 10 | 0 | 3 |
| Champ 3 | 11 | 0 | 5 |
| Champ 4 | 26 | 0 | 10 |
| Champ 5 | 22 | 0 | 11 |
| Champ 6 | 5 | 0 | 6 |
| Champ 7 | 15 | 0 | 6 |
| Champ 8 | 26 | 0 | 1 |
| Champ 9 | 6 | 0 | 0 |
| Champ 10 | 44 | 0 | 4 |
| Champ 11 | 24 | 0 | 16 |
| Champ 12 | 22 | 0 | 16 |
| Champ 13 | 4 | 0 | 7 |
| Champ 14 | 6 | 0 | 8 |
| Champ 15 | 22 | 0 | 3 |
| Champ 16 | 30 | 0 | 3 |
| Champ 17 | 22 | 0 | 6 |
| Champ 18 | 9 | 0 | 12 |
| Champ 19 | 5 | 0 | 2 |
| Champ 20 | 8 | 0 | 3 |
| Champ 21 | 7 | 0 | 9 |
| Champ 22 | 12 | 0 | 8 |
| Champ 23 | 15 | 0 | 11 |
| Champ 24 | 12 | 0 | 1 |
| Champ 25 | 13 | 0 | 5 |
| Champ 26 | 11 | 0 | 5 |
| Champ 27 | 6 | 0 | 11 |
| Champ 28 | 22 | 0 | 10 |
| Champ 29 | 2 | 0 | 5 |
| Champ 30 | 9 | 0 | 0 |
| Champ 31 | 3 | 0 | 1 |
| Champ 32 | 3 | 0 | 2 |
| Total | 445 | 0 | 193 |
| Moyenne | 13,9 | 0 | 6,03 |
| Int. de confiance | 3,29 | 0 | 1,49 |

The histogram of a measurement

A particular measurement (length, width, diameter, perimeter, surface or intensity) can be

analysed individually with a histogram; you must define size classes for the considered measurement and choose which structure falls within the scope of the histogram. The histogram is presented in the form of a "bar" graph; the number of objects in each class can also be displayed.



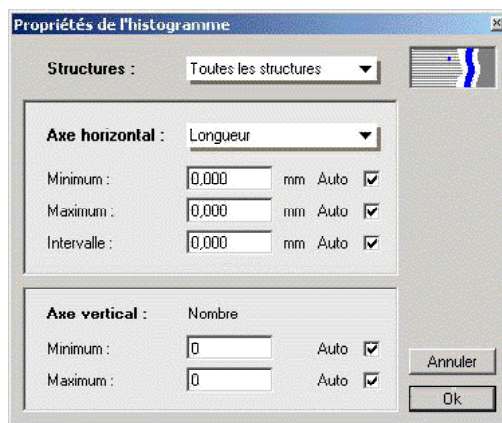
Histogram of the lengths of all structures combined, and the corresponding values

It is possible to display the normal law corresponding to the distribution of the measurement in the defined classes by checking the corresponding option in the lower part of this window.

Other plotting options (colour of the bars and the normal law, graph gridline, curve thickness) can also be chosen under the histogram.



The choice of the structure, that of the measurement as well as limits of size classes are defined by clicking on this button (or via the "Histogram settings" button in the "Measure" menu). The following dialog box appears:



By checking the "Auto" boxes, it is possible to allow Histolab to adjust each of the parameters as best it can.

Exportation

- to presentation software via the clipboard: click on the statistics title bar on the work sheet. When the frame is highlighted, select the "Copy" option from the "Edit" menu. Activate the destination software application and paste in the desired position.
- to a spreadsheet program via a "text" format file: in the "File" menu, use the "Export results" sub-menu, then the "counting..." or "statistics..." item.

The page layout of this document is entirely customisable: the size and contents of the header and footer, the order and title of the printed documents. You access it by

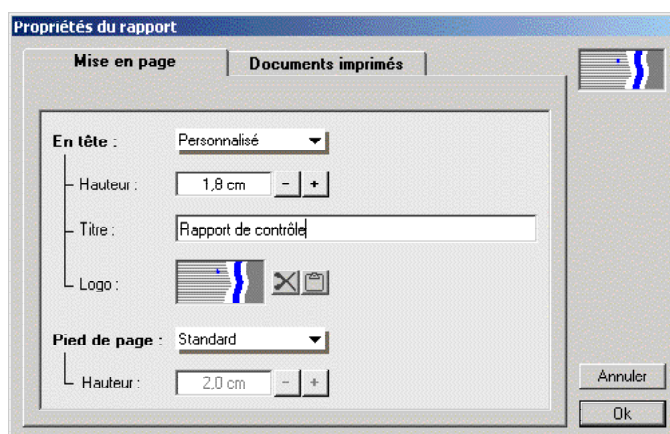


pressing this button or via the "Report settings..." item in the "Results" menu.

The first tab proposes you three types of headers:

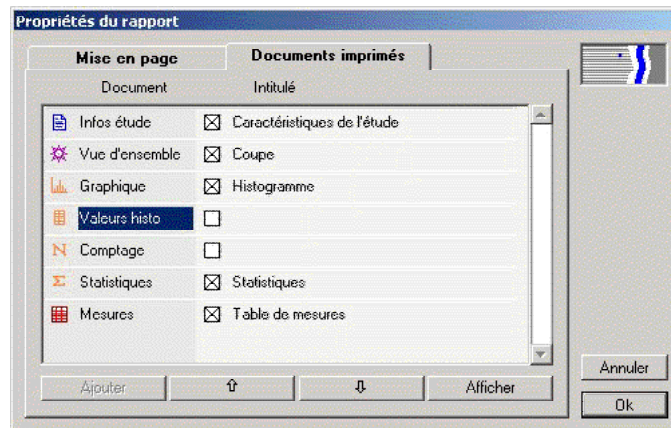
- a standard header not requiring to be set, which automatically includes the name of the software, followed by the study reference.
- a customisable header, for which you can define the height, the title and a logo that you will have already placed in the clipboard.
- a blank header for printing the report onto pre-printed paper. You then only set the height of the space to be left blank.

The footer may include the software reference and page numbering or a given height may be left blank for use with pre-printed paper.



Definition of the header and footer

The second tab is used for defining the contents of the report:



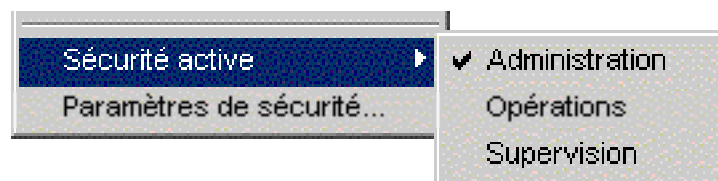
The list shown allows you to choose the documents to be included in the report and to determine the order and title.

The actual print-out is launched via the "Print report" item in the "File" menu.

9. Secure operating modes

For the use of Histolab on slides with very constant shades, it may be interesting to freeze the settings to prevent any accidental changes. Histolab provides the following three operating modes:

- The "Administration" mode is the normal working mode in which all software functions can be accessed.
- The "Operations" mode is a secure mode in which all routine operations are possible. However, the settings are no longer accessible: they remain identical to those made in the "Administration" mode.
- the "Supervision" is a secure mode for which the conditions of access to the software's functions are defined by a distinct software component, specifically developed for certain companies.



The operating modes shown in the "?" menu

The "Security settings..." item allows the administrator to define the software operating mode and to protect access to the "Administration" mode with a password.