Piximeter

Measuring dimensions on images

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www.Piximetre.fr

No measuring instrument is perfect.

It is important to know the limits and assess the margin of error.

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Introduction

Piximeter is metrology software whose primary purpose is to establish the characteristic dimensions of a population of objects from a representative sample. It uses the laws of statistics and probability.

It applies to many areas where precise and easy measurement of dimensions is necessary. From measuring bacteria to measuring galaxies! It applies in particular to mycology which is the domain of its authors.

Piximeter produces a **dimensional formula1** which allows significant progress in the search for whether an object belongs to a target population.

In mycology for example, this formula makes it possible to better answer the question: "are the dimensions of the spores of this mushroom compatible with those of a particular species considered?".

Piximeter has many features, ergonomics and reliability that allow it to be precise and efficient. Its characteristics can be presented along several major axes:

Ensure direct measurement of real dimensions of objects on their images :

o Management of images of very large sizes and various formats (jpeg, bmp, tif, etc.),
o Measurements by tracing calibrated axes on images,
o Grouping of measurements carried out on several images, o
Tracking of axis tracing, o
Automatic detection of axis coupling (length, width), o Modification
(addition, deletion) of axis couplings, o Selective or global
deletion of traced axes, o Permanent calibration
applied to any new image or, failing that,
specific to each image, o
Simultaneous use of several length standards, o Automatic
compensation of the focal length of the cameras
used for shooting,
o Direct connection to a USB camera to acquire images in LiveView,
o Graphical storage of measurements, with backup and restoration, o Display
of metadata included in the images, o Powerful and easy
zoom in and out, and movement on images,
o Automatic management of the measurement scale,
o etc.

[1] In mycology we use the term *sporal formula.*

Visualize and compare the *spatial distribution* of series of measurements:

o Representation of the series of measurements in colors, o Plot of the orientation axis of each series, o Plot of the trend ellipse, o Easy magnifying glass, allowing you to make zooms in and out, o Moving the center of gravity of the series, o Hiding and deleting series, o Possible comparison of four series simultaneously, o Display of the histogram of measurements, o Export of graphs by simple "copy and paste", o etc.

Sequence the measured objects:

o Individual extraction of measured objects,
o Automatic production of representative sequences, in the form pictures,
o Scale normalization,
o Ranking according to different criteria,
o Rotation and positioning,
o Automatic decoration of the images produced (scale, measurements, author signature, comments, etc.),
o etc.

Export decorated images

o Addition of axis plots and corresponding measurements,
o Addition of the measurement scale,
o Definition and addition of author signature,
o Addition of the dimensional formula,
o Adding personalized comments,
o Adjustment of fonts, shading and positions,
o Choice of the size of the exported images and the compression rate,
o etc.

Mix data sources, o Import measurement files by simple drag and drop, o Manual entry of numerical values, o Automatic conversion of measurements into units of length (e.g.: microns), o "Copy and paste" of texts and graphics to external documents, o Saving measurements, o etc.

Presentation

Piximeter is general-purpose metrology software. Its field is that of measuring the spatial dimensions (length, width and height or thickness) of any objects. It uses the laws of statistics and probability to calculate the characteristics of an entire population from a sample.

Its features are as follows:

1. **Collect** series of measurements (samples) representing the dimensions of various objects,
2. **Express** as accurately as possible the *dimensional formula* which reflects the characteristics of the entire population, 3. **Visualize** and **compare** several series of measurements, 4. **Construct** and **export** images enriched with the measurements taken, the length scale, author signature, comments, etc.,
5. **Extract** in the form of images, sequences of objects ordered according to various criteria,
6. **Memorize** the measurements made and the user's choices.

Piximeter offers two distinct operating modes to collect series of measures :

1. In **Digital mode** the user manually enters the **numerical values** of the measurements he has previously carried out on the objects. They are expressed in the unit of the measuring device used (for example the divisions of a graduated ruler). These measurements are converted into the unit of length by *Piximeter,* based on a coefficient provided by the user (typically, the value of each division of the ruler). This mode constitutes a sort of **super calculator.**

2. In **Graphics mode** the user works on **digital images** which represent the objects to be measured. Using the mouse, he draws axes directly on it which correspond to the length and width or thickness of these objects. The measurements of these axes are converted into units of length thanks to a prior calibration. This is **the mode of operation par excellence.**

Regardless of the operating mode chosen, the user has the possibility of:

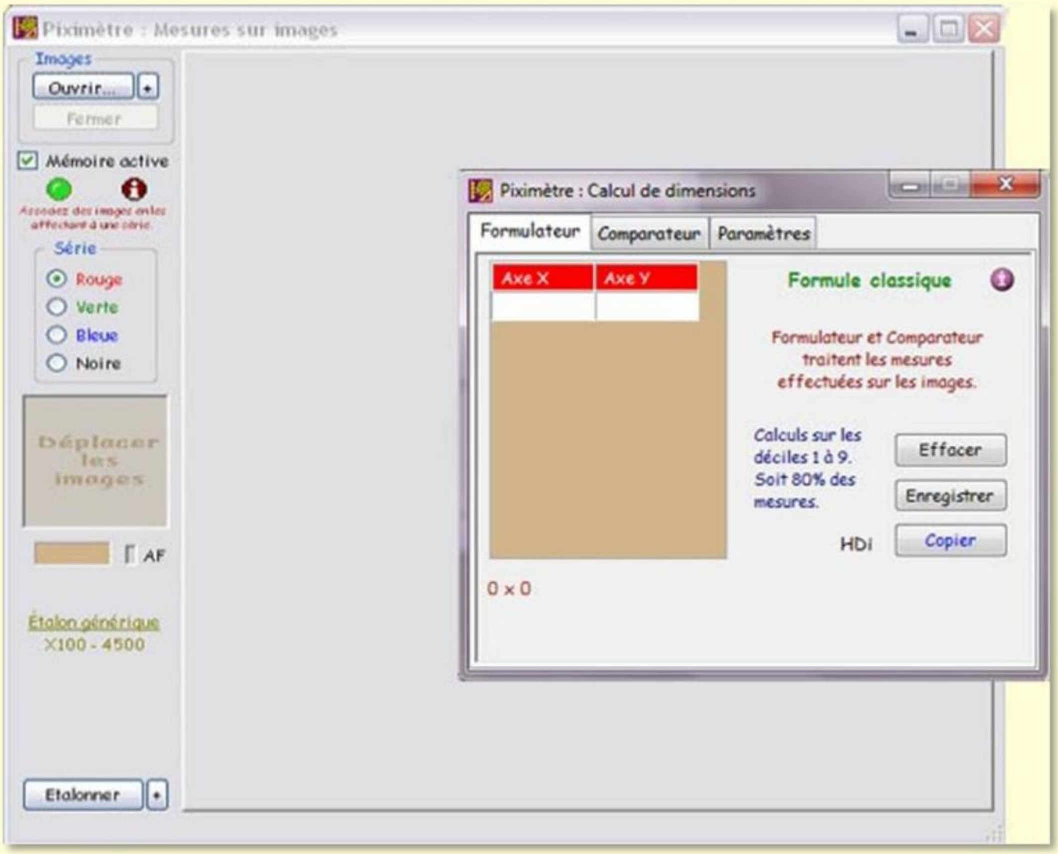
• **Adjust the** Piximeter operating parameters , • **Save the measurements** in files, from where they can be resumed subsequently,
• **Copy the dimensional formula and its accessories** *(Comparator* and *histogram)* to the clipboard for import into personal documents (Word, HTML, XML, etc.).

Piximeter 's automation is advanced, which **optimizes** repetitive tasks. In particular, all parameters, such as digital conversion coefficients, length standards, zoom characteristics of the cameras used for shooting, the size and position of each window, etc., are stored **in memory.** *Piximeter* reopens next time in the state it was closed.

All these characteristics and other operations are detailed below. For now let's see the presentation of the software.

Graphics Mode Overview

When you first start *Piximeter* it opens in **Graph mode,** which has two windows, as shown in the following figure.



Initial view of Piximeter

The smallest, which is placed above, is the main window, also called " Calculator ". It has three tabs:

- Formulator,
- Comparator,
- Parameters.

The second window, the largest, placed below and called " Measer ", is only present in **Graphics mode.** It allows you to work with images representing the objects to be measured and to perform all operations relating to their measurement.

The data processed by this window are automatically transmitted to the *Calculator.*

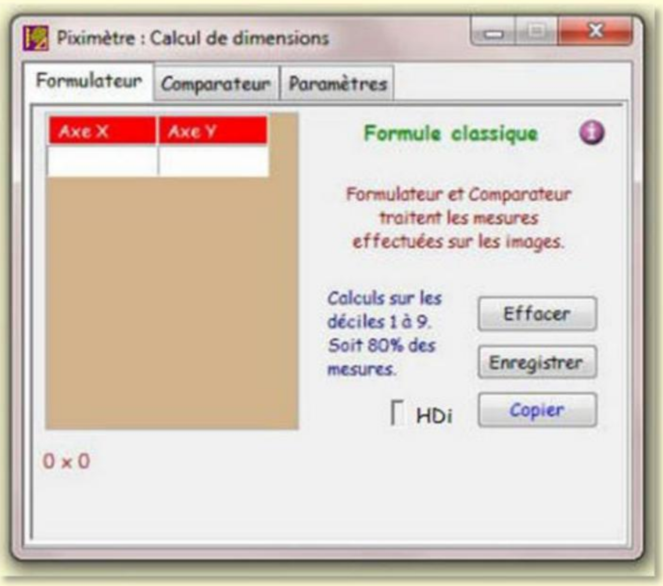
The *Piximeter Formulator* calculates the ***dimensional formula*** (in the case of mushroom spores we use the term *spore formula)* in one or two dimensions of very diverse objects such as mushroom spores or even galaxies. The calculation is carried out on the basis of the numerical or statistical analysis of a representative sample of the population studied.

The main features of the *Calculator* are as follows:

- It is independent of the nature of the objects measured and therefore **independent of units** used,
- It **directly** displays the measurement result in the form of a *formula dimensional,*
- It allows you to graphically visualize the **spatial distribution** of measurements and their **statistical interpretation,**
- The *dimensional formula* as well as the *various available graphics* can be copied into electronic documents by simple **"copy and paste"** via the clipboard.

The two operating modes of *Piximeter* lead to two different presentations of the *Formulator.*

On the ***Formulator*** in Graphics mode, which is selected at the start, we first distinguish the ***input grid*** with its two columns: ***X Axis*** and Y ***Axis .*** It is intended to receive the pairs of measurements (length, width).



These measurements come either from plots made on the images in the *Measurer,* or from files dragged and dropped onto the grid, or from manual entries directly into the cells of the grid.

The calculated ***dimensional formula*** appears below ("0 x 0" when the grid is empty). It is formed automatically, step by step, depending on the introduction of data in the grid.

This *dimensional formula* can take two forms: **classic** or **statistical** which are explained below.

The **Clear** button resets the input grid and formula. The **Save** button allows you to save the contents of the grid and the *dimensional formula* in a text file while the **Copy** button places them in the clipboard to allow them to **be pasted** into a personal document of the user.

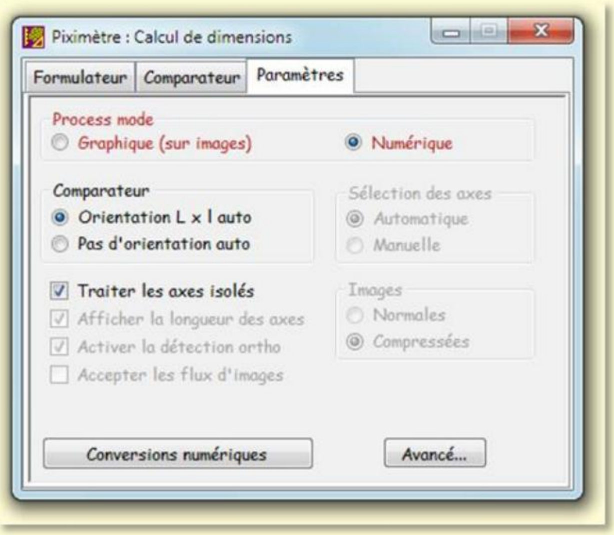
The pictogram provides access to the **pop-up tutorial.**

The **HDi** indicator , which can take on several colors, provides access to the measurement **histograms .** Pressing the mouse on HDi alternately displays or hides them. The histograms are explained here.

Digital mode overview

The choice of operating mode is made on the ***"Parameters"*** tab as shown in the following figure.

In this mode the user no longer works on images. The ***Measurer*** window is closed, only the ***Calculator* window is displayed.**



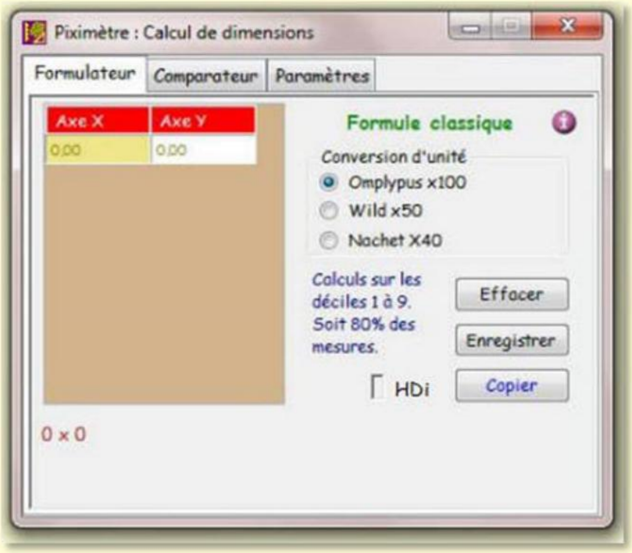
Data are entered manually directly into the *Formulator grid.* Three possibilities:

1. The user manually enters the values corresponding to the length and width of the measured objects into the grid. Moving from one cell to the next is done with the **"Tab"** key on the keyboard, not with the **"Enter" key.**
2. The user drags A (or several) measurement file(s) onto the grid,
3. The first two possibilities can be mixed.

Switching to Numeric mode deactivates choices that are no longer relevant and activates the selection of one of the unit conversion coefficients in the *"Parameters"* tab allows you to (re)define via the **"Numerical conversions" button.**

Below is the *Calculator* window in Digital mode. You will notice the choice of a **Unit Conversion** appear on the *Formulator.* Three conversions have been defined here (see below).

As opposed to Graphic mode where the measurements are expressed directly in units of length thanks to a prior calibration (see "measurements on images"), here they are expressed in the unit of the measuring instrument used (for example the **divisions** of 'an ocular micrometer).

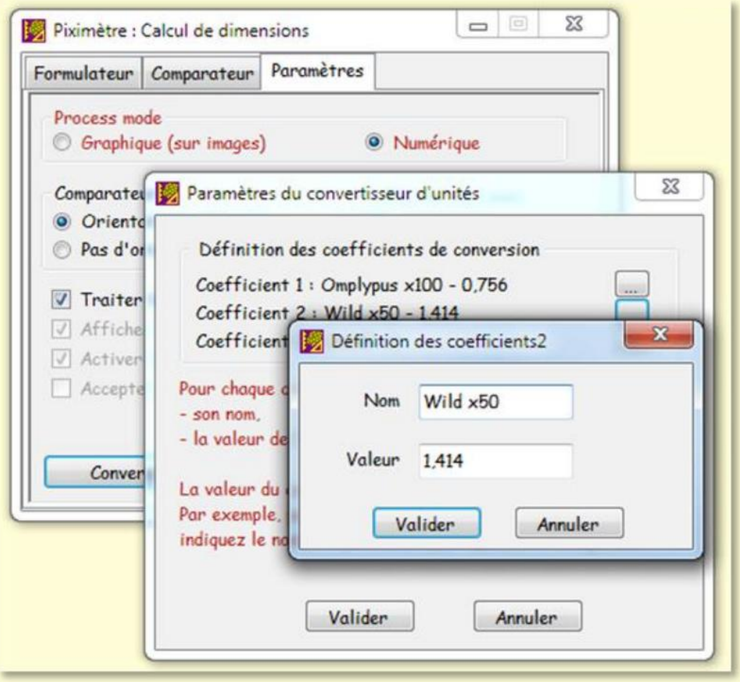


Each of the three configurable conversions corresponds to **a choice of transformation** of this unit into a unit of length. This conversion is **carried out automatically** by *Piximeter* to establish and express the *dimensional formula.*

The user must therefore define **at least one** (three maximum) conversion coefficient by assigning each a specific name and value. Note in the figure that only the name appears in the *Formulator* (example: Olympus x 100).

Set unit conversions in "Numerical" mode

The **"Digital Conversions"** button on the ***Settings*** tab allows you to set the names and values of up to three conversions when Digital mode is selected, as shown in the figure at right.



In the intermediate window entitled **"Unit converter parameters",** the button to the right of each of the three coefficients allows you to define (or redefine) their parameters.

In this example, the coefficient 1 was defined with the name **"Olympus x100"** and the value of **0.756** microns per division.

Coefficient 2 is being defined with the name **"Wild x50"** and the value **1.414** microns per division.

Another example: if the measurement of the dimensions of fungus spores was carried out with an objective x 100 where each division of the ocular micrometer is worth 0.8 microns and that of cystids with an objective x 60 where each division is worth 1.2 microns, the The user would then define two conversion coefficients named **x100** and **x60,** and assign them the values **0.8** and **1.2 respectively.**

Currently the digital converter does not allow you to define a **unit of length** for the coefficients.

Graphic or Digital?

Graphics mode offers the "comfort" of working on the computer screen to carry out measurements on objects. It also ensures the graphic and digital memory of all the measurements carried out on each of the processed images. **This is the preferred operating mode of *Piximeter.*** Its functionalities are described in detail below.

Recommendations for graphics mode

Do not compress images from the camera in order to preserve the metadata they contain. *Piximeter* can work on very large images without any difficulty.

Depending on your image acquisition equipment:

1. If you use a **camera equipped with a variable focal length lens,** it will *be* necessary **to initialize the Piximeter autofocus** in order to automatically compensate for the magnification of images due to changes in focal length.
   You will thus be able to carry out measurements without changing the standard, whatever the focal length used.

   Then start by **initializing the automatic autofocus** of *Piximeter* corresponding to the camera used and its lens (if you use several you must initialize the autofocus for each of them - *Piximeter* can manage any number), which will allow you to use any focal length indiscriminately when shooting of sight, without any worry about calibrating the measuring tool.

2. If you use **a camera** or **camera without a lens** or with a **fixed lens,** the image magnification is constant; autofocus is not used.

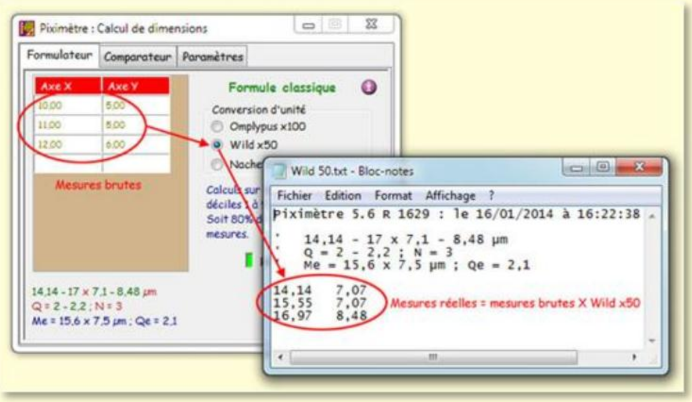
In both cases you must **define at least one permanent** (generic) standard, that is to say applicable to all new images which will be opened by *Piximeter* (which includes one by default, which measures in pixels, but which will generally not correspond not meet your needs). The standards ensure the conversion of the pixels you measure on the screen by tracing the axes on the images, into real dimensions and their unit.

You will no longer have to make **any adjustments** to the *Piximeter,* it retains all the information from one activation to the next and automatically calibrates itself according to the image source used.

You can now work routinely.

Digital mode gives the same numerical and graphical results as its graphical counterpart. It allows you to work outside of photos, with measurements provided by an external measuring instrument such as, for example, a microscope or a telescope. This mode of operation is similar to an **advanced calculator.**

After having defined at least one unit conversion as just indicated above and having selected it in the *Formulator,* simply enter the data manually (read for example through the eyepiece of the microscope or a telescope) in the input grid. *Piximeter* performs the **unit conversion** and the *dimensional formula,* calculated based on this data, is displayed below the input grid.



It is also possible to enter the measurements by **simply dragging and dropping** a text file that contains them. For example one which is built via **"Save" button.** THE

The figure shows the contents of the measurement file thus saved.

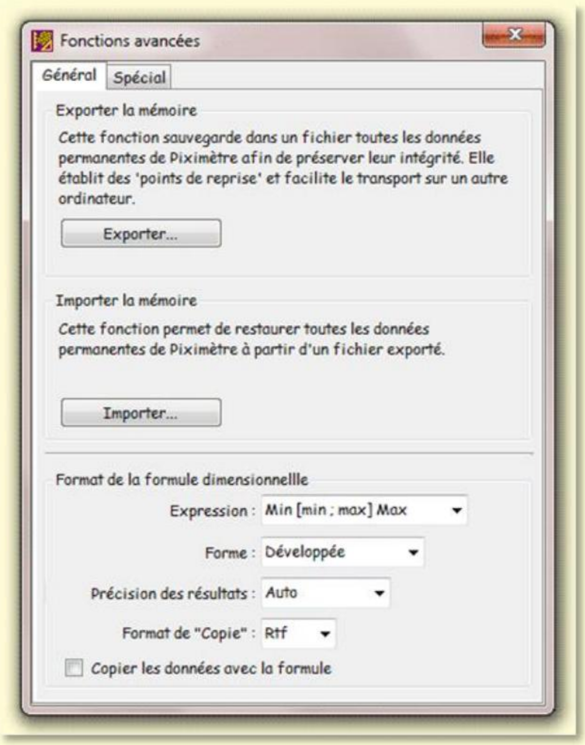
It is important to note that **the recorded values are the actual measurements,** here the raw measurements entered by the user affected by the chosen unit conversion coefficient (here Wild x50).

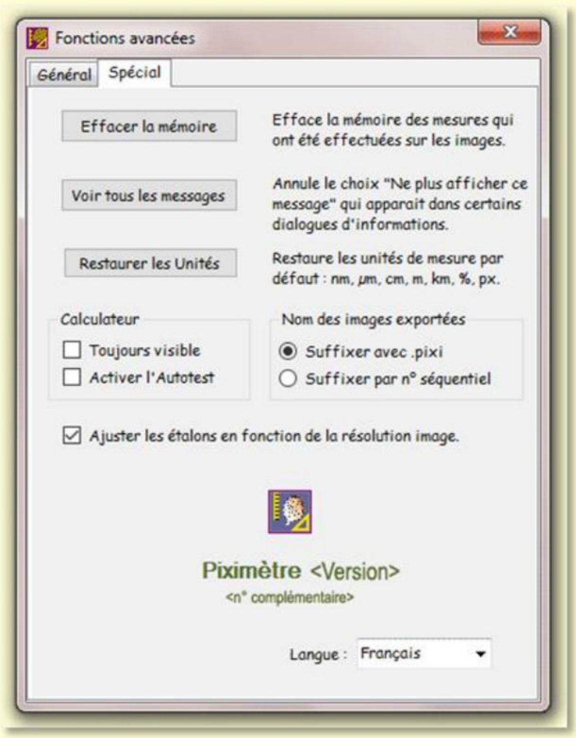
This allows this file to also be used in Graphics mode. The two operating modes are thus homogeneous.

Piximeter does not record the conversion coefficient. When dragging and dropping the file onto the *Formulator grid,* the user must be careful to **select this same coefficient.** Otherwise the measurements will be incorrect.

advanced settings

The **"Advanced..."** button on the ***Settings*** tab gives access to additional settings organized on two tabs, "General" and "Special". Here is a description.





Import-Export of the work context

A first function allows **you to export** to a file all the data managed by *Piximeter* and, in particular, **the generic standards** and **the memory** which contains all the measurements carried out on the images. Symmetrically, a second function allows **you to import** such a file and reload the active memory and other parameters.

This pair of functions ensures the porting of Piximeter **working data** from one computer to another without loss of information .

Along the way, the **change in screen resolution** is managed, which maintains the validity of the data thus transferred and avoids having to redo the calibration. *Piximeter* is then directly operational on your new computer (where it must still be installed to import this data).

These two functions also allow you to carry out **"resume points",** by saving the state of *Piximeter* for example before modifying the calibration, and possibly restoring it afterwards.

To port work data from one computer to another:

1. **Export** to a file of your choice; it will receive the extension **".piximtr",** 2. **Copy** this file to your new computer, 3. **Import** it into *Piximeter.*

Dimensional Formula Formats

This General tab allows, among other things, the user to **change the format of the *dimensional formula.*** Let us repeat here that there is another, quicker way to change the parameters of this formula: *Click on the formula.* A **left click** on the formula displayed by the *Formulator* allows you to switch alternately from the statistical formula to the classic formula. **Right-clicking** allows you to change all display settings described below.

Both formulas are described in detail here. The **"Expression"** list allows you to choose between the **statistical formula** and the **classic formula.** The calculations, and therefore the results and their presentation, are different in the two cases.

1. **Min [min; max] Max** corresponds to the statistical formula.
2. **(Mini) min-max (Maxi)** corresponds to the classic formula. This is the formula **by** Piximeter **defect .**

The **"Form"** list allows you to switch from the expanded form to the concise form of the formula.

The **"Precision of results"** list allows you to choose the number of decimal places displayed (1 or 2) or their **Automatic management.** By default, automatic management is selected.

The **"Info** +" choice controls the integration into the statistical formula of the standard deviations calculated on the series of measurements carried out. This data, which corresponds to a numerical evaluation of the dispersion of the measurements, proves useful in certain cases.

The **"Copy Format"** list allows you to choose the format for saving the formula in the clipboard. By default, this format is the "Rtf" standard.

The **"Copy data with formula"** choice adds **the data from the input grid** in front of the *formula* when copying it to the clipboard ("Copy" button on the *Formulator).* This choice is checked by default.

Special Functions

On the *Special tab,* "Clear memory" allows, exceptionally, to erase all the parameters from the memory, in particular all the axes which have been traced on all the closed images with **active memory** engaged. This operation is not reversible.

The **"See all messages"** button allows you to redisplay messages containing the choice **"Do not show this message again",** which would have been checked.

The **"Restore units"** button allows you to regenerate all the units defined by default (see the list to the right of the button). The calibration function allows you to create, modify and delete units of measurement.

The **"Always Visible"** checkbox forces the *Calculator* to always be displayed above the *Measurer window.*

The **"Enable Self-Test"** box displays a special button on the *Formulator,* which allows the user to generate random series of measurements. These series show different histograms.

The **"Language"** choice allows you to define the working language of *Piximeter.* The choice of language can be made at any time. Data currently being processed is not affected.

The **"Adjust standards based on image resolution"** box allows automatic adaptation of the calibration when image and standard (image on which it was defined) have different X and Y resolutions but **the same ratio.**

This box is checked by default. Learn more.

Measurements on images

The image measurements function allows the **direct calculation of the real dimensions** of objects from their images.

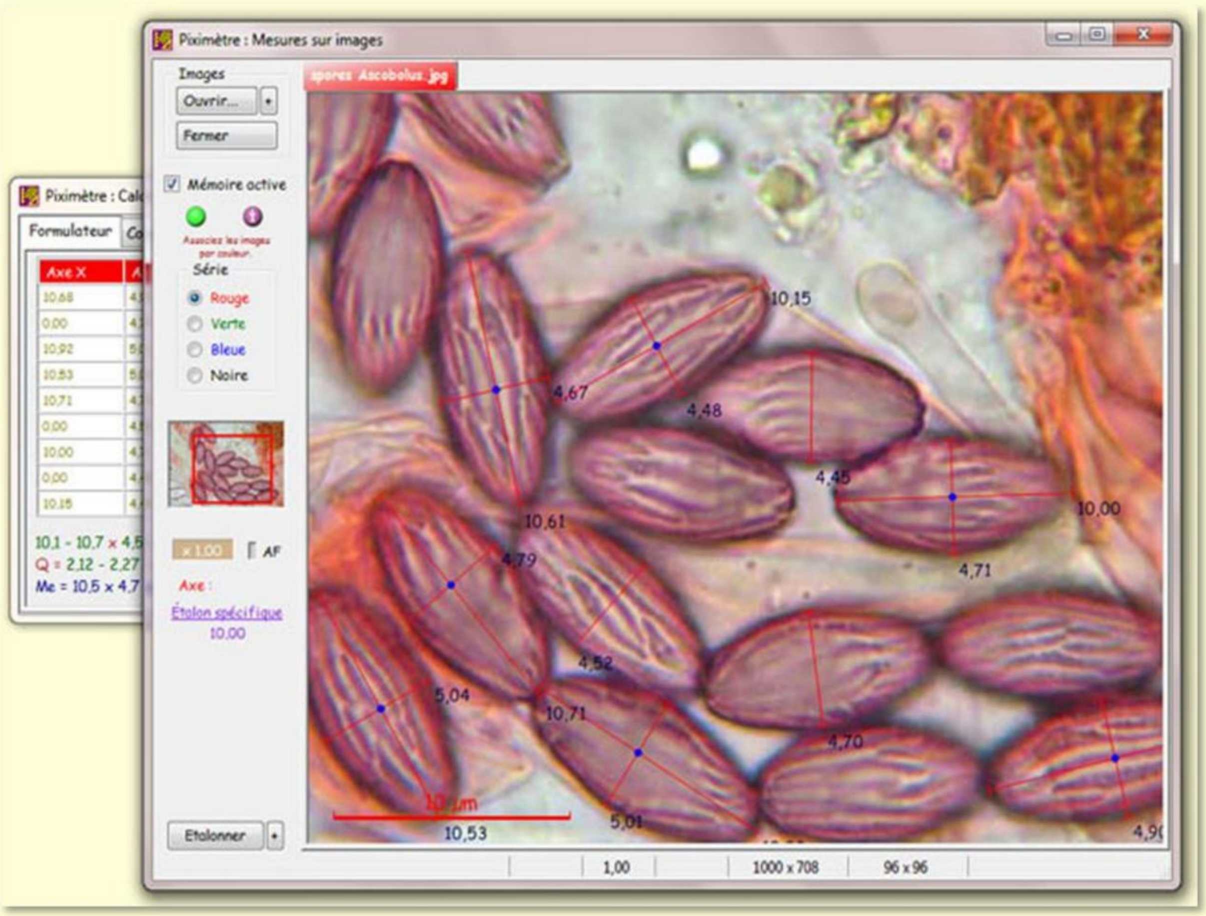
Its **coupling** with the *Comparator* and the *Formulator* makes it possible to visualize and compare the spatial distribution of measurements with that of other series and to express the *dimensional formula.*

Its **active memory** stores the measurements taken and the various image parameters.

Calibration and autofocus guarantee the validity of measurements, without recalibration of images and regardless of the focal length used when shooting.

Principle

The function is activated in **"Graphics"** mode ("Parameters" *tab).*



General view.

The **"Open..."** button allows you to select and open several images simultaneously. Images relating to the **same series of measurements** (a sample) must be assigned the **same color** (red, green, blue or black).

The neighboring **"+"** button allows you to open images while previously specifying a series color. It also allows you to activate **LiveView** capture using a video camera.

The information transmitted to the *Comparator* and the *Formulator* is **grouped by color.** Thus, several images belonging to the same color are treated as a single **virtual image.**

The **zoom** is activated by the mouse wheel, which is placed on the image. The image magnification factor appears under the thumbnail (x 0.73 in the general view example). **A mouse click** on this value (two in the case where the image was taken at zoom, i.e. has an intrinsic magnification greater than one) resets the zoom to 1.00.

The measurements made on the images are **independent of the zoom** used on each of them.

Images are **moved** laterally by **moving the mouse over their thumbnail with** the left button pressed. A **red frame** on the thumbnail indicates the part of the image currently displayed on the screen. Moving is only possible when the image is larger than the display area and the cursor placed on the thumbnail then takes **the shape of a hand.** The movement stops as soon as the mouse is released.
Try moving in small, successive strokes. **The keyboard direction arrows** (up, down, left and right) allow you to move the image in smaller strokes, making it **easier to point** at high magnifications.

The measuring system must have been calibrated before valid measurements can be made. The **"Calibrate"** button activates a quick wizard that guides you through this process.
The **"+"** button to its right activates the wizard with additional possibilities. It is **essential to define at least one generic standard** for the measurement system. This generic standard is applied to any new image opened in the *Measurer.* In the absence, the UNITE standard, always present, is applied.

It is possible, and even desirable, to define **several generic standards.** Each of them corresponding to a particular state of the optical system used (for example to a microscope objective). Generic standards are **always retained** from one use to the next.

The choice of the standard to use to perform measurements on an image is linked to **the state of the optical system** used when taking the image.

Specific calibration of each image is also possible. The standard thus defined is then only applied to this image.

When closing an image with *active memory,* **the image is memorized with its measurement reference frame:** its calibration becomes specific. This makes it possible to find all the numerical values when "reopening" the image with *active memory.*
The complete calibration process is described here.

Under certain **prior initialization** and usage conditions, **autofocus** guarantees that dimensional measurements are independent of the focal length used when shooting.

The operation of the autofocus is indicated by the **"AF"** indicator which lights up green. A mouse click on **"AF"** activates **the autofocus assist.**

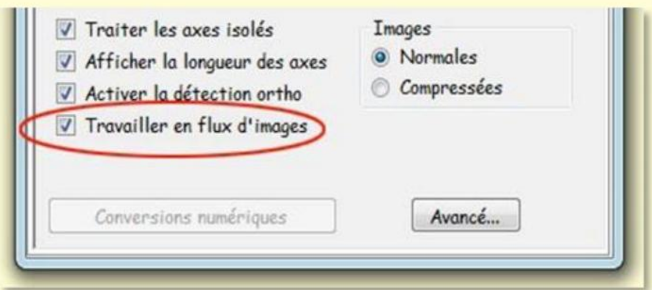
Image stream

In general, **several images** are necessary to carry out a sufficient number of measurements. These images are **opened simultaneously** in the *Measurer.* As soon as an image is closed, the measurements it contains disappear from the *Formulator* and therefore from the final result.

When **many images** are needed to make measurements, it may be easier to reduce the number of images simultaneously open in the *Measurer.*

Image Flow operation is introduced for this purpose: the measurements carried out on the images are **kept by the *Calculator*** *(Formulator* and *Comparator),* therefore in the result, when the images are closed after measurements have been carried out. Other images can then be opened and measured **with the standard that corresponds to them,** possibly different from the previous ones. **The new measurements are added** to those already contained in the *Calculator.* The final result is calculated based on all the measurements taken.

This operation has the particular advantage of being able to mix measurements from images assigned to **different standards** (remember that apart from *Image Flow,* all images in a series are necessarily assigned to the same standard).



Implementing *image streams* is done on the ***Settings*** pane of the *Calculator,* as shown in the figure. It affects all series.

The total deletion of the data, when it is no longer useful, is done by the **"Delete"** button of the *Formulator* or the *Comparator.*

Saving the data in a file and copying it to the clipboard covers all the data displayed in the *Formulator.*

Users' attention is drawn to the **risk of error** due to mixing of data which would occur if measurements relating to new objects were introduced into a *Formulator* still containing **old obsolete data.** You must therefore be careful and not forget **to erase the Formulator** before undertaking a new series of measurements on objects different from the previous ones.

Validity of standards

> **Important :** If you use autofocus (case of photos with different focal lengths), its initialization must **precede** the definition of the generic standards. Otherwise they risk being erroneous.

The photos processed by *Piximeter* come from digital cameras or cameras on which the operator can adjust, among other things, two parameters which influence the validity of the calibration:

1. **Image size :** 1280 x 960, 1600 x 1200, 2272 x 1704, etc. These sizes all have the same ratio **Q = L/l = 4/3.** But there are also other formats, such as the 3/2 format (2272 x 1520 for example).

   A standard defined on an image of size **T** remains valid on an image of size T' with the same ratio Q, at the multiplier coefficient **KT = T'/T** ready which defines the homothety between the two images. In other words, **KT** can be assigned to each of the two components x and y of the image and therefore to their resultant, the standard.

   In the case where **Q** is different from one image to another, the length transformation is no longer identical on their two axes, there is no homothety between them and the standard cannot be validly applied .

2. **The resolution of the images :** when it differs from one image to another (which is not necessarily the case) the problem can become the same as for the size. Adjustment of the standard is then only possible if these resolutions **R** (in x and y) are homothetic between the two images. **KR** is this coefficient of homothety. But there are also images whose dimensions do not change, although their resolutions differ.

The **"Adjust standards based on image resolution." setting,** on the tab Advanced Settings *Special ,* controls the processing to be performed. When selected, and if it is **homothetic,** the transformation is calculated and carried out.
When deselected, no transformation is undertaken and **KR =** 1. By default this parameter is selected.
Whatever this parameter, the lack of consistency between the resolutions systematically produces a warning and turns the general light red.

When a generic standard is applied to an image, the analysis of the **R** and **T** parameters (resolution and size) makes it possible to determine whether or not there is consistency between this image and the one which allowed the definition of the standard and, if so, to calculate the overall magnification factor **K = KT x KR** to be applied to the heel.

The general indicator of the *Measurer* indicates the compatibility of the selected image with the chosen generic standard:

- **Green,** it indicates that the selected image **is compatible** with the chosen generic standard. The homothety factor has been calculated and the measurements can be validly carried out.

- **Red,** it indicates that the selected image **is not compatible** with the chosen generic standard. This image must be calibrated before undertaking any measures.

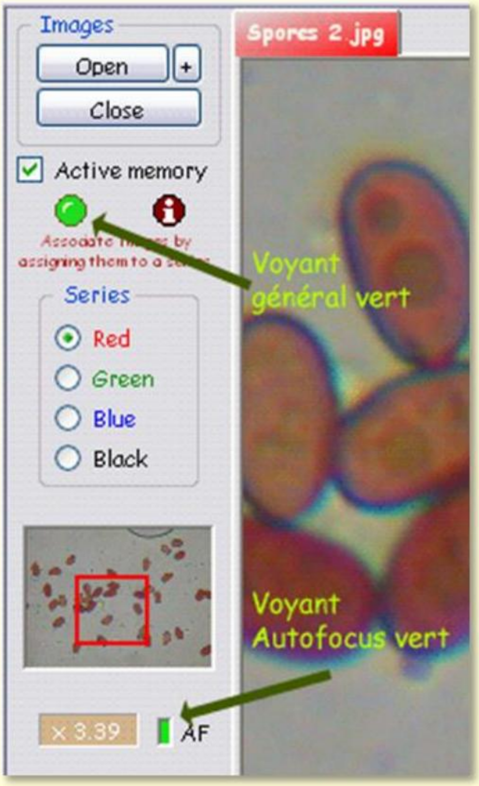
A mouse click on this indicator brings up an information bubble, the color of the indicator, which explains the case if necessary.

In the example opposite, at the top, **the bubble is green like the indicator.** This means that **the selected image is compatible** with the chosen generic standard. The measurements can validly be carried out. The red text here specifies that the series (blue: both images are blue) includes another image for which the standard is not compatible.

In the example below, **the bubble is red like the indicator.** This means that the selected image is **incompatible with the** chosen generic standard. The text provides the necessary clarifications. Here, the size ratios are different, which justifies the red light. In addition, the resolution res = (0 x 0) of the image means that the metadata is absent (this can be confirmed by observing the status bar under the image; if this is the case it contains non-binding fields). informed).
The image was probably compressed too heavily. Its calibration is necessary to be able to carry out valid measurements.

When the case arises, the **red information bubble** appears spontaneously **only the first time** to attract the user's attention. Then a mouse click on the indicator is necessary to make it appear.

Regardless of the image choice made in the *Parameters* tab **(Normal or Compressed),** as long as the image opened in the *Measurer* contains metadata and the autofocus is correctly initialized, the consistency of the measurement system will be demonstrated by the general indicator light and the autofocus indicator light both **lit green** (figure opposite).



In the case of a compressed or **metadata-free** image opened in the Measurer:

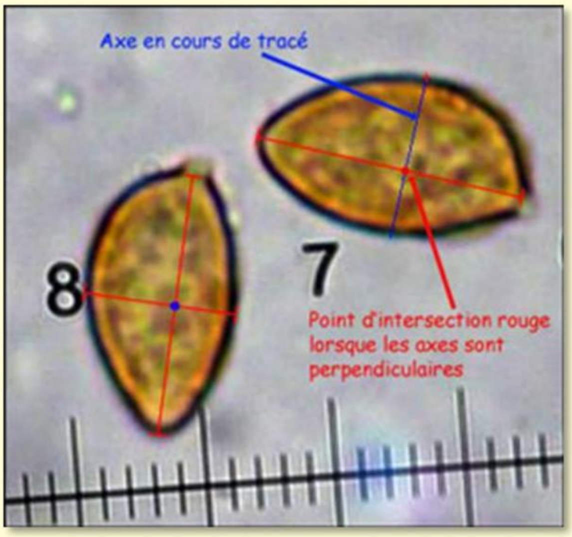
- choosing **Normal Images** in the *Settings* tab will have the effect of reporting an error. The general indicator light will be lit red.

- choosing **Compressed Images** in the *Settings* tab will have the effect of not reporting an error. The general indicator light will be lit green.

In the figure, the measuring system is ready.

Axis coupling

A **first mouse click** on an image sets the first point of a new axis. **To release** the button and **move the mouse** to the position of the second point. The axis is materialized during this operation and follows the movement of the mouse. A **second click** places the second point of the axis which then freezes. **Its calculated length is displayed** right next to this second point if requested (see *Settings tab).* The color of this information can be adjusted via the context menu.



While moving the mouse in search of the second point, it is possible to **vary the zoom** of the image as well as its **lateral positioning ,** per action on its sticker. This mechanism ensures great pointing precision and allows, where necessary, to trace **axes of great length** (for example during calibration where larger axes offer greater precision, since one remains in **the linearity zone of the image).** Very small axes, of the order of a few pixels, are not accepted. You have to **zoom in** to trace very small objects.

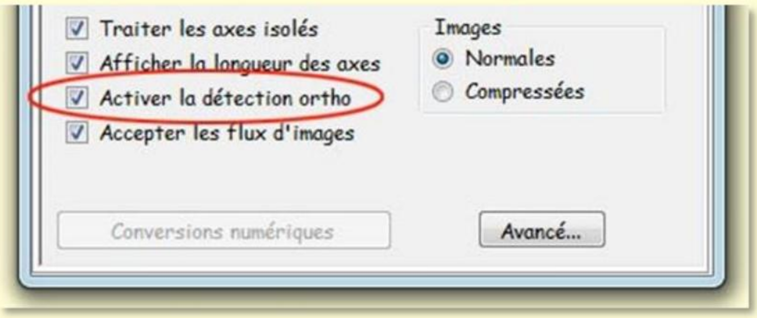
When an object to be measured is seen **perfectly in profile,** i.e. there is no ambiguity about its outline, its two axes can be traced, which measure its length and width. Once traced, their coupling is materialized by **a blue dot** drawn on the image as shown in the figure above. The two dimensions, correctly evaluated, appear in columns X and Y of the *Formulator input grid.*

The axes can be drawn in any order. It is entirely possible to return later to an object for which an axis has been forgotten or to rectify an existing axis.

Plotting orthogonal axes

When the axis being drawn intersects a first axis **perpendicularly , their point of intersection appears in red** during the drawing (figure above). We must therefore systematically look for this red point in order to ensure the perpendicularity of the axes which measure two dimensions of the same object. The axes are **coupled** automatically.

When the **orthogonal detection** function is enabled (as shown in the Formulater *Settings* on tab), **orthogonal coupling becomes automatic** when two axes intersect.



The mouse cursor becomes captive and can no longer leave the **imposed orthogonal trajectory.**

However, it is possible to **occasionally deactivate** this automation by holding down the **Ctrl** key on the keyboard. Symmetrically, when the function is deactivated, this **Ctrl** key activates it as long as it remains pressed. Remember to **release Ctrl** before marking the point otherwise you will link a new segment afterwards.

Ctrl is a "magic key" that has several other functions.

Axis specification

In automatic axis **selection mode ,** the larger of the two represents the length, the other the width.

In manual axis **selection mode ,** *Piximeter* asks the user to specify the nature of the first of the two drawn axes. The user indicates whether it is a length or a width. *Piximeter* automatically deduces the nature of the second.

The context menu also allows you to **subsequently change the nature of an axis** by explicitly specifying "Length" or "Width". In the case where two axes are coupled, the change of one causes the change of the other.

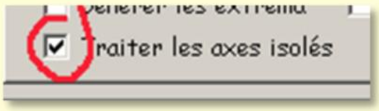
When an axis has been specified in this way, the corresponding item in the **context menu is checked.** Further action on this menu item **overrides the axis specification.**

It is also possible to draw **isolated axes,** that is to say not coupled, as there are in the General View above. They make it possible to improve the precision of length or width calculations by increasing the number of measurements. However, these isolated axes are not counted in the calculation of the **ratio Q = length / width,** which is only carried out on coupled axes, that is to say belonging to the same object.

The **context menu** of the image (right mouse click) allows, if necessary, **to establish or delete a coupling.** The cursor should be placed on the coupling point.
When a coupling is deleted, the two intersecting axes become isolated axes and are treated as such.

Processing isolated axes

Processing of isolated axes is **activated by the user** on the *Settings tab.* When the box is not checked, isolated axes are not processed by the *Formulator.*



When an object does not appear perfectly in profile, it may be impossible to assess its true contour accurately and, in the least difficult cases, only one dimension can be measured. **It is an isolated axis** which is then traced on the image, as also shown in the general view (above).

10,50	21,00
10,00	20,00
11,00	0,00
10,50	0,00

When processing of isolated axes is not activated by the user, **only the coupled axes participate in the final calculation.** The isolated axes are ignored (figure opposite, above, the couples are in green. The sample here has two measurements for each dimension).

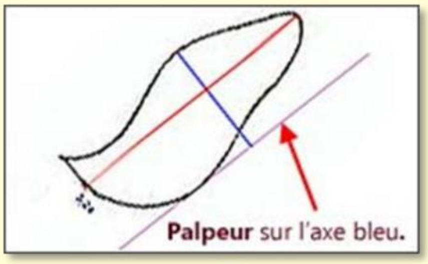
10,50	21,00
10,00	20,00
11,00	0,00
10,50	0,00

When the processing of isolated axes is activated by the user, **all non-zero axes** participate in the calculation of each of the dimensions (figure opposite, below, the sample has four measurements for one of the dimensions and two only for the other). Let us repeat that in this second case, **only the couples participate in the calculation of the ratio of dimensions Q=L/l.**

Note that this principle also applies to the operation of *Piximeter* in "Digital" mode.

The **automatic placement** of the axes, as length or as width, is carried out according to the distance of their length from the average of the lengths of each column X or Y. For example, on the general view, the isolated axes of length *4.45* , *4.52* and *4.70* are automatically placed in the Y column of which they are closer to the average than to that of the X column.

The Feeler



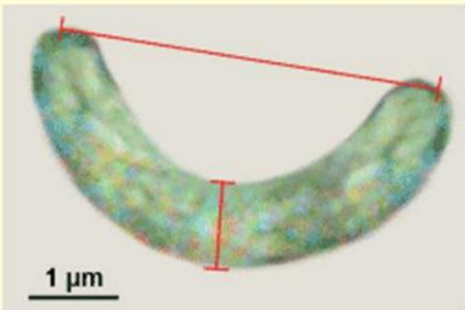
In certain cases, as in the example of a virguliform spore on the left, the geometry of the object to be measured does not allow the two rectangular axes which frame it to be directly traced. **The Piximeter feeler** is *then* a valuable aid which allows you to get as close as possible to the real dimensions.

The *probe* is **a temporary axis, rectangular** to the axis being traced. Its large size allows precise contact with the points of the object to be measured. Furthermore, the magnifying glass on the image allows you to modify its dimensions.

To make the *probe appear,* hold down the **"Shift"** (or Shift) key on the **keyboard** during the drawing of the **second point** of an axis (in the figure, it appears at the end of the blue axis).

Moving the axes on the image

Piximeter automatically couples the intersecting axes, as just explained above. But it is not always possible to draw two intersecting axes on the object to be measured. This is the case, for example, of objects such as the one shown opposite.

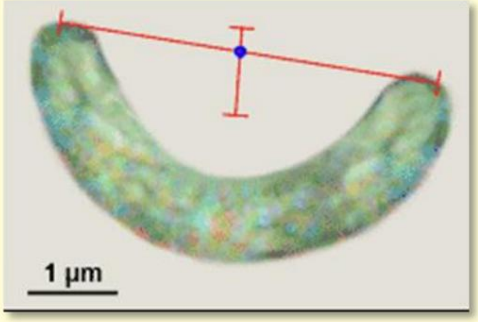


Here the *Calculator* receives the measurements in the form of two isolated axes (one large and one small), as shown in the table below.

It cannot therefore validly calculate their ratio **Q = X /** Y. For this calculation to be possible, it is necessary to **couple the two axes.**

Axe X	Axe Y
0,00	0,97
4,27	0,00

Piximeter offers the possibility of **moving axes on their image,** without of course changing their length!

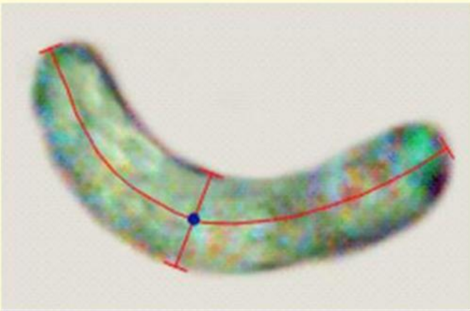


1. Hold down the **"Ctrl"** key on the keyboard while moving the mouse cursor towards the chosen axis. When it gets close enough its shape changes. It transforms into a **hand** that allows you to grab the axis and carry it elsewhere.
2. Grab the axis with a **left mouse click** and drag it while holding down the click. When the axis crosses another, the intersection point materializes automatically.
3. Release the mouse when **both axes are coupled.** And the response from the *Calculator* comes, which allows the calculation of the desired ratio.

Axe X	Axe Y
4,27	0,97

Nonlinear axes

Piximeter allows you to trace **non-linear axes** as shown in the figure opposite. The major **curvilinear axis** of the figure is obtained by a succession of small linear segments drawn by holding down **the "Ctrl" key.**
The trace ends when this key is released.



Measurement with a curvilinear axis.

In the figure below we have drawn **an axis on a map** representing a route. The layout is carried out as for a curvilinear axis. Here the image has been calibrated using the 300 m scale.

The non-linear axes are a generalization of the axes managed by *Piximeter.* They are treated the same way as the others, both numerically and graphically.

The **context menu,** activated by a right mouse click, allows, if necessary, **to delete an end segment** (the first or the last)

Measuring a route.

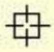
as soon as the cursor comes close enough to one of them. Placed elsewhere on the axis, it allows it to be **completely removed ,** like a classic linear axis

This ease of action on the end segments is especially interesting in the case where **the last traced segment** is supernumerary.

Axis grinding

It may happen that an axis is not drawn exactly where it should be due, for example, to a pointing error; with the consequence that it does not have the correct length and therefore introduces an error in the measurements. In this case, two solutions exist: **delete the axis** (cursor in the vicinity of the axis + right click and *Delete axis menu)* then retrace it, or **resume its trace** and rectify it directly, without deleting it.

To rectify an axis, simply bring the cursor closer to **one of its ends while holding down the "Ctrl" key.** The cursor changes shape and takes this one

 which allows you to grasp the end in question.

At this precise moment, **without releasing Ctrl, press the left mouse button.** The cursor changes again and returns to its initial shape (a cross). We enter the standard axis drawing mode. This takes on the color blue and follows the movements of the mouse as long as you keep the left button pressed.

Two actions are then possible:

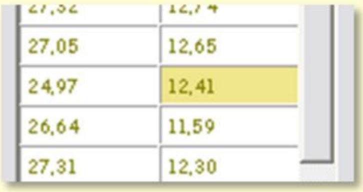
- **Release the Ctrl key** and then move the cursor to its correct position on the image. **Release the left** mouse button. The axis stabilizes in its new position. Its route is finished.
- **Hold down the Ctrl key.** We are then in non-linear axis creation mode as explained above. As soon as you release the left mouse button, *Piximeter* stabilizes the drawn axis and creates a new segment after it, linked to the previous one. To draw the last segment and not move on to a new one, you must release the Ctrl key.

All axes drawn on the images can thus **be rectified at any time,** whether they are linear or not.

In the same way that we trace an end of a linear axis, it is possible to modify a junction point of two segments of a non-linear axis. Move the cursor to the junction point and do the same as above. The two continuous segments are then retraced.

Identification of the axes on the Formulator measurement grid

When the mouse cursor comes close enough to an axis or non-linear axis segment with **the "Ctrl" key pressed,** the mouse cursor changes to the shape of a hand as has been said upper.



At this moment, a **left click** displays the axis **on a yellow background** in the Formulator grid , as shown in the figure above.

In this example **the axis of length 12.41 is pointed** by the user. It intersects with another axis of length 24.97. In intersection with several axes it would be visualized several times. This link between physical axis and numerical value allows **easy verification** which improves data entry.

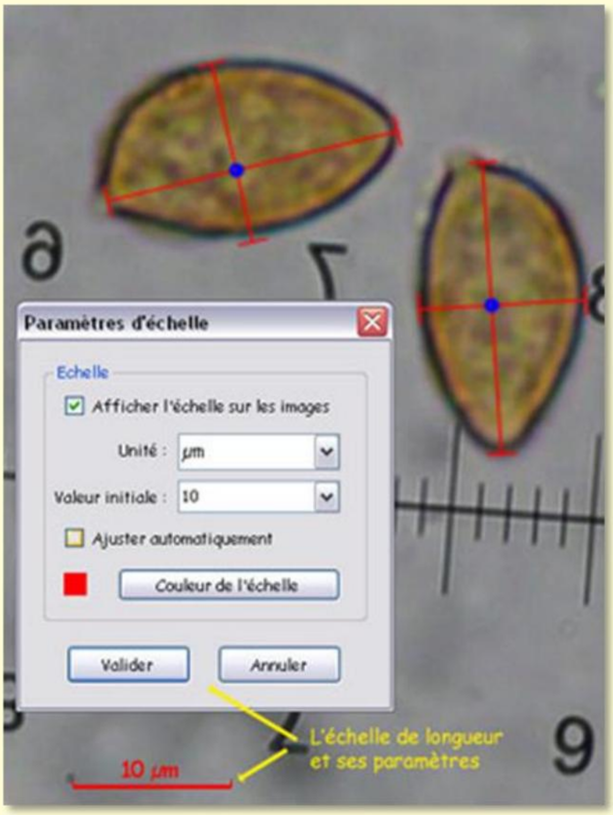
Ctrl and Shift: magic keys

Actions	How to do
Formulator grid which is	**Ctrl** + approach the mouse to the axis until the cursor changes shape and takes that of a **hand.** Left mouse click pressed, the axis appears on a yellow background in **Locate an axis** in the automatically positioned in the *Formulator grid.* to view it. [Details](#)
Draw a **nonlinear axis.**	Place the first point on the image. Hold **Ctrl** pressed and successively place the other points. Release the **Ctrl** key to place the last point. [Details](#)
left mouse button, move	**Ctrl** + approach the mouse to the axis until the cursor changes shape and takes that of a **hand.** Click **Move an axis** on the the axis to the image position. desired. Let go. [Details](#)
Place a **file** If the **Ctrl** other files already	From Windows Explorer, **select and drag** the measurement file onto the *Formulator grid.* key is pressed the file **replaces** any **measurements** in the grid dragged onto the grid. Otherwise the *Formulator.* the file is **added** to the others. [Details](#)
Modify an axis already	**Ctrl** + approach the mouse to the end of the axis to modify.

traced on the image.	The cursor changes to a selection box. Left click to grab this end and move it to the desired location. Let go. It is also possible to modify **a multi-segment axis** by pointing to one of its connection points.
Locally invert the automatic orthogonal axis plotting function .	When the function is activated (Formulator Parameters tab) , *pressed* **Ctrl** deactivates it at the intersection of the axes currently being drawn. Otherwise, pressing **Ctrl** activates the function. Release **Ctrl** before marking the point otherwise you will link a new segment afterwards.
desired point. The Feeler	Draw the first point of the axis normally then hold **Show *Probe* Shift** (Shift) pressed while moving the mouse to the second appears under the cursor. axis on the image. Left click to mark the point.
pressed. the *Measurer.*	**Shift** + mouse move over the image, **left click Move image** in Also **on the keyboard,** by using the arrows (right-left-up-down), with or without **Ctrl** pressed.

Length scales

Each image opened in *Piximeter* can display a length scale like the one shown, in red, in the figure opposite. It is located in the lower left corner of the image.



Comprising homogeneous objects, by definition, all images in the same series are automatically **assigned the same scale.**

Right **-clicking on an image** in the series gives access to its scale parameters which the user can adjust according to their needs:

- **Show or not the scale** on the images from the series,
- **Define your unit.** Several symbols are available for this purpose,
- Define **the scale value to represent** on the image. •

Automatically adjust this value depending on the

image magnification performed manual zooming. by
If the value is fixed, the length which represents it and which changes according to the zoom, can become large. Otherwise, the represented value changes automatically so as to maintain a suitable length scale on the image.

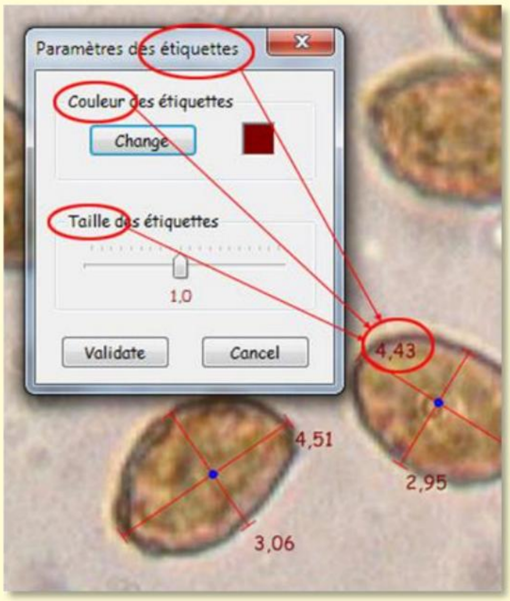
• **Change the color** of the scale.

All of these settings are applied to all images in the series.

Axis labels

Axis labels are the dimensions that are shown near the axes plotted in the images. They can be displayed or not (Parameters tab : **"Show axis length").**

Their color and size can be adjusted using the adjustment panel which is activated by the context menu: Right mouse click on an image then **"Define labels"** (figure below).



The color of the labels is carried over to all images opened in the *Measurer.*

The **"Label size"** slider allows you to vary the character size, more or less, around the central value 1.0.

The actual size of the characters written on the **exported images** also depends on the size of the latter: when their width is between 2048 and 4096 pixels, the size of the characters is multiplied by 2. Beyond that, it is multiplied by 2.4 .

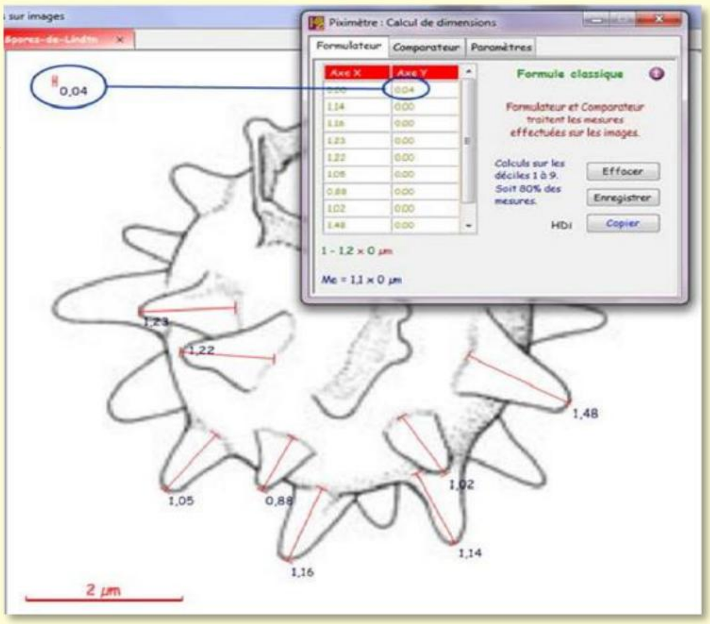
1, 2, 3 Dimensions

Basically, *Piximeter* ensures the measurement of **pairs of axes** corresponding only to two dimensions of space (length x width or length x thickness for example). As just seen above, it automatically breaks down the measurements into one or the other dimension depending on their value or user requirements. If it is not possible to measure the three dimensions of an object simultaneously, **it is however easy to measure one dimension alone** (the third for example) by forcing all the measurements into a single column of the input grid. of the *Formulator.* You will first have taken care to check the **"Process isolated axes"** choice on the Calculator *Parameters* tab .

There are **two ways to do this.**

A first method, the oldest, consists of drawing any axis (only one per series of images) whose length is **very far from the values to be measured,** either very greater than the maximum, or very less than the minimum. All subsequently measured values will then automatically fall into a single column.

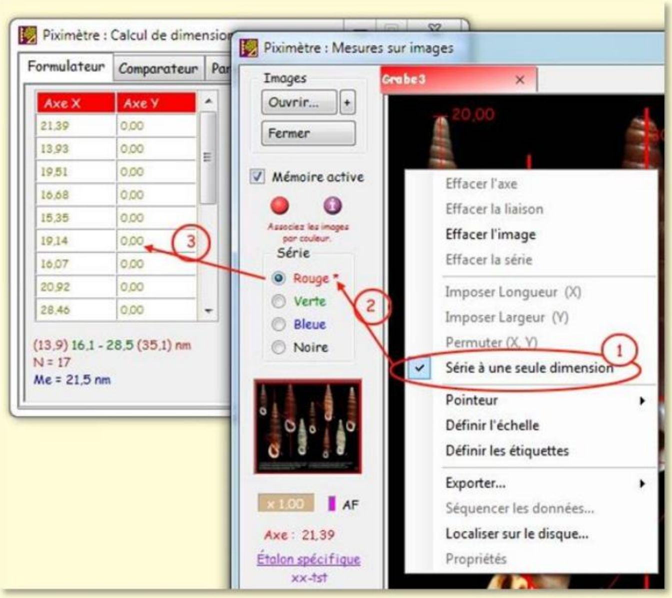
The figure opposite shows such an operation where it involves measuring the height of the spore spines: **the left column bears the valid measurements** while the right column was forced by the drawing of a very small axis of length 0.04 much lower than the values to be measured.



Zero values (in particular here the 0.00 opposite the 0.04) are obviously not taken into account for the calculation. A similar result would have been obtained by drawing a very long axis on the image, but in this case the X and Y columns would have been reversed. To draw a very large axis or a very small one, it may be necessary to reduce or enlarge the image using the zoom.

The *dimensional formula* here gives the length of the spore spines: 1 - 1.2 µm ; Me=1.1.

Version 5.8 of *Piximeter* introduced a **new, more direct method,** via the context menu, to measure only one dimension (figure below):



*Switch to one-dimensional mode using the **context menu.***

The **context menu** opened on an image of the *Measurer* (right click) allows you to specify that the current series, the red one in this example, is to be treated as **a single-dimensional series.** That is to say that all the axes drawn on all the red images will be considered independent of each other and automatically sent to **the X column** of the *Formulator.*

The figure above shows the result of this operation: **The X column contains all the measurements** while the Y column contains only non-significant zeros.
The dimensional formula has only one dimension, the result of calculations on X.

The one-dimensional mode provides a **"classic" formula** as well as a **statistical formula** just like in the two-dimensional mode.

*Note at point 1 of the image **the check mark that appears in the context menu** which means that the series is in one-dimensional mode.*

Also note in point 2 of the image that the name of the series in the Measurer *is **assigned an asterisk for attention.***

In one-dimensional mode, certain operations are no longer available in the context menu: permutation (X, Y), as well as axis specifications.

Clicking on the menu again allows you to return the series to two-dimensional mode.

The series are automatically **in two-dimensional mode when** Piximeter starts .

The multiseries

A series is characterized **by its color :** all the images in a series have the same color. Changing the color of an image means placing it in another series.

It can be useful to work and compare several series of measurements.
To do this, you must assign **a different color** to the images in each series. For example a red series (all its images will have the color red) and a green series (all its images will have the color green).

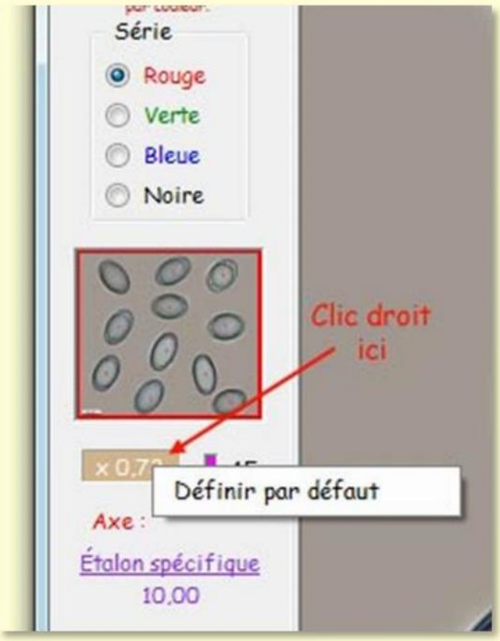
Piximeter can simultaneously manage up to **four series** (red, green, blue and black).

The four series are displayed together in the *Comparator.* The *Formulator,* for its part, displays the *dimensional formula* of the series corresponding to **the selected image** in the *Measurer.*

When several series are present in the *Measurer ,* an arrow appears as shown in the figure opposite. A mouse click on this arrow allows, if necessary, to **circularly permute the color of the series.** Green becomes red, blue becomes green, etc.
The *Formulator* and *Comparator* reflect this permutation.

Opening images

If ***Active Memory*** is engaged when reopening an image, it is displayed in the *Meter* exactly as it was when it was previously closed (provided that *Active Memory* was also engaged at that time).).



However, when **first opened,** obviously no prior information is available for this image. Nor, in particular, the value of the zoom applied to present it, which is set **by default to 1.0.**

This value can be redefined to **automatically obtain a new presentation** more in line with the user's wishes:

1. Frame an image **for the first time** with the mouse wheel (or the right-left buttons on the PAD). In the example opposite, the view (red frame) completely covers the image which is therefore completely visible in the *Measurer* window ; The zoom here is x 0.73.

2. **Right click on the zoom** to bring up the context menu and select **Define by default.**

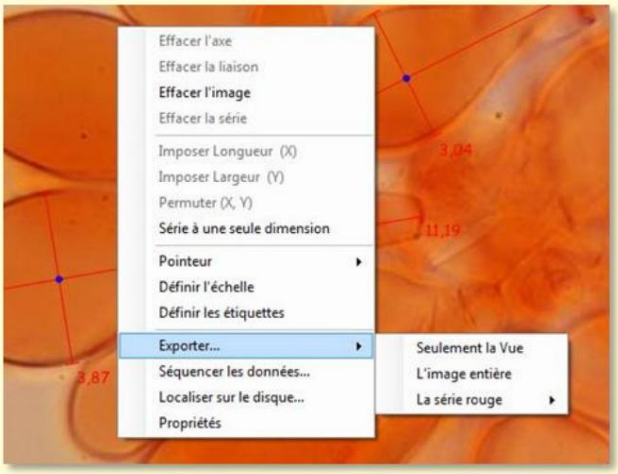
The zoom value is saved and will be applied when opening any new image as well as reopening an old one with *Active Memory* not selected.

Note, however, that the presentation of the images will only be consistent to the extent that they have **the same dimensions** as the image which was used to define the default zoom.

The context menu

To finish with the image measurements function, let's return again to the **context menu,** activated by the **right click of the mouse** placed on an image, which allows:

- **To delete any axis** as soon as the cursor pointer approaches it enough,
- **To delete two intersecting axes** when you click on the coupling point materialized,
- **To erase or reestablish a connection** between two axes when you click on their intersection, • **To erase the image** (all the axes traced on the image - be careful, there is no going back possible ,



- **To erase the series** (all the images in the series, when several of them contain axes), • To force the pointed axis to be **recorded as an** object length, i.e. in the X column of the *Formulator,*
- To require the pointed axis to be **recorded as an** object width, it is up to say in the Y column of the *Formulator,*
- To **swap the two axes** in the *Formulator,*
- To change the **two-dimensional-one-dimensional** measurement mode of the series concerned (see 1,2,3 Dimensions),
- To change the shape of the **pointer,**
- To **define the properties of the scale** of the current series,
- To **define the properties of the axis labels** (their length displayed at their final end),

- **Export all or part of the** selected image as a new file, or **all images** (see here),

- To **sequence the data** (see here),
- To **locate the image on the disk,**

- **To display the image properties** in a specialized window. This can also remain open. It then permanently displays the properties of the selected image (if they are present in the image, therefore if the image is not too compressed).

Note that some items in this menu are only active if they make sense. For example, the two **"Impose..."** elements are only active in the vicinity of an axis, **the permutation** is only active on the coupling point of two axes, etc.

Data mixing

Data mixing is the possibility of adding new measurements to existing data. During a previous session the user **saved results in a file** using the **"Save"** button on the *Formulator.* To improve these results he now wants to add new measurements because he has new data. The process implemented to do this is called **Data Mixing.**

Methods

Two methods are available to perform **data mixing,** corresponding to two different situations:

- When several measurement sessions have been carried out and each saved in a separate file, simply drag *and* **drop** each of them onto the initially empty Formulator **grid .** All the files thus deposited in the *Formulator* are **summed** and the *dimensional formula* is **calculated on all the data.**

- The second method involves using **two data streams simultaneously.**

  One consisting of data introduced as indicated above, by **dragging and dropping** files into the *Formulator,* and the other by data directly **measured on images** opened in the *Measurer.* The flows are added to each other in the *Formulator* and the *dimensional formula* is **calculated on all the data.**

  - Drag the measurement file(s) onto the *Formulator* grid **initially empty.** The selected series (red by default) is initialized with this data which appears **on a gray background.**
  - Open the new images in the *Measurer,* **still in the same series,** and carry out the measurements. These are automatically added to the grid (on a white background this time) and the overall *dimensional formula* appears below (do not forget to check the consistency of the standard applied to the images. If necessary, change it).

The following figure shows the *Formulator* containing two data streams:

1. A drag-and-drop file containing previously established measurements (area gray at the bottom of the grid),
2. A data stream from images opened in the *Measurer* (white area above).

In the figure the small red arrow shows the separation of the two flows.

A new file placed on the grid will be automatically added to the present data, **in the gray part.** New measurements from the images will be included **in the white part** above.

Remember that the data dragged and dropped on the grid, intended for the gray area, can be **modified manually** by the user who may, depending on the needs, correct, add or delete data.

To delete data present in the gray part, simply replace it with 0 (0s are not counted in the results). The row is removed from the grid when both of its cells have the value 0. **To manually add** new data in the gray area, simply enter their value in an empty cell at the base of the grid. We will move from one cell to the next using the **"Tab"** key on the keyboard, not "Enter".

Note that there is another method for adding measurements to a previous result. All you have to do, first of all, is open all the images that allowed you to obtain it. If and only if they were initially closed with **Active Memory** selected, their measurements are found and loaded into the *Calculator.* We then add the new images to the series; The measures taken on them will be added to the first ones.

Mixing Conventions

Several measurement files can be added to the grid, by simply **dragging and dropping.** Holding down the **Ctrl** key during this operation, on the other hand, **clears the grid** and replaces its contents with the new data.

In practice the data can be introduced into the *Formulator* at any time and **in any order :** by the *Measurer* or by the *files deposited* and even *manually in the gray zone.*

Furthermore, when the two columns of the grid are used (general case of measuring two dimensions), *Piximeter* always processes the data under the assumption that column **X represents the length** of the measured objects and column **Y their width.** We therefore always have X > Y. The *dimensional formula* is oriented in this direction.

However, it is also possible to organize the dragged and dropped files in **the reverse order :** X < Y. This is particularly the case for manually constructed files.

When all the files submitted are organized according to this second convention, *Piximeter* preserves it and displays the results in this order. In the presence **of both conventions,** *Piximeter* does not mix the data but **automatically aligns** them following its preferred convention: X > Y.

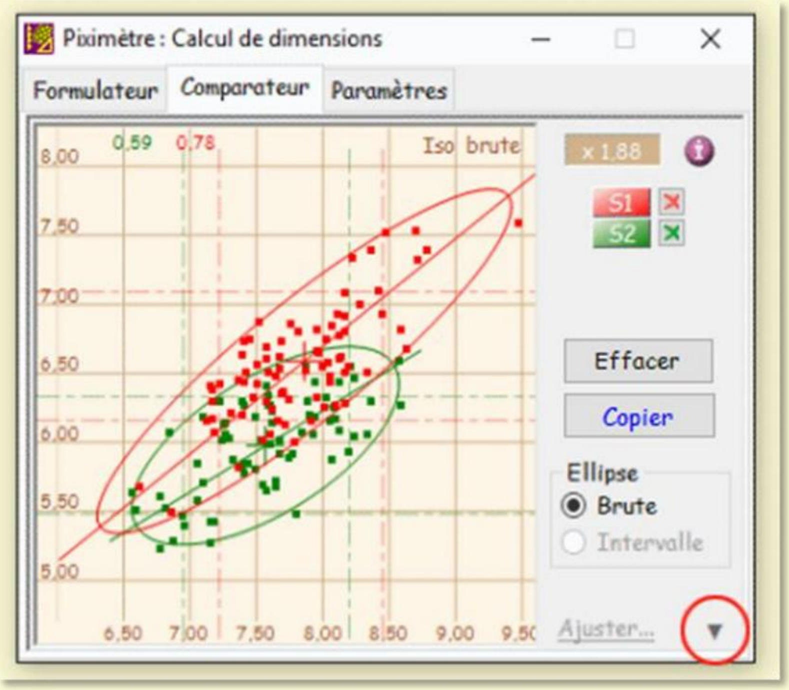
Data mixing is **fully compatible** with *Frame Stream* mode .

The Measurement Comparator

The *Comparator* allows you to **visualize and compare** the spatial distribution of several series of measurements. The *Comparator* image can be copied into a document (Office for example) by simple **Copy-Paste,** via the clipboard.

Principle

The *Comparator* can view **all series simultaneously** (red, green, blue and black). Each is represented by a cloud of points which corresponds to the raw data of **its well-formed pairs (x, y)** (both components of which are present).
Also displayed: their trend ellipse, their major axis, the value of their slope, etc. The figure below shows a red series and a green series present together in the *Comparator.* Also displayed, in light dotted lines, **are the intervals calculated** by the *Formulator.*



The Comparator *in "reduced" presentation.*

The plane (X, Y) is **standardized** and the origins of the axes are automatically adjusted according to the values presented. **It can be translated** by moving the mouse on it with the left click pressed.

A **magnifying glass** allows you to enlarge or decrease the scale. It is operated by the mouse wheel. The magnification coefficient is displayed in the upper right corner (x 1.88 on the image).

The joint action of these two tools makes it possible to frame the series displayed in the Comparator plan as desired .

All elements of each series are plotted in the color of the series. In particular **its slope,** which is displayed at the top of the screen (0.59 and 0.78 in the previous figure).

Several parameters are available to **adjust the displayed elements.** They are accessible via the small triangle (circled in red in the figure) present at the bottom right of the comparator.

The red **S1** and green **S2** buttons (in this example) allow you to hide or display the corresponding series. The **cross,** to the right of these buttons, allows you to delete the series, which has the effect, in Graphics mode, of **closing all the images** open from this series.

The **Copy** button places **the *Comparator*** image on the clipboard. This can then be **pasted** (Ctrl + V) into an Office-type work document for example.

The **Clear button,** as its name suggests, **clears all series** from the *Calculator* and, in graphics mode, **closes all open images** in the *Measurer.* Please note there is no **going back .**

Ellipse settings

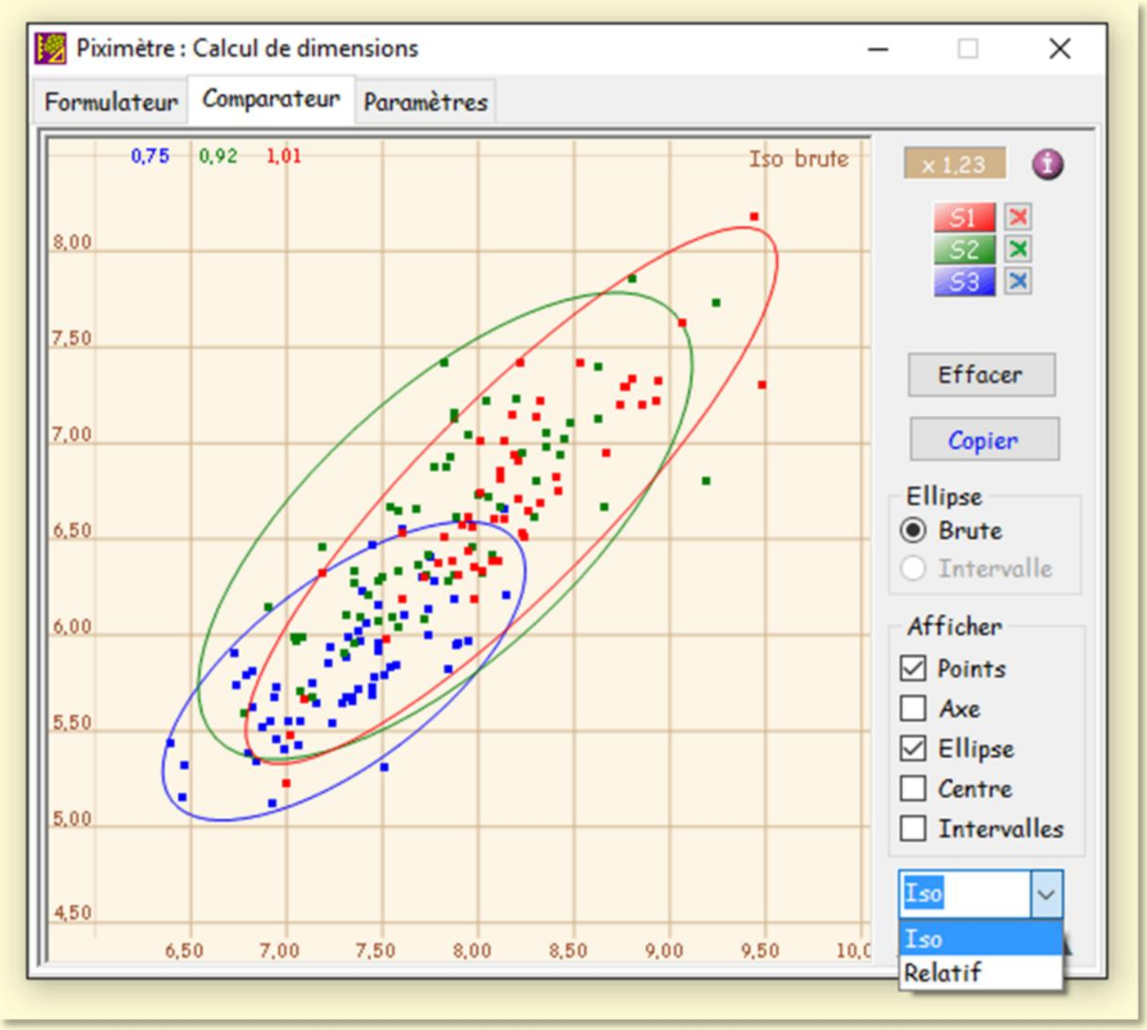
The trend ellipse represents the raw data. It has no particular mathematical meaning. Its characteristics are as follows:

- It is centered on the **center of gravity** of the raw series,
- Its two axes have the respective value of **the length and width** of the series bully,
- Its major axis is superimposed on that of the series; it is a line whose slope minimizes the distance to all points in the series.

Comparator Settings

Two views of the *Comparator* are available: the **reduced** presentation and the **extended presentation.** It is accessed by clicking on **the small triangle at the bottom right** (see figure above).

The extended presentation, in addition to enlarging the *Comparator plan,* gives access to various **display parameters** as shown in the following figure.



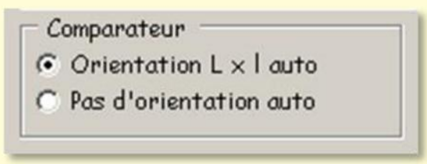
The Comparator *in "extended" presentation.*

We will notice at the bottom the choice of two grid displays: **Iso,** which is the default display and shown here, or **Relative.** In this second case the axes are not encrypted and the series are centered on the center of gravity of the whole (designated by G).

Grayed choices are not active. They are reserved for future developments.

Direction of measurements

At the user's option, measurements can be noted and saved as **L x W** or **L x L** (with L > l). This corresponds to two different orientations of the series. Therefore, the comparison of series with different orientations poses a problem.



To resolve this, *Piximeter* has an automatic measurement **orientation** function. When this function is activated (above, *Parameters tab),* the series entered in the *Comparator* are automatically oriented like the red series which is the **reference series.**

Importing series into the Comparator

The user can directly introduce a series contained in a measurement file, by simply ***dragging and dropping*** this file (from Windows Explorer) onto the *Comparator window.*

The feasibility of the operation is indicated by the mouse cursor taking on a significant shape. To be accepted by the *Comparator,* the data must have been previously recorded using the **Save** button on the *Formulator*
or match the syntax shown below.

It is therefore possible to directly enter **data acquired by any other means**
and recorded in a compatible text file (as created by the "Save" button of the *Formulator).*

The *Comparator* only accepts one file at a time, which must be **text type**
(.txt, .doc, .csv, etc.).

The dimensional formula, result of measurements

Piximeter summarizes the measurements taken in a clear, directly readable and usable form, called a **"dimensional formula".**

For a *quantity* studied (length, width or their quotient) it is interesting to know **the interval** in which its values are located as well as their **average.** An essential solution, when these values become very numerous, consists of taking them from a **sample** supposedly representative of the total population and we traditionally write, for example:

8.1 - ***8.8*** **-** 9.7

However, statistics and probabilities allow **more precision** by limiting the uncertainty linked to the choice of a sample.

Piximeter offers the user the possibility of obtaining results using one or the other method, traditional also called **classic** here or **statistical.** Switching from one to the other is done in the *Formulator,* by **clicking on the dimensional formula** or in the *Advanced* properties of the *Parameters tab.*

From version 5.9 *Piximeter* offers the possibility of adding the calculation of sporal volume.

The classic formula and its calculation

The classic formula is calculated by *Piximeter* using the simplified **decile method.** For each quantity studied:

1. The measurements carried out are ordered by increasing values,
2. The sample thus ordered is divided into 10 groups of the same cardinality (comprising the same number of elements),
3. The sample is then amputated from its two extreme groups,
4. Exceptional values are the extreme values of the complete sample,
5. The interval retained for the quantity corresponds to the extreme values of the amputated sample; deciles 1 and 9.

The classic formula has the general form: **(Mini) min - max (Maxi)** where the values in parentheses are the **exceptional values measured** and min - max are the limits of the interval which corresponds to **80% of the measurements carried out** (deciles 1 and 9). Exceptional values only appear if they are different from the limits of the interval.

In mycology the classic formula offers **direct agreement** with most publications (hence its name).

In practice, *Piximeter* expresses **the classic formula** in one or other of the following forms. Do not hesitate to click (right and left) on the dimensional formula displayed in the *Formulator.*

• The **expanded form,** as in this example:

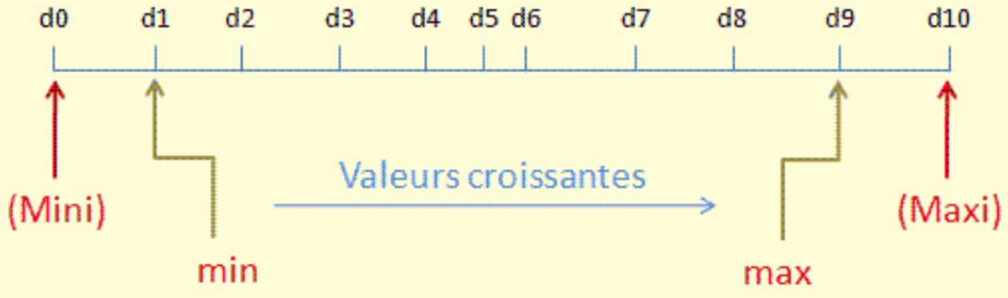
(4.1) 4.15 - 5.06 (5.1) x (2.8) 3 - 3.8 (4.2) µm
Q = (1) 1.1 - 1.5 (1.6); N = 30
Me = 4.62 x 3.45 µm; Qe = 1.35

Me and Qe (e for sample) are the average values of the measurements.

• The **concise form,** like this, at the user's choice:

(4.1) 4.15 -*4.62*- 5.06 (5.1) x (2.8) 3 -*3.45*- 3.8 (4.2) µm
Q = (1) 1.1 -*1.35*- 1.5 (1.6); N = 30

The figure below shows the principle of dividing the sample into deciles. The ten groups have the same number of elements but, obviously, their widths can be different.



Schematic view of the division of the sample into deciles.

The statistical formula and its calculation

On a **sample** of size **n** we calculate the mean and the standard deviation **me** and **ÿe.**

For the **total population** we can estimate the average **m** by **me** from time to time

and the standard deviation by $\sigma_e \sqrt{\frac{n}{n-1}}$

In *Excel* and σ_e are calculated respectively by ECARTYPE and ECARTYPEP.

But we can also estimate this population by an interval, assuming that the distribution of values follows a **Gaussian normal law** whose parameters are **m** and **ÿ**
(Hentic: 178).

Unfortunately **the hypothesis of a Gaussian distribution does not always seem relevant,** especially for samples of insufficient size, because by grouping the values of the sample into classes we see that the histogram, most often, is not not homogeneous (Hentic: 176).

On the other hand, it is quite different for the average of the values: if we take sufficiently large samples (for example of size n > 30), **the average of the sample values approximately follows a normal law,** with parameters **m** and $\frac{\sigma}{\sqrt{n}}$

(Verlant: 124). We then access a confidence interval at the threshold c% **for the population mean.**

Presentation of the results

For example, the length of mushroom spores, with **c=95%, is expressed as follows:**

7.9 [8.7 ; 9.1] 9.9

Resulting in :

- **There is a 95% chance that the length of a fungus spore is in the range [7.9; 9;9].** And
- **there is a 95% chance that the interval [8.7; 9.1] contains the average length of the fungus spores.**

Generally speaking, in the ***statistical dimensional formula*** of *Piximeter,* each quantity (length, width and quotient) is noted: Min [m; M] Max

Or :

[Min ; Max] is an interval centered on the sample mean. At the threshold of c%, it has a radius and the $k(c)\,\sigma_e\sqrt{\frac{n}{n-1}}$ Min and **Max** values are its limits. He

means that there **is a % chance that the value of the quantity studied is between Min and Max.**

[m; M] is the confidence interval **on the population mean.** It is centered on the sample mean and, at threshold c%, it has radius . The values **m** and **M** are $k(c)\frac{\sigma_e}{\sqrt{n-1}}$ its limits. It means that there **is %**

chance that this interval contains the average of the values of the quantity studied over the entire population.

Let us again emphasize the need to work with **large sample sizes, for example 30.** Below this, the method no longer provides reliable results at the same confidence threshold.

In practice *Piximeter* expresses the **statistical formula** in one or the other form next.

- The **expanded form,** as in this example:

  11.4 [14.6; 15.4] 18.6 x 11.4 [14.6; 15.3] 18.6
  Q = 0.9 [1] 1.1; N = 100; C = 95%
  Me = 15 x 15; Qe = 1

  This example shows for Q an interval of the population mean (in green) whose two limits are equal. In this case *Piximeter* replaces these two terminals with one. On the third line Me and Qe (e for sample) are the average values of the measurements, as in the case of the classic formulation.

- The **concise form,** like this, at the user's choice:

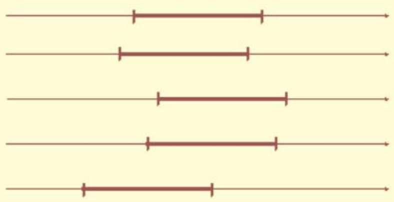
  11.4 [14.6 *-15-* 15.4] 18.6 x 11.4 [14.6 *-15-* 15.3] 18.6
  Q = 0.9 [-1-] 1.1; N = 100; C = 95%

Here the limits of the population mean interval (in green) only appear if they are different from the mean value.
The expression for Q in this example is characteristic and replaces the form [1 -1- 1]. Increasing the number of decimal places in the result can change this expression.

Noticed
With other samples of the same size we would obtain new confidence intervals of this mean m, with the same confidence coefficient. All would have the same amplitude:

$$2 \times 1{,}96 \frac{\sigma}{\sqrt{n}}$$

Here are some examples :



If one were to take a very large number of such samples, about 95% of them would contain the unknown mean **m** of the population. In fact, we only take one and we cannot know with certainty whether or not it contains the number m, but the method implemented makes it possible to obtain a "good" interval in 95% cases. A "good" interval contains m.

Mathematical formulas

1. Sample

Let **n** values of the quantity studied (length, width and quotient) we obtain the arithmetic mean and the standard deviation m_e by the formulas σ_e

classics:

$$m_e = \frac{1}{n}\sum_{i=1}^{n} x_i \qquad \text{And} \qquad \sigma_e = \sqrt{\frac{1}{n-1}\sum_{i=1}^{n}(x_i - m)^2}$$

2. Total population o

Point estimate

Average $m = \frac{1}{n}\sum_{i=1}^{n} x_i$ and standard deviation ÿ $= \sqrt{\frac{1}{n}\sum_{i=1}^{n}(x_i - m)^2} = \sigma_e\sqrt{\frac{n}{n-1}}$

o **Interval estimation**

k(c) = 1.96 at the 95% threshold and **k(c) = 1** at the 68% threshold.

ÿ Confidence interval for the magnitude values:

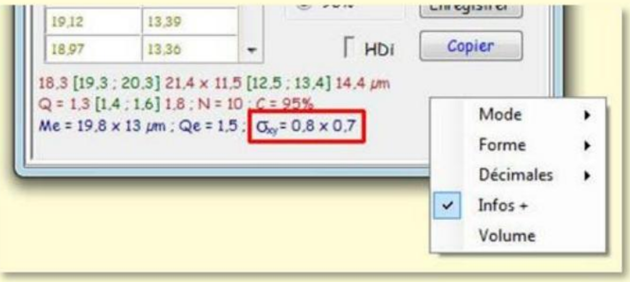
$$\mathbf{m_e} \pm \mathbf{k(c)}\boldsymbol{\sigma} = \mathbf{m_e} \pm \mathbf{1,96}\ \boldsymbol{\sigma_e}\sqrt{\frac{n}{n-1}}$$

ÿ Confidence interval for the mean of the magnitude values:

$$\mathbf{m_e} \pm \mathbf{k(c)}\ \frac{\boldsymbol{\sigma}}{\sqrt{\mathbf{n}}} = \mathbf{m_e} \pm \mathbf{1,96}\ \frac{\boldsymbol{\sigma_e}}{\sqrt{\mathbf{n-1}}}$$

Access to statistical parameters

Piximeter also provides **the value of the standard deviation** calculated on each sample. This information is added to the dimensional formula as shown, boxed in red, in the figure opposite.



This information is only available on the statistical formula, by **Right Clicking on the formula** and **"Infos** +". It is copied with the formula.

Sporal volume

Concerning **the study of mushrooms,** certain recent publications refer to other parameters, including **sporal volume.** Jean-Louis JALLA, experienced mycologist, drew our attention to the interest of adding it to the dimensional formula calculated by *Piximeter.* It is certain that this data is very significant and varies a lot between close species.

The formula for calculating the sporal volume currently used in mycology tends to assimilate the spores to **ellipsoids of revolution,** which is of course only a theoretical view. This volume will be even more accurate as the shape of the spores comes as close as possible to this perfect structure. For real shapes, the calculated volume will therefore be **subject to a significant ± error,** depending on their distance from the ellipsoid.
However, it can still serve as a **reference** provided of course that it has always keep in mind the limitations of the method.

The formula for calculating this volume is $\frac{4}{3}\ \pi\ (\frac{l}{2})^2\ \frac{L}{2}$ where l and L represent respectively

the small and large dimensions of the objects measured.

Be careful however, an error in measuring dimensions has significant repercussions on the volume. For example, for a spore measuring **8.5 x 10 µm** the calculated volume is **378 µm3 ,** it becomes **445 µm3** for simply **0.5 µm** of excess error on the two dimensions. The interest of a study involving a large number of measurements is obvious here, the random component of the measurement error is weighted by the average. It is also obvious that systematic measurement error (for example Piximeter calibration error) always exists, hence the interest in the most rigorous calibration possible.

Piximeter calculates this sporal volume and processes it according to the chosen display method **(classic** or **statistical)** to add it to the dimensional formula which takes, for example, the following form:

The volume **V** is added, here in classic form, with the average volume **Ve** calculated on the sample.

The **context menu** (right click on the formula) allows you to display or not the volume, as shown in the image.

All information displayed by the *Formulator*

The buttons are copied or saved by

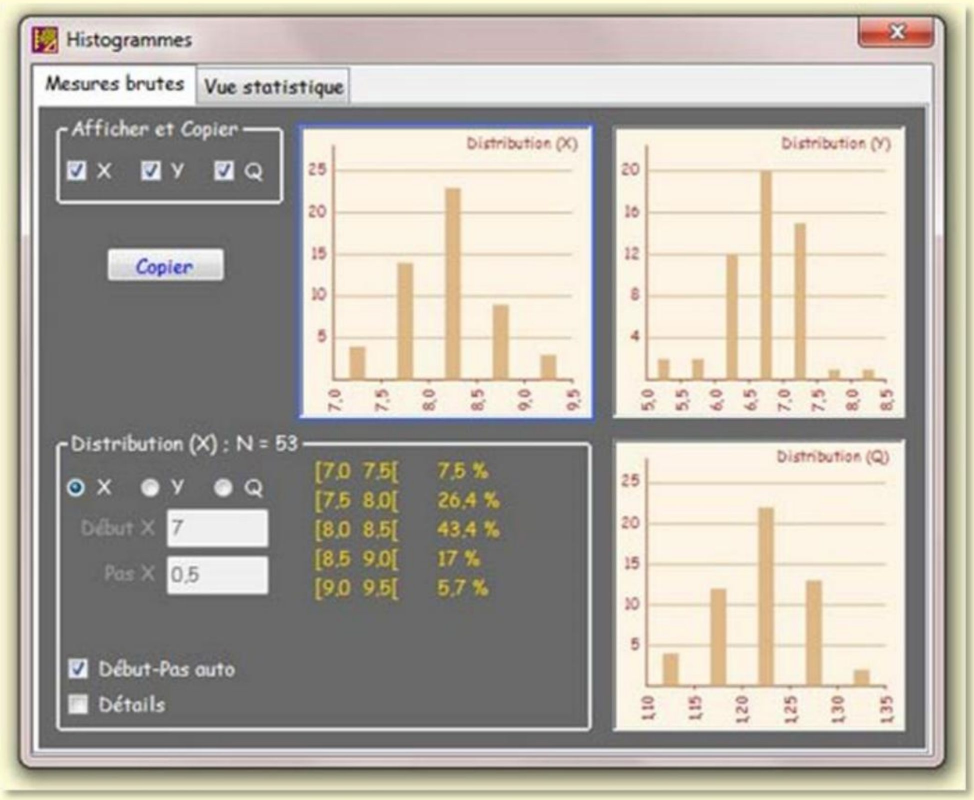
correspondents, like other data.

Histograms of measurements

The floating window below is activated by pressing the mouse on **"HDi".** It provides additional data on the measurements carried out:

- The graphic and numerical presentation of the **raw measurements** of the X and Y axes and their Q ratio,
- Their classification for **statistical interpretation.**

Raw measurements



***Histogram** of raw measurements.*

The **"Raw measurements"** tab shows the distribution histograms of X, Y and Q. By default the three histograms are displayed but it is possible to select only some of them. The **"Show and Copy"** frame is reserved for this purpose.

At the bottom, the **"Distribution"** frame displays the characteristics of the selected distribution (framed in blue – here X). It is possible to select a distribution by clicking on its histogram.

When the **"Start-Auto step"** box is checked, *Piximeter* automatically chooses the data range explored and the resolution step. When unchecked, the user can change these settings.

In yellow are the selected intervals and their population.

The **"Details" box,** when checked, displays the numerical values within each interval.

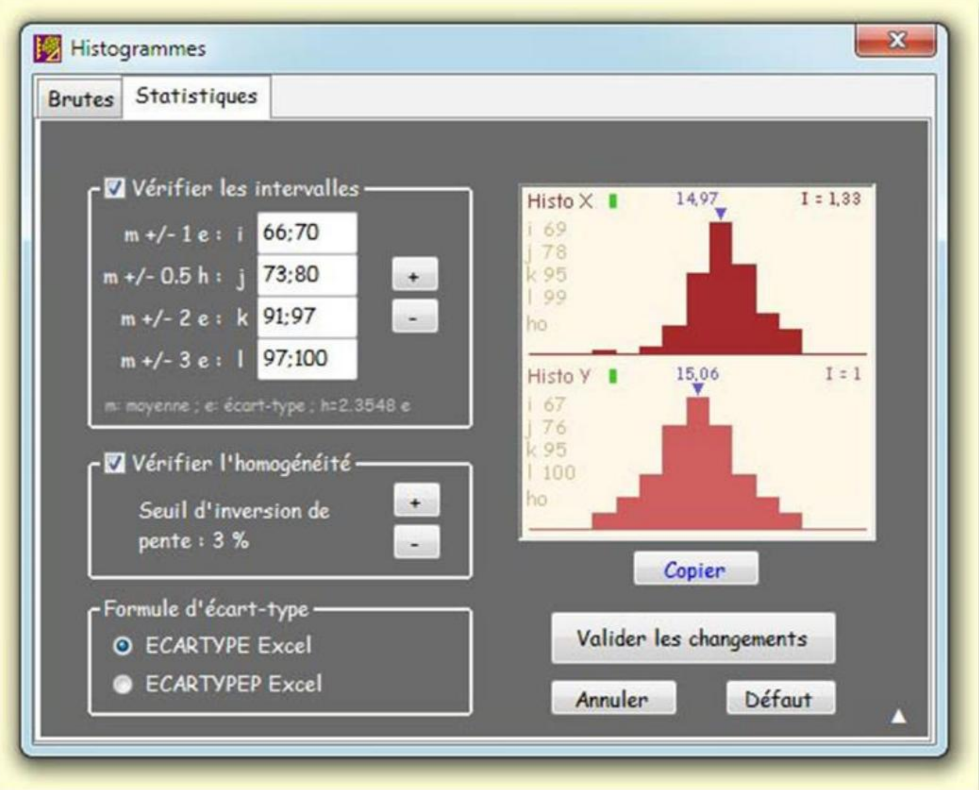
Finally, the **"Copy"** button places the selected histograms and numerical data on the clipboard. The data can thus be pasted into a Word document, for example. Below is an example of a document after layout on three columns in Word.

The **"Details" box,** when checked, displays the numerical values within each interval.

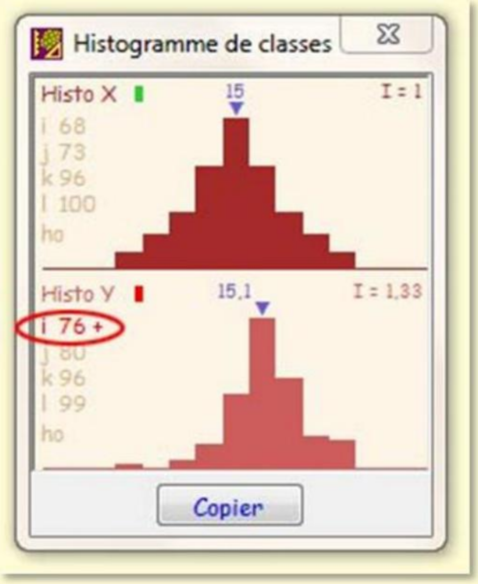
Finally, the **"Copy"** button places the selected histograms and numerical data on the clipboard. The data can thus be pasted into a Word document, for example. Below is an example of a document after layout on three columns in Word.

Statistical results

Expanding the **Statistics** tab (ÿ) provides access to several elements that control histogram conformance testing.



Statistics and normality tests.



In the figure on the left the histogram is **normal** in X but **not** in Y.

Experience shows that in mycology, for the measurement of mushroom spores, it is not uncommon for carrying out a large number of measurements (100 - 200, or even more) to result in **the famous bell curve of the normal law,** which further validates the results of the statistical formulation. *Piximeter* is capable of easily managing a very large number of measurements.

The samples obtained are divided into **classes** (typically nine classes) materialized by histograms **(figure** opposite).

As indicated above, the red interval [Min, Max] **of the statistical formula** is only valid in the case of a **normal distribution** of the sample values, while the green interval [m; M] him, is **valid** whatever this law.

***Piximeter* checks the normality** of the distribution law of the samples and turns on the **green light** from the *Formulator* when this is the case. It implements **two complementary methods** to verify this normality:

1. **The so-called interval method.** It is established that a normal distribution law contains :
   - o 68% of measurements in the interval m ÿ ÿÿ
   - o 76% of measurements in the interval m ÿ ÿÿÿÿÿÿ ÿ,
   - o 95% of measurements in the interval m ÿ ÿ ÿ,
   - o 99% of measurements in the interval m ÿ ÿ ÿ.

   Where **m** is the mean value of the sample and ÿ its standard deviation.

   In practice *Piximeter* accepts a certain tolerance on these values 68%, 76% etc. and considers the normality acquired if the content of each interval is located within a determined range, around the target value. By default, these intervals are: [64%, 72%], [72%, 80%], [91%, 99%] and [95%, 100%].

2. **Checking the homogeneity** of the histograms. This check ensures that the content of the classes is regularly increasing, then decreasing beyond the median class. Here too *Piximeter* allows a certain tolerance: a reversal of growth (or decrease) is only taken into account beyond a
   certain threshold, corresponding to a defined percentage of the sample (by default

5%). Clearly, a slope reversal of less than 5% (in this example) is not counted as such.

The result of the checks is **reported on the histogram** (above). The contents of the four intervals above are denoted i, j, **k** and l respectively. Homogeneity is noted **ho.** These ratings are reported in red when they are outside the defined tolerance limits. The light in each histogram is only **lit green if the sample is normal.**

The General *Formulator* LED **only turns green when the X and Y samples are NORMAL together.** It lights up orange if only one of the two is normal and, otherwise, remains off.

On the Y histogram in the figure above, **i 76 +** (in red) means that the interval i (m ÿ ÿÿ of sample Y has **76% of the sample values,** which is more (+)
as defined (default [64%, 75%]). The corresponding law is probably not normal although the histogram is homogeneous (ho is not red). As a result, **the histogram light lights up in red,** which means that the general light of the *Formulator* does not light up in green but in orange (in our example, since X is green).

Please note: The interval provided in red by *Piximeter,* in its statistical dimensional formula, is only valid in the context of a normal population attested by a normal sample. It is understood, on the other hand, that **the interval on the average is always valid** since there is **a normal law for the averages.**

In the case of **measuring a single dimension** (X or Y) *Piximeter* displays the indicator based on the only significant sample.

The interested user can refer to detailed developments presented on Wikipedia.

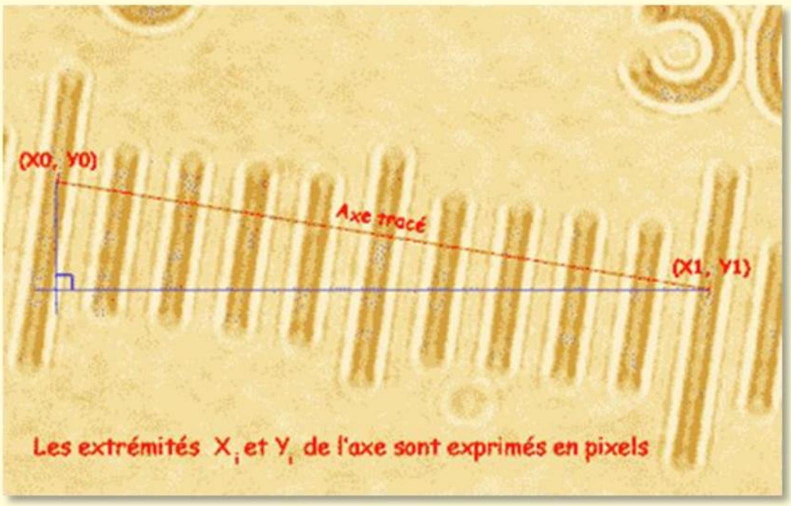
It is also possible to choose the standard deviation calculation formula used. *Piximeter* uses the Excel formulas: ECARTYPE and ECARTYPEP explained above, respectively when it comes to analyzing a sample of the total population, which is the general case in mycology, or the entire population in other cases. By default, **EARTYPE** is used.

Note: We advise uninformed users not to modify these settings.

Piximeter calibration

Convert pixels to length unit

The calculation of the length of an axis drawn on an image on the screen is first carried out in pixels. The two ends of this axis are determined by their coordinates (x, y) relative to the screen (in reality relative to the window which supports the image, but this is only a question of origin because the The unit remains the same: **the pixel).**
The axis is therefore **the hypotenuse of a right triangle.** Its length is calculated by a square root (Pythagorean theorem).



The goal of calibration is to establish a **relationship between pixels and chosen unit of length** (for example micron). To do this, simply measure an object in pixels whose length we know perfectly in this unit!

The introduction of a **magnifying glass** which allows the scale to be expanded or contracted corresponds to the modification of this relationship by application of the multiplying coefficient of the magnifying glass.

Very precise length measurements, commonly with less than 2% error (often with only 0.1 to 0.2%) can be carried out with *Piximeter* provided that a precise prior calibration has been carried out.

When images are obtained using variable focal length (zoom) cameras, **two independent operations** are necessary to fully carry out this calibration:

1. **Automatic autofocus initialization** for each camera used.
   This operation aims to allow the calculation of the image magnification for each zoom position used when shooting,
2. **Length calibration** itself, which aims to establish the pixel-length unit relationship.

These two operations, of a different nature, are independent and can be carried out in any order. You will only perform the first operation once for each camera. The second can be repeated to define several standards (see below).

When images are obtained from **fixed focal length** devices (lensless cameras or certain video cameras) or when they do not contain the data necessary to **identify the shooting conditions** (metadata), only the Length calibration is possible and necessary.

The ***Parameters*** tab of the *Piximeter* calculator allows you to specify the nature of the images used, **Normal** (with metadata) or **Compressed** otherwise.

It is strongly recommended to read this note relating to optics and measurement errors.

Initializing automatic autofocus

Routine measurements, as well as length calibration, are carried out on photos taken with a digital camera(s). The **intrinsic magnification** of the image depends on the zoom position used when shooting, all other things being equal as to the shooting conditions.

The aim of initializing *Piximeter* 's **automatic autofocus** is to precisely establish the **mathematical function** which allows the calculation of this magnification (see this operation here).

Piximeter uses the metadata contained in the photos (but invisible to the eye) to know the focal length used when shooting. We should therefore be careful **never to compress the images** intended for it. In fact, this operation can delete the metadata they contain if the compression rate chosen is high. Furthermore, *Piximeter* easily manages very large images (the only limitation is the memory capacity of the computer used).

This autofocus initialization operation being carried out once and for all, for each camera used, all photos can now be correctly interpreted, **whatever the focal length used** for their shooting.

Piximeter 's autofocus handles **any number of different cameras.**
Also, to work on images from several of them, it will be necessary to first perform this initialization for each of them.

Piximeter also offers the possibility **of directly importing the mathematical functions** in question, calculated and exported elsewhere (see here). This possibility is useful when a user wishes to work on images that have been taken by others, with a camera that they do not have.

Remember also that *Piximeter* **visually** indicates the operation of its autofocus on the images it processes (see here).

Length calibration

Which **reference object** to choose and how to measure it to calibrate *Piximeter ?* The answer to the first part of the question is simple: **any object** visible through the optical system used (for example the microscope) and **whose real length is perfectly known !** This reference object will be chosen such that its length is **in scale** with the subsequent objects to be measured. For example, if you want to measure mushroom spores of the order of magnitude of ten microns, you will choose as a reference standard a ruler or an object of this order of magnitude rather than a double decimeter.

As for the second part of the question, it is enough to photograph this object **for a defined state of the optical system.** The *Piximeter* calibration is then carried out:

1. By drawing an axis on the image of this object of known length (figure above),

   And,

2. By manually indicating the exact length that this axis represents.

This operation is carried out using the Piximeter **calibration wizard .** Note that if you have previously initialized autofocus for the respective camera, you can use **any zoom** for shooting here. The correction will be carried out automatically. You can also use **the magnifying glass and side-to-side image movement,** essentially to magnify and position the image on the screen and thus increase pointing accuracy.

Consider for example the case of a microscope:

1. You photograph through a calibrated micrometric eyepiece, meaning **you know precisely the value of each division of its ruler**
   for the microscope objective used. The image of this ruler appears in the photo and the calibration of *Piximeter* is done by drawing an axis on all or part of this ruler (figure above) then indicating the exact length that it represents.

2. You photograph with a microscope without an eyepiece or through an eyepiece without micrometric, that is to say that you do not have a graduated scale on the image. *Piximeter* must then be calibrated independently of these images, using a graduated reference ruler **placed directly under the** microscope objective. This is a different rule from the previous one. It is called *an object micrometer.* It generally comprises a series of divisions spaced 10 µm apart and is classically used to calibrate optical microscopes. Here also the interval between graduations is precise and perfectly known. We therefore take a photo of this rule and we proceed as in the case above.

It is important to note that in either case, whether you are photographing through an eyepiece, micrometric or not, or directly behind the lens, and all things being equal, in the photo the value d 'a division of the graduated reference rule **depends on the objective used on the microscope,** hence the notion **of state of the optical system** introduced previously. Calibration is only valid for a specific lens.

Pimeter offers the possibility to **define and use any number of standards** corresponding to different states of the optical system, for example the use of different objectives on a microscope.

The Calibration Wizard

The calibration wizard is activated by the **"Calibrate"** button in the image measurements window or by the **"+"** button to its right. This **"+"** button initiates the first step of the process. The **"Calibrate"** button is a shortcut which leads directly to the second step. The first step (figure opposite) triggered by the **"+"** button specifies **the operations** to be carried out:

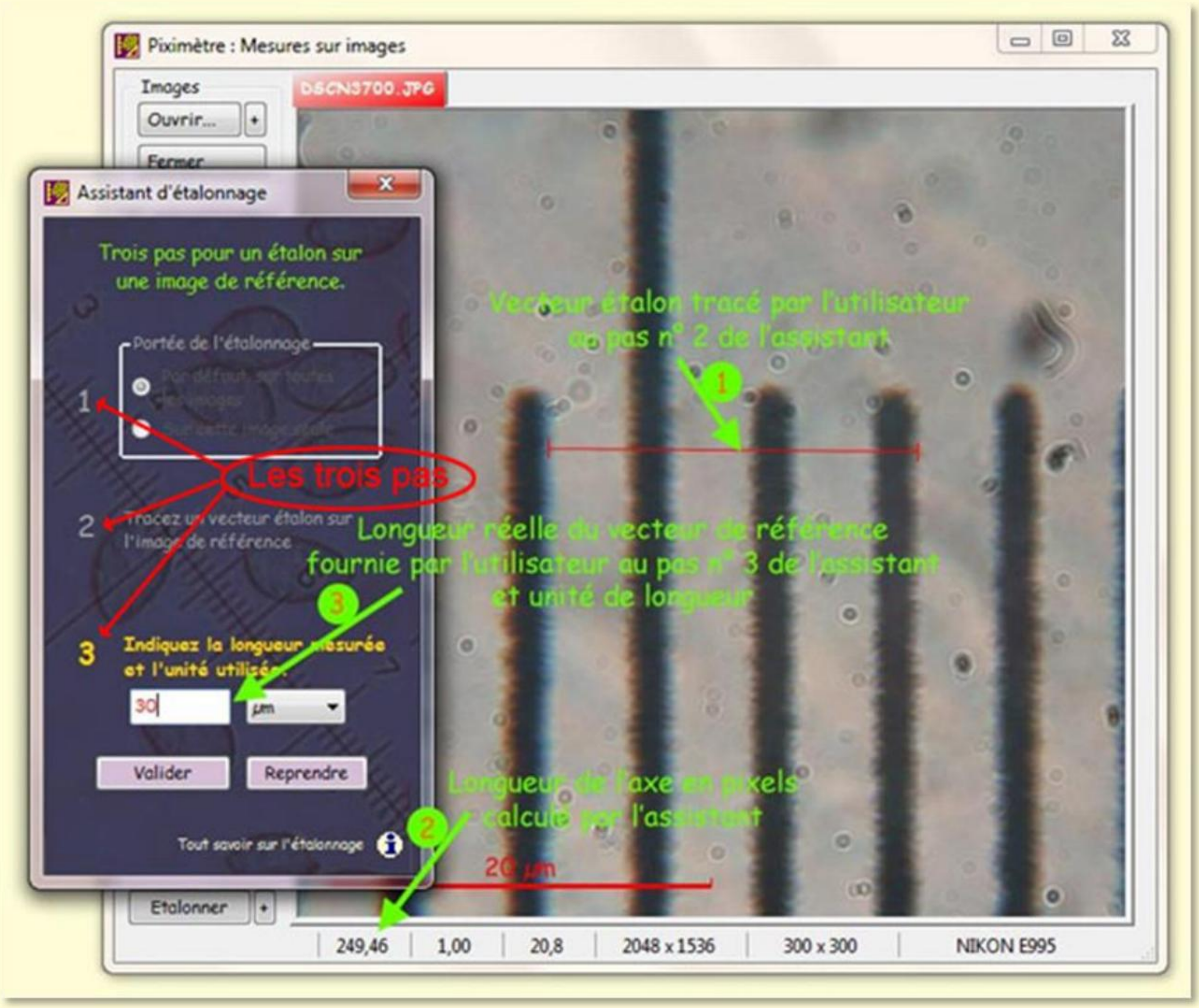
Create or **modify a standard.** You will see later that there are two types of standards and that it is possible to name some of them in order to *facilitate their use and to be able, if necessary, to modify them.*

***Delete an* existing standard.**

activates the tutorial for Calibration icon.

The second step depends on the choices that are made. If create or modify is selected, the wizard helps you define a standard. If deletion only is selected it takes you directly to this operation. If both choices are selected, the wizard first helps you create (or modify) one standard and then delete another.

Let's see the calibration step, **in three steps.**



The calibration process in step #3.

The calibration process involves three successive steps as shown in the figure.
You can **open the reference image** before or after activating the wizard, or even change it during the process and, possibly, resume it at step 1 ("Resume" **button).**

- In step number 1 **you choose the scope of the calibration.** You can decide that the standard will be specific to this single image or, on the contrary, generic, that is to say applied by default to all images, **open or future,** which do not already have a specific standard (see below). below). This is where the two types of standard appear: **specific** or **generic.**

- In step number 2 **you draw an axis** on the ruler or the object of known length, present on the image. Note the display of the length of this axis in pixels (249.46 in the figure). Also note that better precision will be obtained by choosing **an axis of great length** (reduction in the relative error inherent in the measurement), subject however to perfect linearity of the image.
  See here about it. You can also **use the magnifying glass** which allows you to improve the

pointing precision as well as **lateral movement of the image on the screen** between each of the two points.

- In step number 3, finally, **you indicate the real length** of this axis in the appropriate unit (30 microns in this example where the object micrometer is worth 10 µm per division).

The calibration wizard, by default, offers **different units of length** but here you have the possibility of defining new ones if those proposed are not suitable. The **"- Edit -"** choice available in the drop-down list gives access to this functionality.

Validation completes this **process** when it comes to a specific calibration, **on that image alone.** The new standard is assigned to the image and the axis is cleared on it.

In the case of a generic calibration, **applicable by default on all images,** the **"Validate"** button presents a new step in the wizard asking you to **name this standard** (figure opposite).

Managing **several generic standards** requires their naming, which allows their identification.

For example, you can define a different standard for each microscope objective.
Here, the new standard is named **"X100-995"** because the image was taken under an X100 microscope objective, with a Nikon 995 camera.

A list shows **the generic standards** already defined. One of them is permanent **"Unit (UN)",** the others have been defined by the user. The selection of an existing standard allows its redefinition.

The **"Finish"** button validates the standard and completes the process. The new generic standard is assigned to all the images concerned and, of course, the *Formulator* and the *Comparator* are updated.

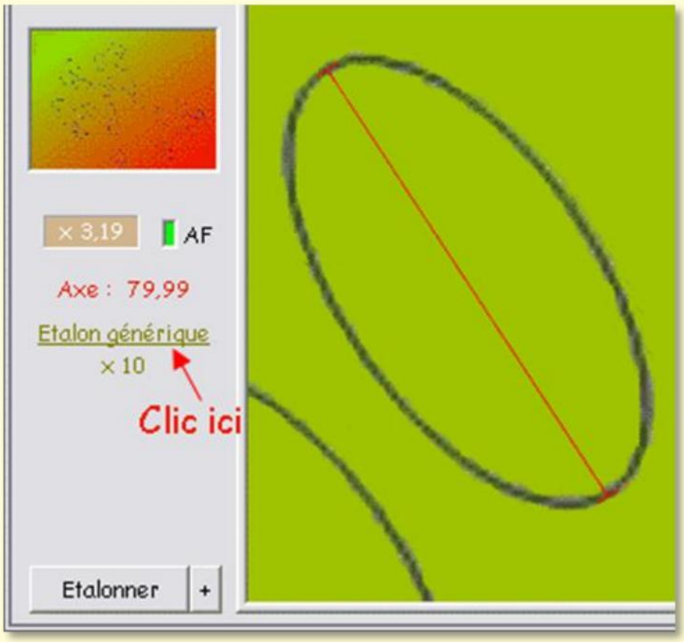
If the named standard already exists, the wizard can, under the user's control, **replace its value with the new one.**

The **"Delete"** button allows you to delete a selected standard from the list.

Choosing a generic standard

Standards are created as shown above.

It is **in the image measurements window** (figure below) that the user chooses the generic standard which will be applied to all images currently open or future and not already referring to a specific standard. Remember that this standard must be chosen **depending on the state of the optical system** used when taking the image, in other words, in the case of a microscope, the objective used (one for the x40 and one other for the x100, not to mention the eyepiece or the camera used).



As shown in these figures, a mouse click on the **"Generic Standard"** (or "Specific Standard") link brings up the list of available standards and allows you to choose one (right view).

It will be applied to all open or future images which use a generic standard, i.e. which do not refer to a specific standard.

The selected generic standard **remains valid** until changed by the user

Generic standard, specific standard

When an image is **first opened ,** *Piximeter* has **no information about the optical system** that created it. By default therefore, this image is assigned the **generic standard** which is currently selected. This corresponds to the general case where most of the images come from the same optical system (for example they were all photographed with the same microscope objective).

The user must **change the standard** or perform a **calibration specific** to this image if this is not the case.

Piximeter 's **active memory ,** when activated, allows you to memorize all the parameters of an image when **it is closed :** its position on the screen, the magnifying glass used, all the axes drawn, etc. and, in particular, the name and value of the standard applied to it and which allows the exact calculation of the length of the axes.

When **it is subsequently reopened,** if the active memory is still engaged, the image regains all its parameters and is displayed on the screen in the exact state it was in when it was closed. The length standard which allowed the calculation of the axes is necessarily restored, regardless of the standards then defined in the system. **It becomes SPECIFIC to the image.**

Thus, when it is first introduced into *Piximeter,* **an image is assigned by default a generic standard,** which the user can keep or, if necessary, change.
But, upon its subsequent reopening, this standard, whatever it was, **becomes specific to the image** and can no longer be affected by a possible modification of the generic standards; his name is preserved. This principle makes it possible to **guarantee the preservation of the measurements** carried out on the images when they are subsequently reopened.

In the case where the user **deletes or redefines a generic standard** that he has previously created and used, all the images in active memory that refer to it are transformed: the value of the standard is of course preserved but **its name is modified by the addition of the prefix "ex",** since it no longer exists as is. When it is reopened with active memory engaged, **the image regains the value of its initial standard** but the name of this standard is then prefixed by "ex".

LiveView Video Assistant

Principle

The LiveView video assistant allows *Piximeter* to connect directly to the **cameras** installed on the computer, to view the images they produce, to make the main adjustments of light, contrast, etc. and, finally, to capture images to then carry out the desired measurements.

Images captured by the wizard are **automatically opened in the *Measurer*** which ensures all standard metrology operations, which are here identical to those carried out on images from other media, such as cameras for example.

It is necessary to establish a **generic standard** for each optical system implemented - for example for each objective used on the microscope and for each video camera with a given form factor (width/height ratio of images).
Let us repeat here that, to be valid, the generic standard must be **created and used under identical conditions** (same lens, same axial camera position, same image form factor, etc.).

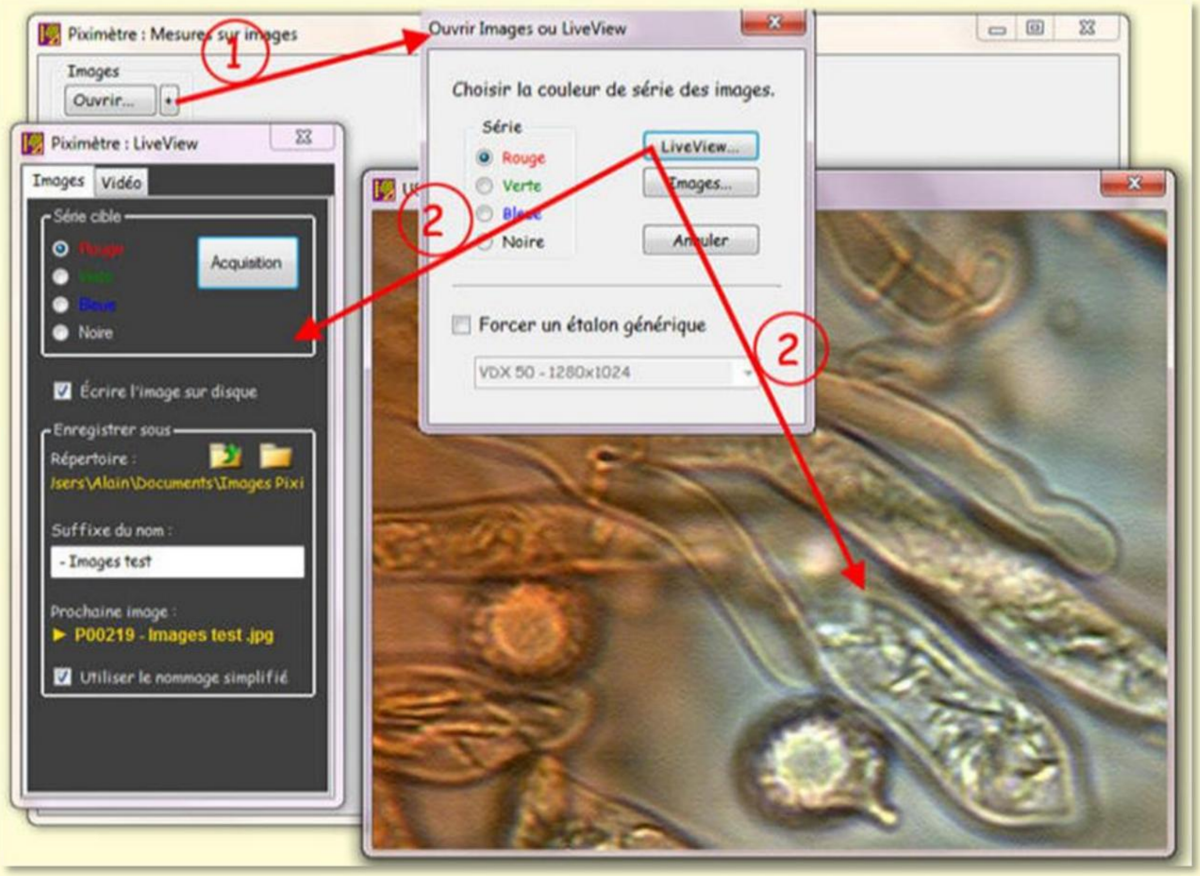
The main advantage, and not the least, of *the LiveView video assistant* lies in the **simplification of image acquisition :** no need to manually transfer images from the camera's memory card or to use image editing software. separate video capture. *Piximeter* provides **a fully integrated image capture service.**

LiveView video assistant supports cameras **installed on the computer.** Their installation must, of course, have been carried out beforehand using the specific software provided by their manufacturer. Furthermore, to be usable by *Piximeter,* the cameras must meet **recent standards,** in particular in terms of their integration "driver" under Windows. A camera that is too old or does not meet these standards will not be usable. *Piximeter* and its *LiveView video assistant* are **32 and 64 bit compatible.** But be careful, not all drivers are! Some cameras only work on 32-bit versions of Windows.

The images produced by the cameras generally do not contain the metadata which specifies their acquisition conditions. However, so that they can be processed by the *Measurer, Piximeter,* from version 5.3, **adds the necessary metadata to the images :** name of the camera, image dimensions and fixed focal length.

The advantage of this camera identification lies in the **consistency check** carried out by *Piximeter* between measured images and standards used. Images from different sources cannot be unintentionally measured by the same standard without giving rise to a warning message. Reducing sources of error is always for the benefit of the user.

Implementation



Opening of the ***LiveView video*** assistant *by the Measurer.*

The LiveView video assistant is opened using the **"Open** +" button on the *Measurer* which activates an **intermediate window,** as shown in the figure above (1).

This intermediate window allows you to **specify a target series** for the images that will be captured. Otherwise, the images will be sent in *the active series* of the *Measurer.*
It also allows you to **choose a generic standard** different from the one currently selected.

When these parameters are set, the **"LiveView..."** button activates *the LiveView video assistant* (2).

The wizard has **two windows** which remain in the foreground: The smallest (black), of fixed size, is the control window which allows you to specify the capture parameters (see below). The second, of **variable size, adjustable with the mouse wheel,** receives the video from the camera. These two windows can be moved as desired on the screen.

The wizard closes when the user clicks on the red cross typically located at the upper right edge of one or the other of the two windows.

The wizard **control window** has **two tabs** which allow you to choose the camera, for one, and to acquire the images for the other. All settings are retained from one activation to the next.

The "Video" tab , opposite, allows you to **select the desired camera** among those connected to the computer and recognized by the assistant. If the camera is not connected at this time, simply close the wizard, connect the camera and reopen the wizard.

The **"Image size"** choice allows you to choose the size of the images that will be produced by the camera (they can generally produce several image sizes). Note, however, that **the captured field remains the same** regardless of the image size chosen. Only the definition of the images changes. Also note that the standards are only valid for a given form factor (width/height ratio).

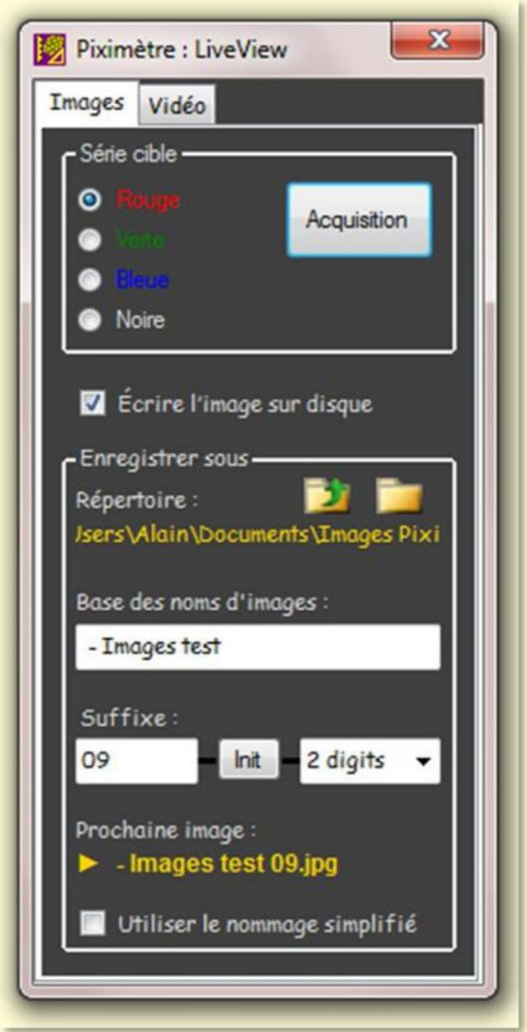
It should also be noted here that the wizard **shows exactly the image** provided by the camera, regardless of the size of the video window. The proportions are preserved.

The **"Adjust settings"** button gives access to the settings available on the camera: light, contrast, gamma, exposure, etc.

The "Images" tab below allows **images to be acquired,** named and saved to disk. Images are captured using the **"Acquisition" button.**
They are **transmitted to the** Pixímeter , *in* the target series which is specified (Red, Green, etc.).

In mycology, for example, one series may be devoted to measuring spores while another may be devoted to measuring basidia or cystidia.

Note in passing that a check box specifies whether or not the captured images must be **saved to disk** by *the LiveView video assistant* before their transmission to the *Measurer.* In the event that recording on disk takes place, the images can be re-opened later by *Piximeter* and, as with all images processed by it, the measurements carried out on them will be instantly found. Otherwise, the images not written here on disk will be presented to the user before their closure by the *Measurer* in order to allow a final backup



The **"Save as"** box ensures the naming of the images and that of their destination directory (we use folder interchangeably).

Opens the directory selector **from current of** there **position** : *"...Alain\Documents\Images **Pixi"*** in the example in the figure opposite (or *"My documents",* in the absence of a defined directory).
The user has the option of conventionally creating new subdirectories or navigating through the accessible directory structure of his workstation.

Offers the same possibilities as above but opens the directory selector from the position **preceding the current position** (its root) : *"...\Alain\Documents* " in the example.

The wizard offers **two image naming modes** (how image names are constructed): a **"standard"** mode and a **"simplified"** mode which is activated by the **"Use simplified naming"** check box (see below). against).

The tab presents, behind the symbol, **the name of the next captured image,** i.e. **"- Images test *09.jpg"*** in our example, which will be saved in the selected directory ***"...\Alain\Documents\Images Pixi".***

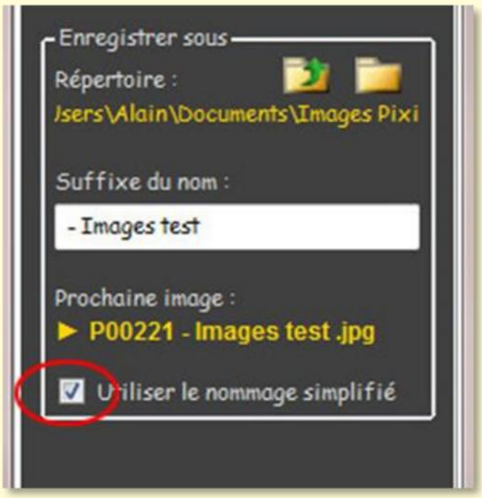
Standard naming

The name of the created image is composed **of a typically alphabetical base** followed **by a numeric suffix** as shown in the Wizard figure above. It is possible to modify these two parameters by replacing them with new values.

The number of digits of the suffix is adjustable. It can't be zero. The **"Init"** button resets the suffix to value 1.

This naming method is also found in *the Image Export Wizard* , which allows you to save enriched images to disk. Both assistants refer **to the same selected directory ,** which ensures their complete homogeneity.

Simplified naming



The figure opposite shows the presentation of the **"Save as"** frame in simplified naming mode.

The destination directory remains the same. The composition of the image name changes: the name is formed by a **unique prefix** (P00001, P00002, etc. - P00221 in the figure), cannot be modified by the user and which progresses automatically with each image capture, followed by a **suffix of the user's choice** (- Test image in the example).

This simple mechanism ensures both the uniqueness of names and precise identification of images.

When operating in this naming mode, the wizard, when opened, offers the user the choice of continuing to save images in the same directory as previously, or changing it. This mechanism is very useful for saving images in a good place on the disk.

Trick

To facilitate the capture of images while manipulating an external device, microscope, telescope or other, the assistant also captures images by simply pressing any key on the keyboard, instead of the "Acquisition" button.

Compatibility of standards

Version **5.3** of *Piximeter* marked a significant development regarding the processing of *LiveView* images : the cameras used are now identified by **EXIF metadata** that *Piximeter* introduces into the captured images, as cameras do. This allows it to carry out a **consistency check** between the measured images and the standards used.

All *LiveView* images captured before this version, and in particular those used to create the standards, did not include this metadata since the cameras do not deliver them. *Piximeter* was then unable to carry out the consistency checks in question.

From version 5.3 ***Piximeter* introduces EXIF in the images,** the cameras are identified, it then becomes necessary to **redefine their standards** on the basis of this new type of images. This is **done automatically by *Piximeter*** when *LiveView images are captured.* A message requests user approval.

If the user refuses to update the standards concerned, their use remains valid but there will be no consistency check possible.

For all practical purposes, here is a quick reminder on how to proceed to create a standard from a microscope and a camera installed on it.

Create a standard in LiveView

Calibration consists of introducing **a length standard** (a standard ruler) into the optical system (i.e. an object micrometer in the case of a microscope) and measuring it in pixels in order to obtain a reference called **a standard.**

A generic standard can apply to all images with the same width/height ratio as that of the image which allowed its definition.

The first step consists of **obtaining an image of this standard** for each state of the optical system (i.e. an image for each objective used, in the case of a microscope).

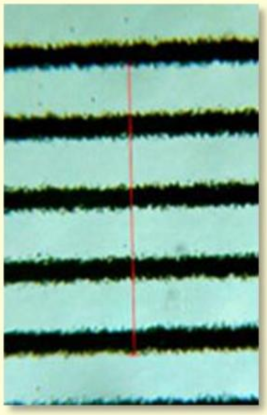
When acquiring images by the camera, **two recommendations** must be strictly followed:

- The **axial position** of the camera on the optical system (i.e. its height) must remain strictly invariable for all images,
- The **width/height ratio** of the images provided by the camera must remain constant (in general several sizes are proposed), or define a generic standard for each of them.

Perform calibration

1. Place an **object micrometer** on the microscope stage and make the adjustment " at the eye ",
2. Open *Piximeter* in **Graphics mode** and choose “Normal Images” (tab Formulator *Settings),*
3. Activate **the Video Assistant,** refine the sharpness settings if necessary and acquire an image of the object micrometer. The image appears in the Measurer.
4. Close the Video Assistant,
5. Activate *the Piximeter* Calibration **Wizard** (“Calibrate” button):

    - Step 1 : choose **“On all images”,**



- Step 2 : precisely trace **a reference axis** on the standard rule visible in the image (figure opposite),

- Step 3 : indicate **its real length** (which you must know - on an *object micrometer* each division corresponds to 10 microns), and **validate,**

The Assistant then asks you to **name this standard :** give a name, for example *test,* or better for mycologists, a name related to the camera, the objective of the microscope that is used and the size of the image, for example **"CX40 1280-1024 Q=1.25"** and **finish.**

6. Close the image.

You have just defined a **generic standard** which will apply by default **to all the images that you will** subsequently open and which will be taken in the same state of the optical system (the same lens and the same camera in the same position).

In practice, for a microscope, define **a generic standard for each objective** and for each image size likely to be used, for example:

CX10 1280-1024 Q=1.25 for lens 10, with Q=1.25 image X/Y ratio.
CX40 1280-1024 Q=1.25 for lens 40
CX100 1280-1024 Q=1.25 for lens 100
CX100 1280-960 Q=1.33 for 100 lens on 1.33 aspect ratio

Note for ***MOTICAM cameras***

The driver from the ***Moticam* camera** manufacturer is an exclusively 32-bit driver.

On certain 64-bit PCs, *Piximeter* , which then operates in 64 bits, does not detect it and the connected cameras are not recognized.

To resolve this 32-64-bit compatibility problem, download the special 64-bit version of the driver (available on the Piximeter online site) and **manually copy the file** into the **Windows/ System32 system directory,** without changing the name (MUCam32. dll). You need “Administrator” rights .

Unfortunately, this operation cannot be carried out automatically by *Piximeter,* for security reasons.

Exporting images

The aim of export is to save on disk images (or parts of images) identical to the initial images but **enriched with additional information :**

- the plot of the measurements carried out on the images (axes and digital data), • the scale associated with the standard used,
- the author's signature,
- the dimensional formula corresponding to the measurements taken,
- the name of the image,
- free comments.

Principle

When at least one image is open in *Pixímeter,* the context menu (right click on the image) gives access to the **"Export..."** function :

1. **Only the view :** the view is the **part of the image** visible at a given moment in the *Measurer window,*
2. **The whole picture,**
3. **The entire series of images** (all images in the series will then be exported automatically).

The export involves **three successive phases that** are largely automated, in the sense that the settings established for an image apply by default to the following images:

- **Comments phase :** The measurements are already written on the image but here the user adds the textual information they want. Some are available directly at the touch of a button (image name, dimensional formula, author signature - once saved as such). Others are free texts. Many options are offered to define text styles (choice of font, size, color, shadow, opacity, etc.). Adjusting **the scale bar** allows you to adjust it to the actual dimensions of the images.

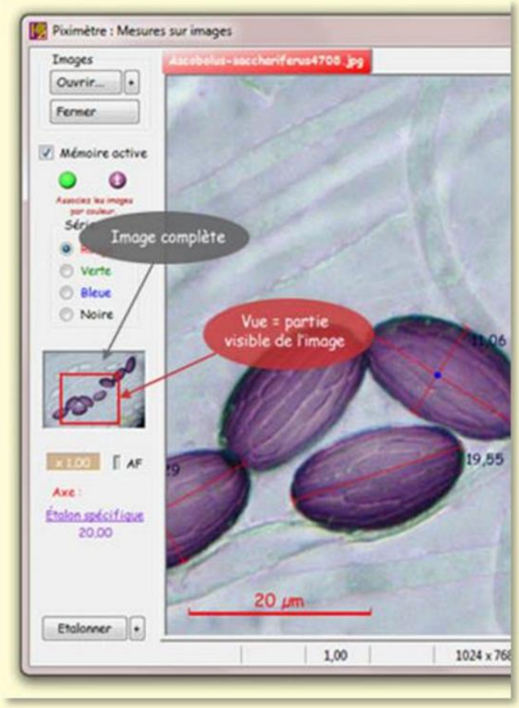
- **Compression phase :** The compression process in JPEG allows you to adjust **the desired level** as well as **the physical dimensions** of the image.
- **Recording phase :** The enriched image is recorded on the disk, under a specific name.

An **Export Assistant** is in charge of executing these three successive phases.
It is activated directly when it comes to exporting **a single image or its view.** A second wizard, called **Group Export Wizard,** supports the export of several images. He appeals to the first.

Initial images are not affected by their export.

Images and Views

Piximeter processes large (very large) dimensional images. These images are displayed in a window (the *Measurer)* of generally much smaller dimensions which therefore only presents part of each image.
This part of the image is called **the view.** It is represented by a red frame in the left *pad* , as shown in the figure opposite.



Moving the view in the *Measurer* window is done by moving the **red frame** on the *pad* using the mouse (keep the left click pressed while moving).

The magnifying glass allows you to enlarge or reduce this frame. It works either with the mouse wheel or by right or left clicking on the red frame.

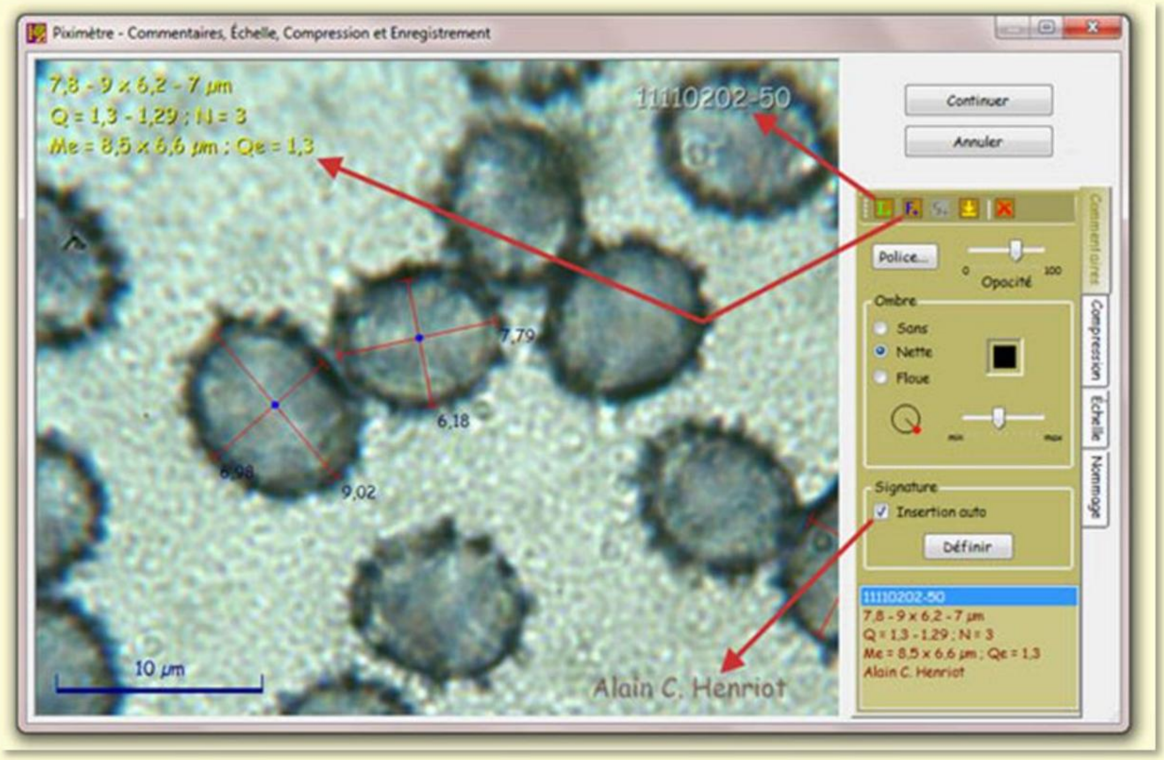
The scale displayed at the bottom left of the window is automatic (20 µm in the figure). It is not integrated into the image, because it would not be visible at all times on the screen, but it **is superimposed on the window,** that is to say on **view** of the image.

The combination of magnifier and view acts as an image crop and makes it easy to isolate visual objects that can be saved via view export.

The elements which will enrich the exported image must have been **previously defined,** that is to say their location on the image must have been specified as well as their format (font, size, color, shading, transparency, etc.). This is the subject of the so-called **comments phase,** carried out by *the Export Assistant.*

We will successively see the Export Wizard (for a single image) and the Group Export Wizard (for several).

The Export Wizard



The Export Wizard *in* the Comments phase.

From the *Measurer :* Context menu, *Export...* **> Image or View.**

The Export Wizard opens in the **Comments** phase as shown in the figure.

On its right side, there are several tabs: ***Comments*** and ***Compression*** which deal with the phases of the same name, **Scale,** which allows you to adjust the scale to the image (see here) and, finally, **Naming,** which allows you to define the naming mode for exported images (see here). The transition from the **Comment phase** to the **Compression phase** is done using the **Continue** button or by directly selecting the **Compression tab.**

Abandoning and closing the wizard is done by the **Cancel** button or by the red cross in the upper right corner of the Wizard.

When the image is larger than the Assistant's working area, it is moved by **holding a left click + moving** the mouse.

The exported image always contains the drawn axes, corresponding to the measurements made in the *Measurer,* their length and their point of intersection as well as the length scale from the standard used for the measurements.

The user is then ready to enter their textual comments as we will see below, or to go directly to compressing the image by pressing **"Continue".**

Add comments to the exported image

A **left mouse click** on the image (without moving) opens a new text for editing which receives all the characters entered on the keyboard. Any text element can also be entered from the clipboard, using **Ctrl+V.** In particular, any special characters, not available on the keyboard, will be placed in the clipboard from a specialized word processing application (for example Word) then entered here using Ctrl+V.

The "carriage return" or "Enter" key ends the current text, as well as clicking outside the text box. Each text can therefore only have one line. It is perfectly possible to **create several texts.**

The **editing cursor,** represented by a yellow vertical bar, advances with each new character. Its position can also be modified using the **left and right arrows.** The **"Back"** key deletes the last character entered (to the left of the cursor) while the **"Delete"** key deletes the one to its right. The **"Start"** (sometimes marked Home) and **"** End" keys move the editing cursor to the beginning or end of the text, respectively.

The text typed on the keyboard is also displayed in **the lower part** of *the Comments tab,* in a list which allows, among other things, to select one or the other text of the image.

Left clicking on a text selects it. Moving the mouse, left click pressed, then moves **the text** to the desired location in the image. A left click on a text, released without moving, **opens it for editing.**

The exported images do not all have the same dimensions, so **the position of the texts** is anchored in two ways, depending **on their proximity to the edges of the image,** namely:

- fixed in relation to the edges of the image, which is materialized by a double line which appears when they are moved or,
- relative to the dimensions of the image, which is materialized by a simple line which appears when moving.

This mechanism ensures **correct placement of texts, regardless of the size of the images.** For example, texts placed on one view only will remain well positioned on the entire exported image, which is in principle larger.

The tool palette located at the top of the *Comments* tab offers several facilities:

-  Automatically inserts **the image name** by creating new text.

- Inserts **the dimensional formula** in the form of three texts corresponding to its three lines.
- Inserts **the user's signature.** This tool is only active if a signature has been defined and the choice for its automatic insertion is not made.
- Applies **the style of the** previously selected text to the selected text. This tool therefore allows you to repeat text **formatting .** It is only active if the two texts have different styles.
- **Clears the** selected text.

These different tools are active or not, depending on the context.

In the *figure,* the name of the image has been inserted by the button (red arrow). The author's signature, previously defined, has been inserted automatically **("Auto insert"** checked).

The ***Comments*** tab also offers various adjustable parameters for each text:

- the font to use,
- the opacity of the text,
- the nature of the shading to be applied,
- his color,
- its orientation,
- its distance from the text.

Finally, the current selected text, with its style and its location on the image can be defined as the **author's signature** and this can be automatically inserted in each image, in the exact place where it was defined, whatever are the dimensions of the image. It is necessary to **define an author signature**
before you can insert it into an image.

Texts created on one image remain **applicable to all subsequent images .**

Examples of creating texts

The **"Comments"** tab of the wizard is selected.

Creating free text

- **Mouse click on the image.** An empty text appears which invites the user to enter characters on the keyboard,
- Enter the text using the keyboard (special characters, not available on the keyboard, will be copied **from the clipboard** using **Ctrl+V),**
- Use the **"Font"** button to choose a font, its size and its color,

- Add **a drop shadow** to the text: sharp, blurry, without; its color, its distance from the text and its position,
- Adjust **the transparency** of the whole,
- With the mouse, **move and position this text** towards the precise location of the image where it will be inserted,
- It's over.

Creating the author signature

- Proceed as for a free text to create and set up the text of Signature,
- If you wish, use the @ button in the *Signature* frame to insert the Copyright character,
- Select the text (left click) and use the **"Define"** button in the *Signature* frame to declare that **this text is the signature.** The signature is then recorded,
- Optionally, check the **"Auto Insert"** box so that the signature is **automatically inserted** on each exported image,
- It's over.

Inserting the image name

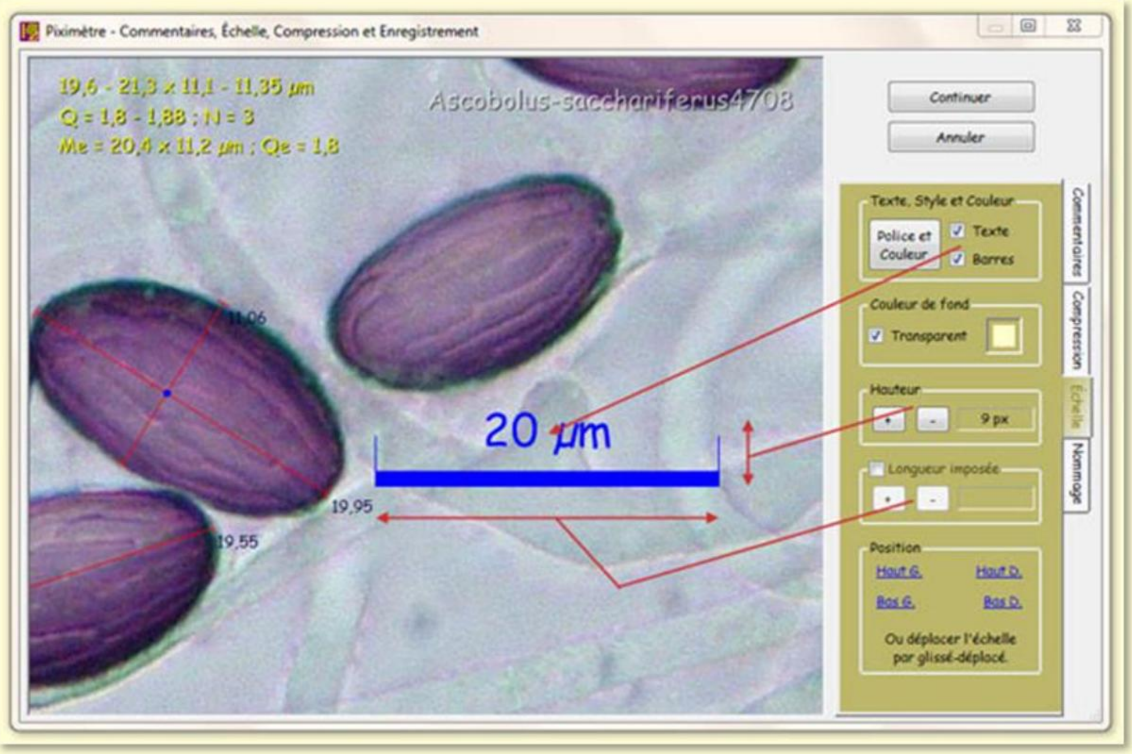
- Mouse click on the image to open empty text,
- Press the tool from the tool palette: **the name of the image is written at the cursor location,**
- Format as described above for free text,
- Using the mouse, **move the text to the desired position** on the image,
- It's over.

Inserting the dimensional formula

- Mouse click on the image to open empty text,
- Press the tool from the tool palette: **the three lines of the formula appear,** one under the other, at the cursor location,
- Click on a line in the formula to select it,
- Format it as described above for free text,
- Just make **a single click** (not a double) on another line of the formula and press the tool from the tool palette. It formats the line like the previous one,
- Do the same for the third line of the formula,
- Using the mouse, **move each line** to its assigned final position on the image,
- It's over.

Positioning of texts and scale on images

Adjust the scale to the size of the images



As has been said, **the scale** which is visible on the initial image, in the ***Measurer window,*** is adapted to permanent vision in this window, that is to say adapted to **the view** of the image, not to the entire image.

However, when exporting an entire image, it is necessary **to adapt this scale to the actual dimensions of the image.** This operation is carried out on the **"Scale"** tab of the Export Wizard (figure above), and only on this tab.
The scale cannot be changed in any other way.

Here the scale can be **resized in height and length** in such a way that it is clearly visible and correctly placed on the exported image. These settings obviously maintain its accuracy. The figure opposite shows the buttons to use for this purpose.

The length of the ladder is only adjustable if the **"Imposed length"** choice is checked. Otherwise, it cannot be modified and keeps the length it has on the initial image, in the *Measurer window.*

The accompanying text (20 µm in the figure) is **adjustable in size, font and color** ("Font **and Color" button).** It is hidden if the **"Text"** choice is not checked.
The **"Bars"** choice draws or not the two vertical bars at the ends of the scale line.

A **background color** is applicable to the scale. It is **Transparent** by default. Otherwise it can be chosen from the standard color palette (here in yellow in the figure).

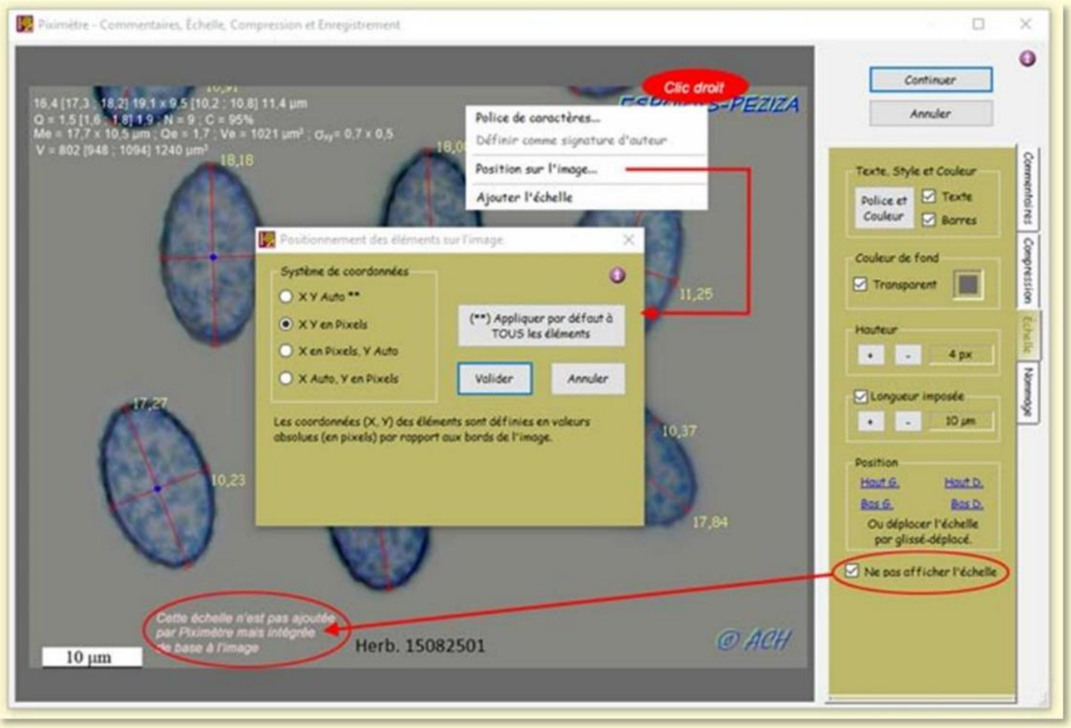
The position of the scale on the image is adjustable. **Four positions are defined by default,** in the *Position* frame but the user can **move the scale at will,** like text, with the mouse.

But the exported images do not all have the same dimensions, so **the position of the scale, like that of all the other texts** placed on the images, is anchored in two ways, namely:

1. **Fixed** in relation to the edges of the image, which is materialized by a double line which appears when it is moved or,
2. **Relating** to the dimensions of the image, which is materialized by a simple line which appears when moving.

As with texts, the scale remains correctly positioned on the images, whatever their dimensions.

Advanced functions



Version 5.9 of *Piximeter* introduced several new features:

1. On the Comments tab , the scale added to the image by *Piximeter* can be **moved directly** on the image with the mouse, without going through the *Scale tab,*

2. On the ***Scale*** tab , a new choice is available **"Do not add scale"** to the image (because the initial image may already include one). By default this choice is unchecked, the scale is added to the image.
3. A **context menu** on the elements added to the image (text and scale) is available which allows you to change certain parameters detailed below.

The context menu on texts and scale

The **context menu** is activated by right-clicking on one of the elements added to the image by *Piximeter, namely :* one or the other line of the *Dimensional Formula,* the name of the image, the signature of the author, the scale introduced by *Piximeter* or even a free text (here, for example, the herbarium reference *"Herb. 15082501").*

The context menu presents **possible actions,** for example changing the font or color of the text selected by the right click, adding the scale if it is not there or, conversely, removing it if it is not useful, etc.

The designation of a text as **an author signature** is only possible on a free text or on the previously defined signature. It is not proposed, obviously, on the elements of the formula as well as on the name of the image, which are eminently variable elements.

Positioning elements on the image

The processed and exported images are of **eminently variable dimensions** (height, width). The positioning of the texts and the scale, added automatically, must be **independent of their dimensions.** The context menu gives access to the coordinate systems which define this positioning.

There are **four different coordinate systems . Each element** added to the image (text and scale) can have its own coordinate system:

1. **XY Auto :** The X and Y coordinates of the element are calculated in pixels when positioned in one corner of the image and in relative value compared to the size of the image when positioned elsewhere. The positioning adopted is materialized on the image when the element is moved: by two parallel lines for positioning in pixels, only one for relative positioning.

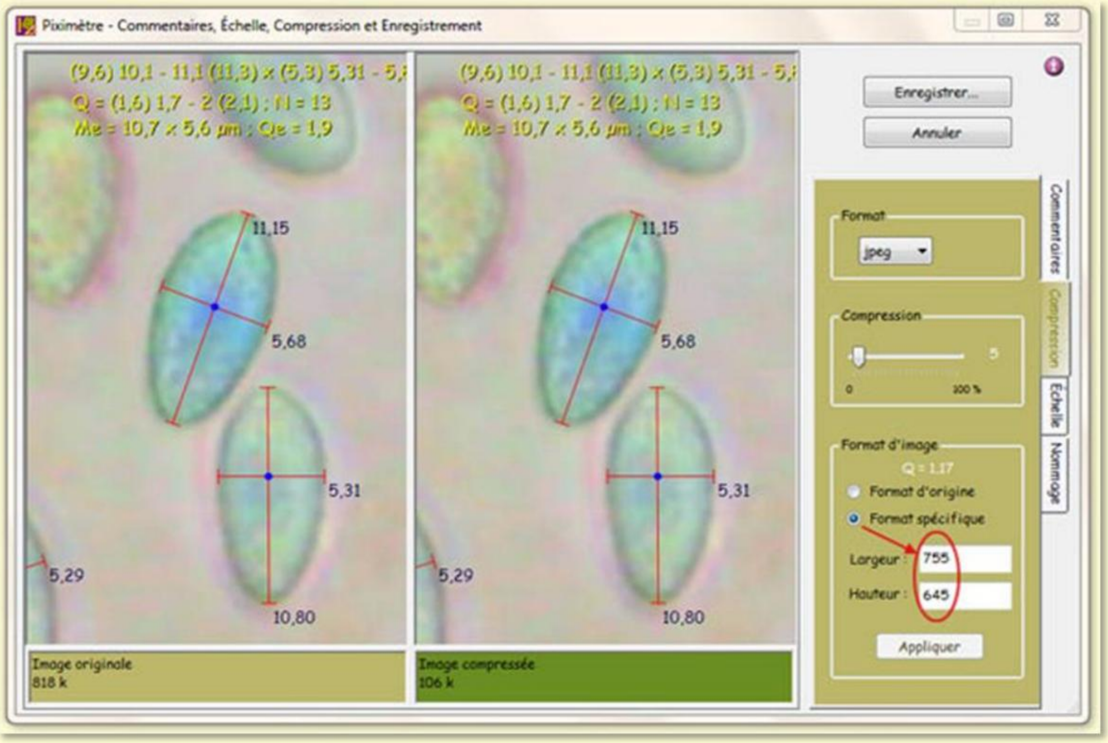
2. **XY in Pixels :** Elements can no longer be positioned outside the corners of the image. A check is carried out so that they never come out of the images, whatever their dimensions.
3. **X in Pixels Y Auto** or **X Auto Y in Pixels :** Are a combination of both previous possibilities.

By default, the coordinate system is **XY Auto** for all elements.

A button allows you to redefine the default system (marked with two asterisks - see figure above).

Compress the exported image

From the Comments phase , pressing the **Continue** button gives access to the next Image *Compression* phase (figure above). This button then changes to the **Save...** button which gives access to the next phase, recording to disk.



The wizard shows the **two versions of the image,** on the left the uncompressed image and, on the right, the compressed image with the compressor settings.

It is possible to **drag images** in their frame by **left clicking + moving** the mouse.

Only **jpeg** compression format is available.

User can adjust **compression level** and change **image dimensions** produced. Note that in this case the proportions are preserved. **The wizard does not modify the geometry of the images.** The figure above explains how to change the size of the images produced. Changing one value automatically changes the other.

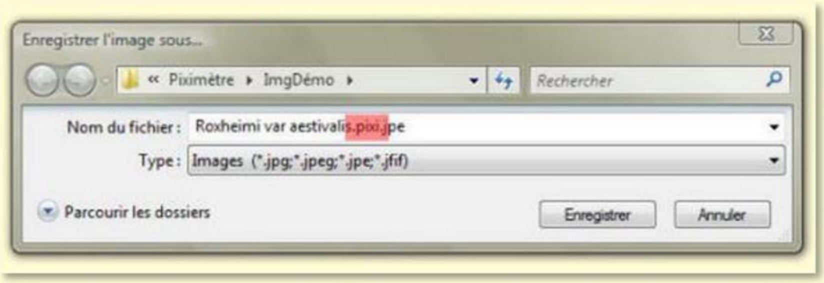
Finally, it is possible to return to the *Comments* phase by selecting the corresponding tab.

Save the exported image

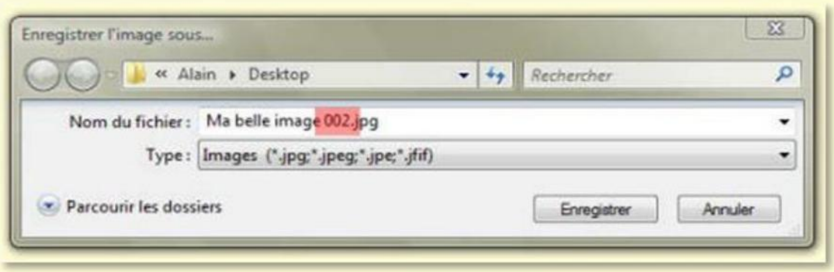
It is essential to understand that the image, or only its view, thus exported **must not be confused** with the original image. Indeed, the original image contains no axes, no intersection points, no scale, no comments; these are only drawn on the screen. Conversely, the exported image contains all of these elements. It is the ***active memory*** of *Piximeter* which makes it possible to find the elements and trace them on the original image when it is reopened.

It is therefore important that the exported image, or just its view, is **named differently** of the original image. *The Export Wizard* offers two methods for automatically naming exported images:

1. Added a **".pixi" suffix** to the end of the image name, just before the extension. For example, if the original image is named " Roxheimi var aestivalis.jpe ", *the Wizard* will suggest naming the exported image " Roxheimi var aestivalis.pixi.jpe ".

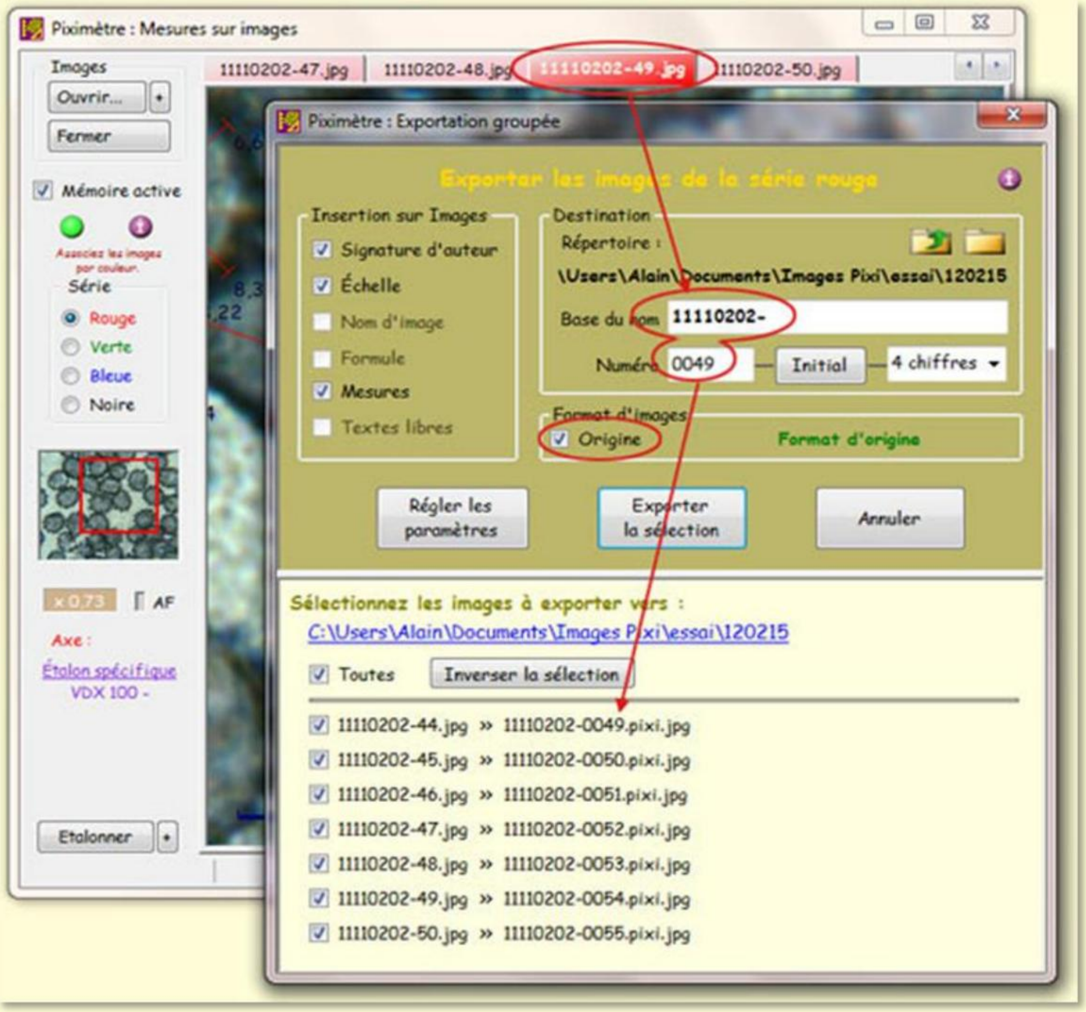


2. Added a **sequential numeric suffix** in front of the extension. For example, if the last image exported was named " My beautiful image 0001.jpeg ", *the Assistant* would suggest naming the following one " My beautiful image 0002.jpeg " (figure below). For this process to work, you must **have specified at least the first time** a name ending with a number. *Piximeter* keeps the number of digits of the number.



The selection of one or the other method is done on the **Naming tab of the Wizard** and also on the **Parameters** tab of the *Formulator,* Advanced... option , Special pane .

The Bulk Export Wizard



The Bulk Export Wizard automates the export of multiple images from those belonging to the series selected in the *Measurer.* For example all the images from the red series.

It is activated by the context menu **"Export... > Series".**

The figure above shows the Wizard opened from the Measurer image **11110202-49.jpg .**

All images from the red series have been automatically integrated into **the Assistant list** (11110202-44.jpg , -45.jpg, ... ,-50.jpg). A checkbox allows the user to **choose which ones will actually be exported.**

Exported where and under what names? The **"Destination"** box allows you to define it.

Buttons  And  allow you to choose **the destination directory.**

Opens the directory selector from the current position: *"...\Pixi Images\essay\120215"* in the example in the figure (or *"My documents",* in the absence of a defined directory).

Opens the directory selector from the position preceding the current position "in the root) :"...*Pixi Images\test* example. (its

The name of the exported image is composed **of a base and a** suffix number taken from the name of the selected image as shown in the figure. It is possible to modify these two parameters by replacing them with new values. The **"Initial"** button resets the suffix to the value 1.

The number of digits of the number is adjustable. It can also be zero. The list of exported images is updated based on these two parameters as can be seen in this figure.

If the user chooses **"Without" as the number of digits,** the naming process changes: the exported images take the name of the initial images. Note that in all cases a suffix **".pixi"** is added in order to differentiate these images from the initial images.

If an image with the same name already exists in the destination directory, the Wizard automatically suggests another valid name by suffixing it with a numerical value in parentheses.

How this **Destination frame** works can be found in *the LiveView Video Assistant* which allows you to record video images. Both Assistants refer to the same directory, which ensures complete homogeneity.

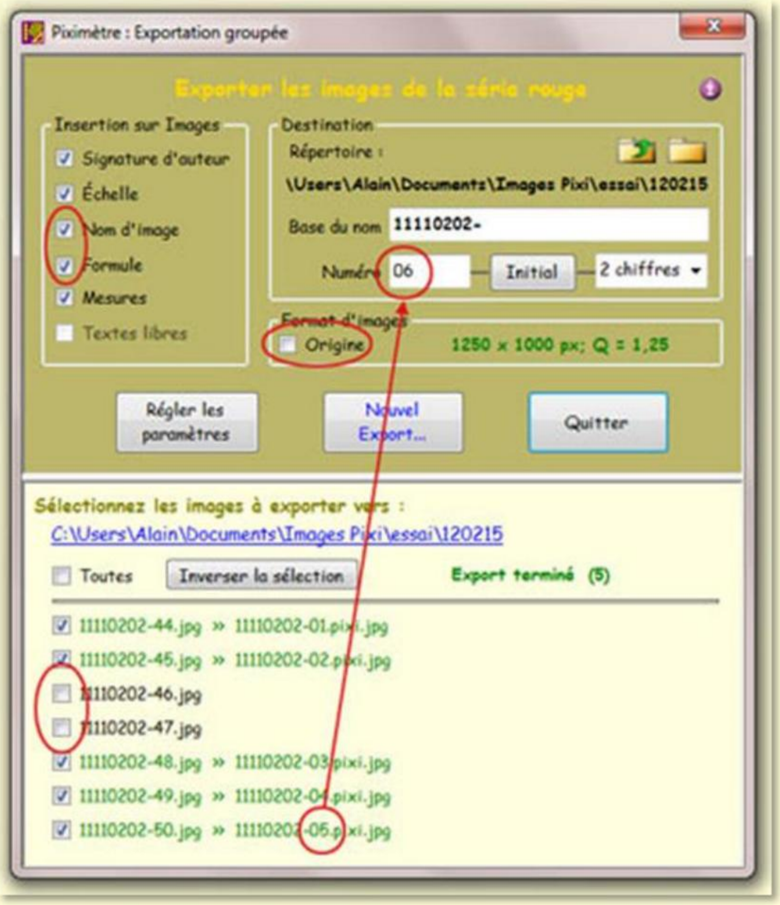
Also note the **Image Format** that is selected here: **Original.** This format is always offered by default. It means that the images will be exported without modification of dimensions, each image being able to have its own dimensions.
However, when the views are exported and not the entire images, then all have the same dimensions, those of the view.

When a specific format has been defined, the **"Origin"** choice is enabled, which allows the user to select that format.

The **"Insertion on images"** frame offers automatic insertion of various **objects** available at this time. In the figure above, **"Author Signature, Scale"** and **"Measurements"** are available and therefore can be selected by the user. Other objects are not yet defined and therefore cannot be selected to be integrated into images.

The name of the image as well as the dimensional formula are however known at this moment. For example the name of the first exported image is "11110202-44.jpg". However, the Assistant does not yet know at this moment **where to place this name on the image** that he is going to build and does not know the font to use, nor its color, etc. Thus, for **the name of the image, the dimensional formula, the author's signature** and **the texts free ones,** it is necessary to first define their shape and their position on the images before being able to select them.

These definitions of shape and position as well as the image format are made once and for all, but can be modified at any time with the **"Adjust parameters"** button which activates the first Assistant.

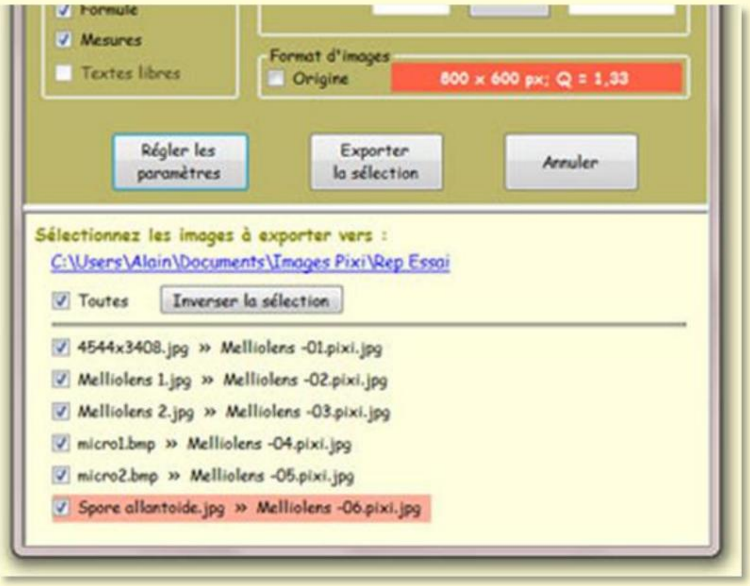


The figure shows *the Bulk Export Wizard* after:

1. **Define the objects to be inserted** on the images (except free texts), 2. **Define a new image format** (1250 x 1000),
3. **Deselected** two images from the list,
4. **Changed the number** of the first image (01). Note that the last exported image has the suffix 05. The number of the next one is then 06.

After export, the **"Export selection"** button changes and becomes **"New Export...'.** Pressing this button has the effect of renumbering the selected images which are then ready to be exported. And the "Export" button the selection" then reappears.

Geometry of exported images



The wizard does not modify the geometry of exported images.

It systematically checks **the compatibility of their format** with the specific format chosen.

Here, in the figure, the image *"Spore allantoide.jpg"* has a format that is not compatible with the selected 800x600 format. This image **will not be exported** by the Assistant.

To export this image, the operator will have to **define a new specific format** compatible ("Adjust settings" button) or keep the original format.

The Graphic Object Sequencer

Piximeter performs dimensional measurements on various **"graphic objects"** present on images (mushroom spores as well as galaxies in the Universe, it is simply their size that changes). Their orientation and distribution in the images are generally arbitrary, sometimes making their visual comparison difficult.

The objective of the *Sequencer* is to **facilitate the visual comparison** of objects. He **extracts** images all or part of these objects, it **normalizes** them in order to respect their sizes and proportions, it **orients** them in space at the same angle, it **classifies** them according to different criteria, it **presents** them on the screen and, finally , it **saves** them in the form of images independent of the source images from which they come.

The **Sequencer** is a new *Piximeter* assistant that performs these tasks **automatically** in just a few mouse clicks. And hop !

Principle

The *Sequencer* uses the axes drawn on the objects when measuring them. It therefore occurs **after** measurements have been made on the images with the *Measurer.*

Take measurements with the *Measurer :*

1. **Open** the image series that contains the objects to be measured. Assign the appropriate length standard to it.
2. Classically **draw** the two rectangular axes which delimit the objects. THE *Formulator* calculates and displays the dimensional formula.

The *Sequencer* then intervenes:

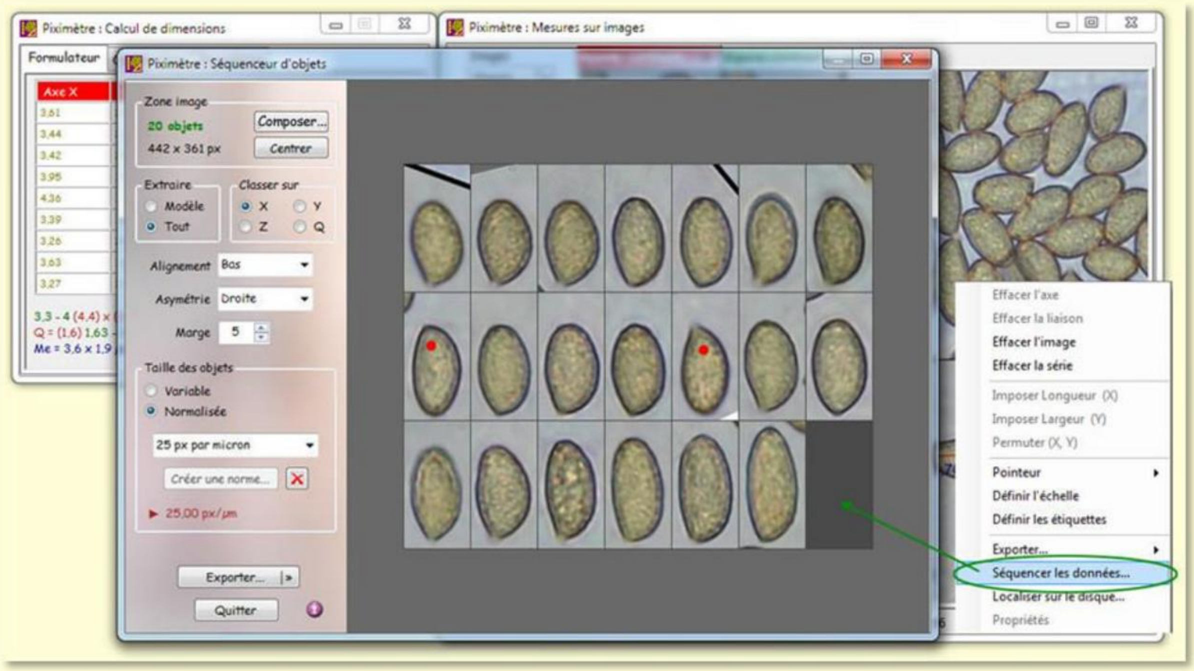
- In the *Measurer,* **right-click** on any image in the series to display the **context menu** and select **"Sequence data...".** The *Sequencer* opens (figure below).

- If they are not already there, **adjust its parameters,** possibly drag the edges of the window to modify its shape. The sequenced objects appear in the **image area** (dark gray in the figure).

- Press the **"Export..."** button to save the **image area** in a jpeg file.
- That's it, exit the *Sequencer.*

We will see later that recording, which is in fact an **export of images,** may require additional settings.

Functioning



Opening the *Sequencer* using the **context menu.**

The figure above shows the *Sequencer* open on a series of images of mushroom spores on which measurements have been made. It has a adjustment panel on its left side and an image panel, of variable size, on its right side. The *Sequencer* presents the objects (here spores) in the image panel, in an area called **the image zone** (dark gray in the figure). This area can be saved as a jpeg image using the **"Export..." button.**

The sequenced objects are contained in **frames of identical sizes,** calculated to accommodate the largest one. The **"Margin"** parameter of the adjustment panel (here at the value 5), allows this size to be increased more or less.

The geometry of the image zone (here three lines, the last of which is incomplete) can take several shapes defined with the **"Compose..."** button located at the top of the adjustment panel, in the **"Image zone" frame.** Its operation is described below. By default, **the arrangement of objects** in the image area is linked to the size of the *Sequencer window.* It changes automatically when the user drags the edges of their window. The **"image area"** frame on the adjustment panel presents its characteristics (here 20 objects and a size of 442 x 361 pixels).

The measured objects are thus presented **in the form of a sequence,** from left to right and top to bottom, in which they are:

1. **Classified in ascending order** of one or the other of their parameter defined in the **"Classify on"** box where X corresponds to the height of the objects, Y to their width, Q to the X/Y ratio and finally, Z is the order of entry of measurements (of the coupling points of the two axes).

2. **Aligned vertically** in relation to their support frame. The position is adjusted by the **“Alignment” choice,** here at the **Low value.** Other possible values are **Center** and **Top.**
3. **Oriented from right to left :** in this example, all the spores (except two marked in red in the figure) have their apicle at the bottom left. The right-left orientation is set by the **“Asymmetry” choice,** here at the Right value . Other possible values are **Auto** and **Left.**

Note : *We will see later that the vertical presentation of objects (by default) can give way to a horizontal presentation. In this case the possible values for* ***Alignment*** *and* ***Asymmetry*** *are replaced by others, adapted to the horizontal presentation.*

Also note that embedded in the icon gives direct access to this **tutorial** *Piximeter.*

Orientation of objects

Piximeter cannot know a priori the geometry of the objects represented in the images. The notion of top and bottom is then determined explicitly by **the orientation of the route** of the main axis (the major axis). Thus the user must always **respect the same convention** and draw the main axes which measure the objects, for example, from their base to their summit (or vice versa).

In the example in the figure above, the two spores pointed in red have deliberately been drawn in the opposite direction to the others. Of course, the orientation of the axes does not alter the measurement of their dimensions.

Fictitious measurements

Piximeter also measures complex objects, such as curved objects for example, by tracing non-linear axes made up of successive contiguous segments which match their shape (see here). However, these **non-linear axes** do not currently make it possible to completely circumscribe the objects they measure. As a result, the *Sequencer* can only process objects defined by two rectangular linear axes. So how do we sequence objects measured by nonlinear axes?

Miguel Angel Ribes Ripoll, Spanish mycologist, suggests the solution: **fictitious measurements.** The method consists of replacing the non-linear axes which precisely measure the objects, with the two linear axes which make it possible to circumscribe them.

This method obviously generates erroneous measurements in the *Formulator* which we will therefore refrain from considering and integrating into the final image. But it has the great advantage of allowing the extraction and visual comparison of all kinds of objects, whatever their shape.

Choice of sequenced objects

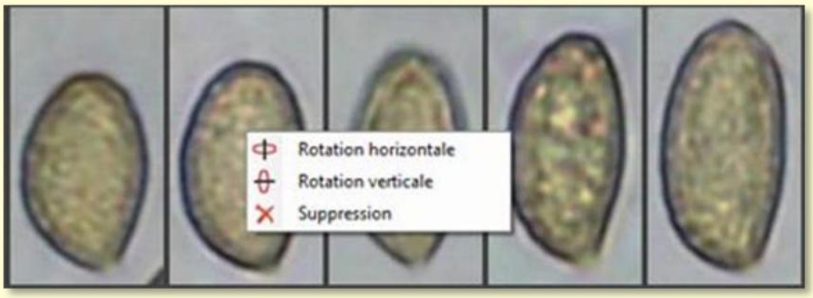
The **"Extract"** box presents two possibilities: **Model** or **All.**

"All", as the name suggests, means that all objects measured on the series of images will be introduced into the sequence.

The **"Model"** choice , for its part, calls for some comment: what model is it?
In fact, all the numerical values from the measurements (X, Y and Q) are organized in ascending order and divided into **classes.** The number of classes depends on their width and the amplitude of the data. Each dimension therefore has a specific number of classes. These numerical data as well as their graphic representation are accessible via the **"HDi"** button on the *Formulator.* The model developed by the *Sequencer* includes **one representative image per class.**

Contextual menu

As shown in the image opposite, **right-clicking** on one of the objects in the image area brings up a context menu which offers a few simple actions allowing you **to adjust the sequence** if necessary:



- The rotation of the object around the vertical axis,
- Rotation around the horizontal axis,
- Pure and simple deletion of the object.

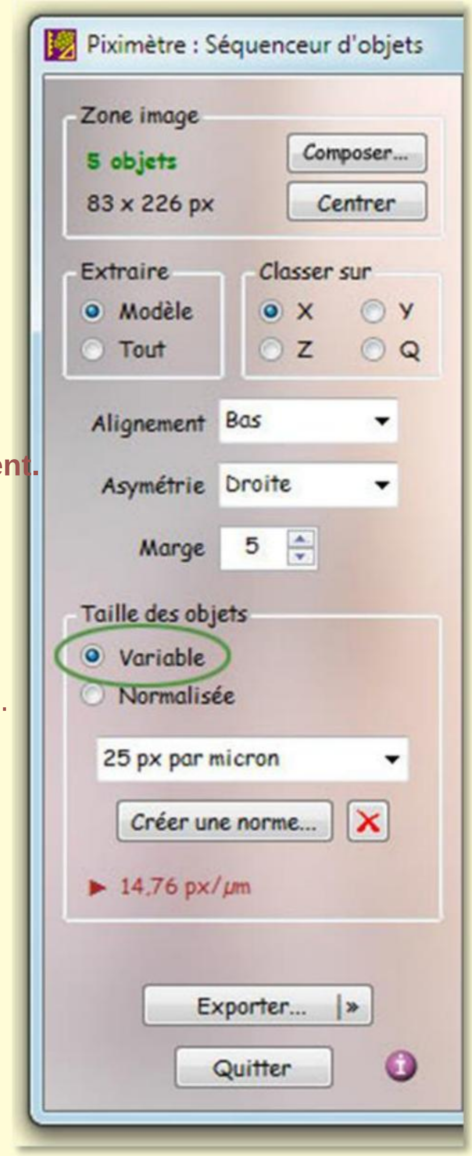
Standardization

One of the important characteristics of the *Sequencer* is **respect for the scale of objects.** Thus, the image of an object measuring 10 µm will be half as small as that of another measuring 20 µm. Normalization is the operation which allows you to fix and maintain the scale of the different **Sequencer** objects .

Standards are not created by default in the *Sequencer.* **The user must create at least one standard** that matches their needs.

The **"Object Size"** frame of the adjustment panel has two operating modes: **Standardized** or **Variable.**

In **"Variable" size,** the user can vary the size of the objects represented in the image area using the **mouse wheel.** There is no active normalization, the size of the images is arbitrary.



The **"Variable"** size gives access to the **"Create a standard"** button (figure opposite) which associates an image size with the actual size of the measured objects. From then on, **a length standard** (a conversion scale) is established and memorized by the *Sequencer.* The size thus defined therefore serves as a **reference** to calculate that of the objects which will be sequenced subsequently.

Normalization guarantees the representation of objects with a size consistent with their measurement.

In **"Normalized"** size it is no longer possible to vary the size of the images present in the image area. When opening a new sequence of objects, **the size of the images is calculated** according to the selected standard and the actual size of the objects it represents.

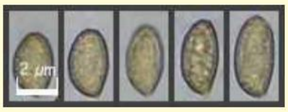
In order to be able to sequence objects of very different sizes, it is possible to **create several standards,** each adapted to the subject.

It is also possible to **redefine a standard** at any time, by returning to **"Variable" size mode,** then changing the size of the images (mouse wheel) and finally by pressing the **"Create standard" button again.** However, in doing so, we will lose consistency with previous images...

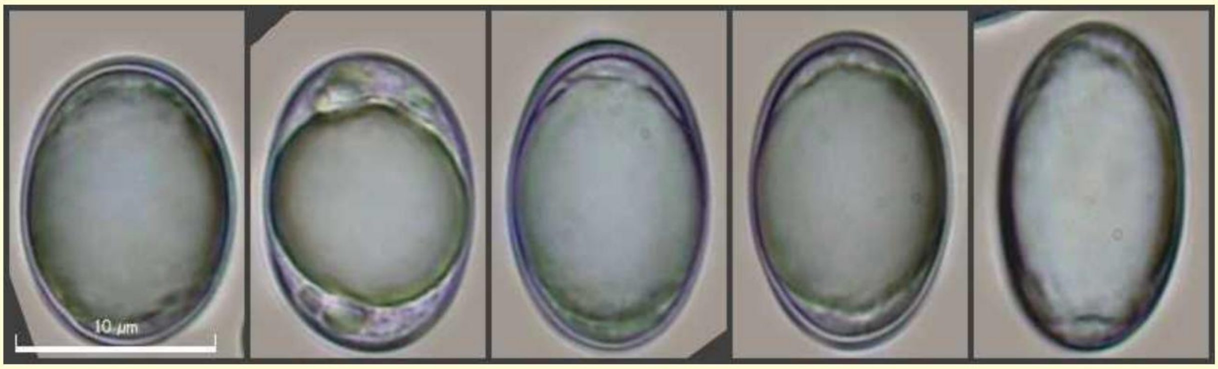
The button with **a red cross,** to the right of the "Create standard" button, allows you to delete one or more of the saved standards.

Note also that the ÿ field indicates the current size of the objects (here 14.76 px / µm).

As an example, here are two images of mushroom spores of very different sizes. Normalized images are **automatically in the same size ratio.**



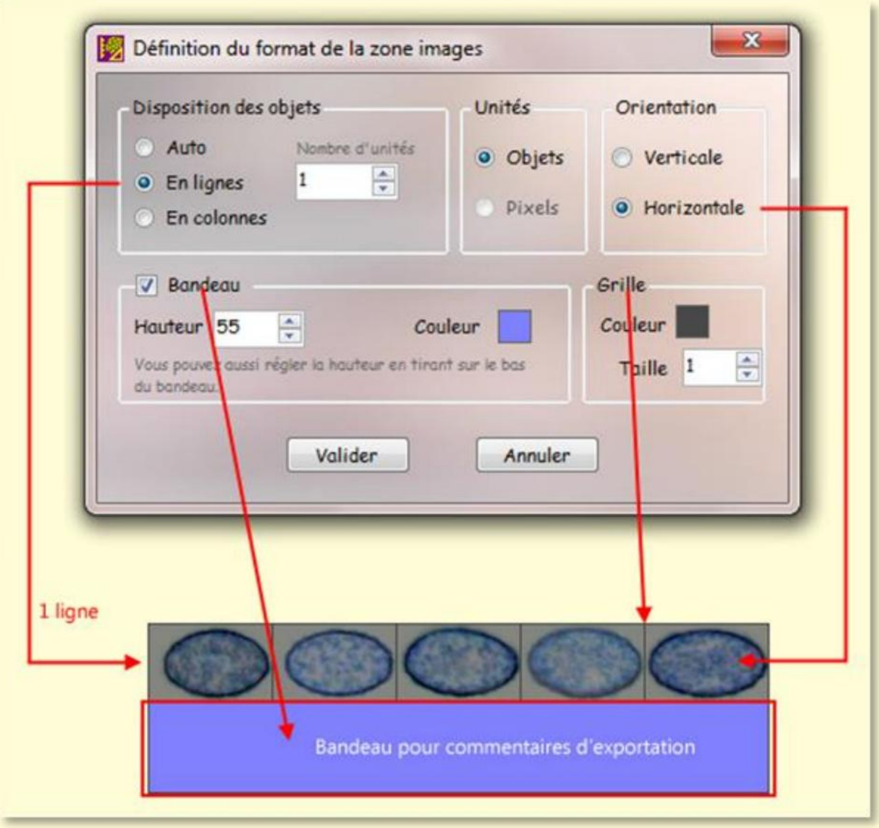
3.3 - 3.7 (4.1) x (1.8) 1.9 - 2 (2.1) µm
Q = (1.6) 1.63 - 2 (2.1); N = 15; Me = 3.5 x 1.9 µm; Qe = 1.8



(14.8) 15 - 16.4 (16.7) x (9.2) 9.6 - 10.7 (11) µm
Q = (1.4) 1.42 - 1.7 (1.8); N = 11 ; Me = 15.8 x 10.2 µm; Qe = 1.6

Image area format

As said above, the **"Compose..."** button in the *Image Zone* frame allows you to specify the presentation of objects in the **image zone.**



The available options are shown below.

The arrangement of objects can be done either automatically, depending on the geometry of the image area, or in rows or columns. In these last two cases, we will specify the number of rows or columns desired.

Units are not covered here but will be developed in a later version .

The vertical (default) or horizontal orientation of objects is chosen here. Note again that the possible values of the **Alignment** and **Asymmetry** choices in the adjustment panel depend on the orientation chosen.

The **Banner** frame allows you to specify the presence, height and color of a horizontal banner added at the base of the *image area.* This banner allows you to add information and other comments when exporting the image before saving it to disk.

This different information is added to the banner by the export process.

Finally, the **Grid** frame allows you to adjust the size and color of the border which surrounds the image and which separates the objects from each other.

Registration

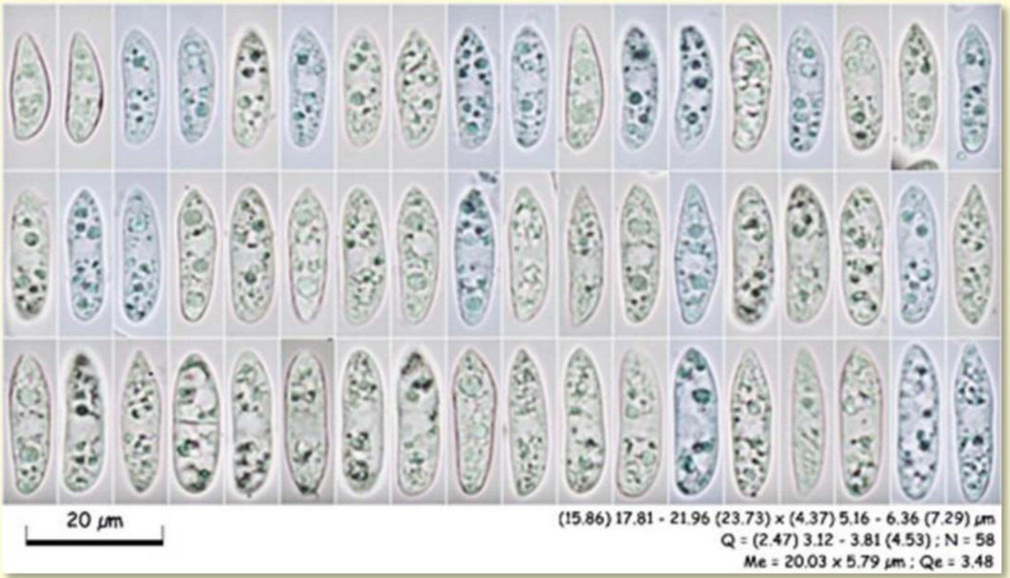
The **"Export..."** button saves the displayed sequence to disk. Either in the form of a **global image** represented by the image zone, or in the form of a **sequence of images,** each object giving rise to a different image.

The "Export..." button has, on its right, the **">>"** sign which allows you to choose the desired recording mode: **one or more images.**

Images are saved using the ***Export Wizard*** which offers the possibility of enriching the images (scale, text, author signature, etc.). Please refer to the corresponding chapter of the tutorial for a description of how it works.

Examples

As an example, here are two images produced by *Miguel Angel Ribes Ripoll* with the *Sequencer.* The first shows a sequence of spores classified in increasing order of size. It was exported with comments which are automatically written on the banner. The other is a sequence of paraphyses obtained using several photos, in different reagents and mounting liquids.


20 µm
(15.86) 17.81 - 21.96 (23.73) x (4.37) 5.16 - 6.36 (7.29) µm
Q = (2.47) 3.12 - 3.81 (4.53) ; N = 58
Me = 20.03 x 5.79 µm ; Qe = 3.48


10 µm

How autofocus works

In the absence of autofocus, the use of **generic photo calibration** implies that they are taken with a constant focal length and all things being equal in terms of the shooting conditions. Otherwise, a **specific calibration** is necessary.

Piximeter 's **autofocus** aims to **automatically compensate for the change in focal length** used when shooting, thus removing the need to recalibrate photos taken with different focal lengths.

Autofocus improves the user experience by saving **time** and **reducing the risk of error.**

Principle

The autofocus provided by *Piximeter* is associated with the graphic measurement function on images. It supports **intrinsic magnification** of photos taken with varied focal lengths (zoom).

The apparent size of an object in its photo depends on two parameters:

1. the **distance** from the camera to
the object, 2. the **focal length** used for the shot.

By making the first of the two parameters **fixed ,** the size of the object in the photo only depends on the focal length used when shooting. This information is included in the images delivered by digital cameras. It is used by *Piximeter* 's autofocus to **precisely calculate** the image magnification induced by the use of the camera's variable focal length.

The automatic calculation of this intrinsic magnification, specific to each camera, is indicated by the **"AF"** indicator on the *Meter,* lit green. The light off or in another color indicates that this calculation could not be carried out. In this case a manual calibration must be carried out before carrying out the measurements.

The operation of the autofocus requires **prior initialization** which aims to set up the *mathematical function,* **specific to each camera and its lens,** which allows the precise calculation of the intrinsic magnification of the image for any focal length. This initialization is carried out using a **wizard** activated by a mouse click on **"AF".**

Initializing the autofocus also makes it possible to define the **reference focal length** of the device, for which the intrinsic magnification factor of the image is **fixed at ONE.**

The **overall magnification** of the image on the screen, which is shown in the frame to the left of the autofocus (x 3.00 in the figure above) is the product of the intrinsic magnification of the image by the manual zoom of *Piximeter,* implemented by the mouse wheel.

Initializing autofocus

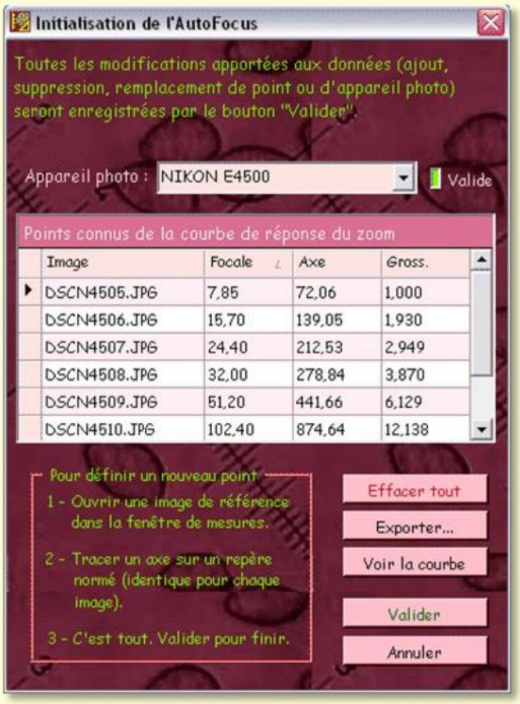
Autofocus initialization must be performed for each camera and varifocal lens that may be used.

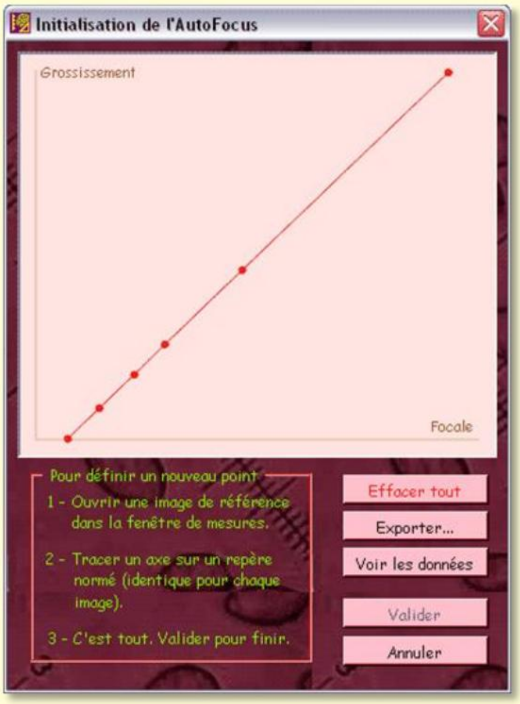
The Autofocus Assistant, activated by a mouse click on **"AF",** helps with this operation. It has two operating modes:

1. **Viewing** recorded autofocus data: all operations with the assistant are possible in this mode but changes made cannot be validated. The validation button is missing. Current measurements in the *Measurer* are not affected.

2. **Creation-Modification** of autofocus data: in order to guarantee that the image measurements necessary for this operation will be carried out under **identical conditions,** the assistant:
   1. **Closes all images** possibly open in the *Measurer,* 2. Forces the generic standard to "Unit (UN)", 3. Deactivates its memory.

The figures below show the autofocus assistant in the data modification phase.





The **“See curve”** or **“See data”** button allows you to switch alternately from one view of the assistant to another.

The curve which is presented on the right corresponds to the points which have been determined on the left. It provides the intrinsic magnification of the image depending on the focal length used by the camera. The camera corresponding to this example here is a Nikon E4500.

Setting up the autofocus curve

The method to use to define the points of this curve is simple, but first of all see this note on optics and measurement errors:

1. First choose **a length standard** to photograph, that is to say a **precise and unique** object (for example the interval between divisions 50 and 60 of a graduated ruler),

2. Make a series of photos (for example 10) **by varying the focal length** of the camera,

3. **Open the Autofocus Assistant** in “Edit-Creation” mode,
4. Open the series of photos in the *Measurer,*
5. On each of them, **draw an axis** which is superimposed on the chosen length standard (as if measuring its length). The wizard **reads the focal length used** in the image metadata and **associates it with the apparent length of the standard** represented by the drawn axis. It records a new point on the curve.
6. **Validate** when all the images in the series are thus processed; It's finish.

The assistant chooses the smallest **reference focal length used in the series of photos as the reference focal length .** It also checks **the consistency of the measurements** taken, which results in the monotony of the curve: the length of the axes must be increasing, like the focal length. The **"Valid"** indicator materializes this consistency: **green,** it means that the measurements are valid, **red** it indicates a probable error. Such an error can occur when the measured object **(the standard)** differs from one photo to another.

In this initialization phase, the points can be defined in **any order,** i.e. the photos can be opened in any order in the *Measurer.* The wizard takes care of putting the focal lengths in ascending order. New points can also **be added later.**

If a measurement is performed several times on the same image, **the last measurement replaces the previous one.**

The **“Validate”** button controls the saving of the data and the closing of the wizard. The **"Cancel"** button closes it without saving the data, but the user is asked for confirmation if the data has been modified. The button

“Clear all” erases the points on the board, allowing a new one to be rebuilt. The modifications are only final after validation.

It is possible to simultaneously define or redefine **several cameras and lenses.**

Finally, the **"Export..."** button allows you to save the points of the curve in a file which can, for example, then be transmitted to other users and **integrated into *Piximeter*** by the assistant, by a simple **drag and drop . deposited from this file onto the table of values or onto the curve.**

Photograph the length standard

We will use one of the following two methods to obtain the **sequence of photos** necessary :

- Mount the camera on the microscope, choose an objective (it doesn't matter which one) and take several photos of the ocular micrometer or object micrometer placed on the microscope (or telescope).
- Mount the camera on a tripod and photograph a double decimeter.

We will **vary the focal length** (zoom) between each photo and we will be careful, in method 2, not to vary the distance between the camera and the ruler.

Important note

The accuracy of calculating the intrinsic magnification of images is linked to the care taken in constructing the curve. Defining **an insufficient number of points** leads to a greater approximation and therefore a greater calculation error in the event of non-linearity in the camera response.

Also note that the focal length used when shooting is provided by the camera to **two decimal places.** The precision of the measurements, when using an intermediate focal length, cannot therefore be greater than 1%.

Using autofocus

In routine measurements, autofocus operation is **automatic** as long as the camera used is described in the Piximeter database . Otherwise, autofocus is disabled.

The AF indicator characterizes the operation of the autofocus on **the image which is selected** in the Piximeter measurement window . **A mouse click** on this indicator provides information on its status:

- The **light off** means that (several cases):
  - o The image comes from the ***LiveView Piximeter.*** The sensor is in principle fixed focal length; autofocus is disabled,

- o In Compressed Images mode , the image is **heavily compressed** and no longer contains the information necessary for its identification (metadata),

- o Always in *Compressed Images* mode , the image contains metadata but **autofocus is not initialized** or is invalid for the camera used,

- o **No images** are open in the meter.

• The indicator lit in purple means that: o In *"Normal Images"* mode , the image has been **heavily compressed** and no longer contains the information necessary for its identification (metadata), it is then necessary to use a less compressed image.

• **The indicator light is lit red** means that:

- o In "Normal Images" mode , **autofocus** initialization is invalid for the camera used.
  This is unknown or its characteristic curve is not monotonic but presents a cusp.
  It is necessary **to restart its initialization** with the AF assistant.

When the light is off, **purple** or **red, autofocus is inactive** on that image; its intrinsic magnification is **fixed at ONE.** It may then be necessary to carry out specific calibration of the image depending on the zoom.

• The indicator lit in Green means that autofocus is active on this image: o The camera used for shooting **is correctly described** and

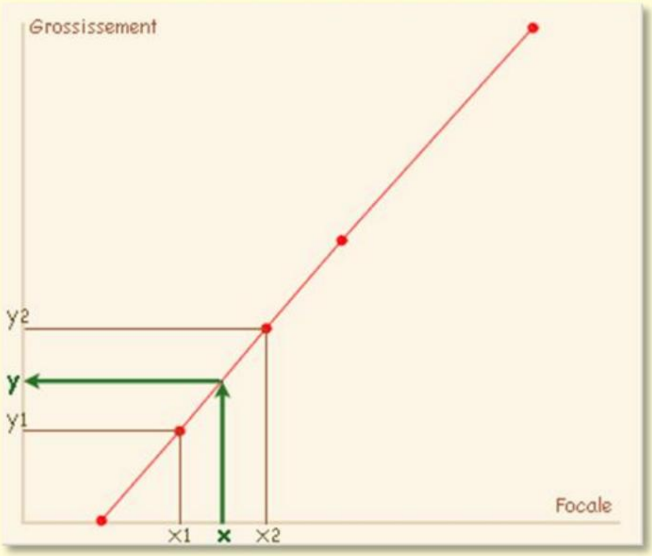
- o The image metadata made it possible to determine its **intrinsic magnification.**

When the indicator light is green, the intrinsic magnification **y** of the image, corresponding to the focal length **x** used, is calculated by linear extrapolation of the curve between the known points **x1** and **x2** which frame the value x.

If the focal length **x** is less than the *first* known point or greater than the last (a case which may occur if the initialization has not integrated these extreme points), the calculation is carried out by extrapolation respectively of the first or last segment of the curve.

This calculation method, associated with the multiplicity of points, makes it possible to obtain a very good approximation of the **real magnification** of the images even with a non-linear curve.

Object **dimension measurements** are then carried out normally.



Optics and measurement errors

Optics and image distortion

Carrying out valid length measurements on objects present in an image assumes **perfect linearity** of the image. However, unfortunately, the images provided by optical systems are generally not free from distortions. This phenomenon, well known to photographers, is due to **defects in the optics used,** which are in fact not perfect.

Concerning photos taken using a microscope, at least two optics are involved: **the objective of the microscope and the objective of the camera.**

The geometric defects of the images produced by cameras vary greatly with the quality of their lenses and the focal length used. **They generally increase at short focal lengths** and are maximum at wide angle (which should therefore be avoided here for this reason).

Microscope objectives, for their part, vary greatly in quality, and therefore in cost. Geometric corrections are obtained with **plan objectives.** Chromatic aberrations, which depend on the wavelength of the incident light, must also be corrected. The **plan apochromatic** objectives are thus corrected for three characteristic wavelengths. They give excellent results but are obviously very expensive.

As a result, the images provided by a camera mounted on a microscope contain more or less significant **geometric deformations .** They can be spherical or cushion-shaped or even take more complex shapes. These distortions are generally more significant towards the periphery of the images. The central zone can be considered linear, that is to say free of deformation.

Carrying out measurements using a **linear scale,** over the entire surface of such images, therefore exposes oneself to errors inherent in the geometric deformations of the images. There are two ways to reduce these errors:

1. **Correct image geometry.** But currently no measurement software and *Piximeter* does not carry out this correction either.
2. Carry out measurements **only towards the center of the images,** avoiding their edges as much as possible, this is what is easiest to do.

It is easy to **check the quality of the images** obtained by photographing through a microscope:

1. Place an object micrometer under the microscope objective and focus point,

2. Set the camera to a short focal length and take a photo of this micrometer. The photo shows the ruler graduated in equidistant divisions, spaced 10 microns apart (standard for object micrometers),
3. Introduce this image into *Piximeter* or into a quality graphics software, such as *Photoshop* for example,
4. Draw an axis superimposing two divisions in the center of the image,
5. Then move this axis towards the divisions which are located at the periphery of the image and note that it no longer perfectly superimposes two divisions, their spacing being altered by the deformation of the image.
6. Evaluate the error made (length of the axis that measures the difference).

The use of a video camera, in addition to allowing direct vision and capture of images, **largely eliminates distortions.** Generally, on the one hand, these cameras do not have a lens but a simple sensor protection glass and, on the other hand, their field is reduced and centered on that of the microscope lens. Which eliminates the two sources of errors indicated previously.

Piximeter provides connection to **many video cameras installed** on the computer (with some reservations, however).

Calibration and autofocus

In mycology, *Piximeter* calibration is done on an image of the **object micrometer** (this is generally a 200 µm ruler made up of 20 divisions spaced **10** µm apart).
We will take a photo of this micrometer with each objective likely to be used.

Taking into account what has just been explained above, and without certainty of the perfect linearity of the images, we will take as a reference the interval limited to only **two or three divisions located in the center of the image,** avoiding choosing too large an interval, possibly altered by geometric aberrations.

Furthermore, establishing the camera's autofocus curve must be done carefully. Experience shows that it is preferable **not to take a photo at the longest focal length** (minimum zoom, maximum error) but at the focal position just below. We will use as a **length standard** an interval located at the approximate center of the image such that it is not **too long** at the smallest focal length (maximum magnification).

Measurement and statistics

The objective of statistical studies is to determine the characteristics of an **entire population** based only on the known elements of a **representative subset** called a sample (series in *Piximeter).*

The least we can say is that measuring mushroom spores poses a real problem. On the one hand, **the spore population of a specimen is not always**

homogeneous and the image accessed by the mycologist is very limited. On the other hand, **the methods used** by authors vary greatly and are generally not indicated in the publications. These two reasons lead to variable results.

Due to their very small size in general (20 to 30) compared to the total population (several 10*106 to 100*106), the spore samples are not always sufficiently homogeneous for the laws of statistics and probabilities are applied with all necessary rigor. *Piximeter* introduces **a new measurement**
perfectly valid under these laws. This new measure consists of **a confidence interval** which relates to **the average value** of each dimension (length, width, thickness and their ratio) for the entire population.

Concerning the sample retained for the measurements, the attention of users must be drawn to the need to consider only the **spores representative** of the total population and not all those which fall under the objective. There as elsewhere, the quality of the result depends first of all on that of the operator, which translates here into the choice of the sample. We will eliminate those which are **out of norm,** that is to say clearly too large or too small or unripe and we will take care to measure only the spores seen perfectly in profile, exactly as specialist mycologists do.

In the case of **multimodal populations,** that is to say comprising several distinct subsets of spores (generally two, beyond that they become difficult to discern), it will be appropriate to carry out as many series of measurements in order to account for this aspect. .

Ultimately, **the choice of spores measured** on the images must be identical to that made for spores traditionally measured by eye, through the eyepiece of the microscope.

Furthermore, in order to increase the number of measurements, and therefore the precision of the result, *Piximeter* allows you to measure only the length on some and only the width on others. Of course, it only calculates the ratio of these two dimensions when they emanate from the same spore.

The drawing of the axes which measure the dimensions of the objects is assisted in order to facilitate the operation: when the axis being drawn intersects a first axis **perpendicularly , their point of intersection appears in red.** It is therefore necessary to systematically **look for the red point** when drawing the second intersecting axis or use the automatic detection of orthogonality of the axes.

Measurement software in general, and *Piximeter* in particular, does not provide an answer to the relevance of the choice of sample. They do nothing more than **facilitate measurements,** which is already no small thing, compared to the work “by eye” traditionally carried out with the eyepiece of the microscope. The choice of objects to be measured as well as the interpretation of the results remains, fortunately, within the domain of **the users' expertise.**

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