

Piximetre

Measuring dimensions on images



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www.Piximetre.fr

*No measuring instrument is perfect.
It's important to understand its
limitations and assess the margin of
error.*

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Introduction

Piximetre is a metrology software program 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 fields where precise and easy measurement of dimensions is necessary. From the measurement of bacteria to galaxies! It is particularly relevant to mycology, which is the field of its authors.

Piximetre produces a **dimensional formula** that enables significant progress in determining whether an object belongs to a target population.

In mycology, for example, this formula helps better answer the question: "Are the dimensions of the spores of this fungus compatible with those of a given species?"

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

Ensure direct measurement of real object dimensions on their images:

- Management of very large images and various formats (jpeg, bmp, tif, etc.),
- Measurements by tracing calibrated axes on the images,
- Automatic tracing (assisted by AI) for certain objects,
- Grouping of measurements taken from multiple images,
- Tracking of axis tracing,
- Automatic detection of axis coupling (length, width),
- Modification (addition, deletion) of axis couplings,
- Selective or global erasure of traced axes,
- Permanent calibration applied to any new image or, failing that, specific to each image,
- Simultaneous use of multiple length standards,
- Automatic compensation of the focal length of the cameras used for shooting,
- Direct connection to a USB camera to acquire images in Live View,
- Graphical storage of measurements, with backup and restoration,
- Display of metadata included in images,
- Powerful and easy zooming in and out, and panning within images,
- Automatic measurement scale management,
- etc.

Visualize and compare the spatial distribution of measurement series:

- Color representation of measurement series,

- Plotting of the orientation axis of each series,
- Plotting of the trend ellipse,
- Easy magnifying glass, allowing zooming in and out,
- Shifting of the series' center of gravity,
- Hiding and deleting series,
- Comparison of four series simultaneously,
- Display of the measurement histogram,
- Export of graphs by simple "copy and paste",
- etc.

Sequence measured objects:

- Individual extraction of measured objects,
- Automatic creation of representative sequences, in the form of images,
- Scale standardization,
- Classification according to various criteria,
- Rotation and positioning,
- Automatic decoration of produced images (scale, measurements, author's signature, comments, etc.),
- etc.

Exporting decorated images

- Adding axis plots and corresponding measurements,
- Adding the measurement scale,
- Defining and adding an author's signature,
- Adding a dimensional formula,
- Adding custom comments,
- Adjusting fonts, shading, and positions,
- Choosing the size of exported images and the compression rate,
- etc.

Mixing data sources,

- Importing measurement files with a simple drag-and-drop operation,
- Manually entering numeric values,
- Automatically converting measurements to length units (e.g., microns),
- Copying and pasting text and graphics into external documents,
- Saving measurements,
- etc.

Overview

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

Its features include:

1. **Collect** series of measurements (patterns) representing the dimensions of various objects,
2. **Express** as accurately as possible the dimensional formula that reflects the characteristics of the entire population,
3. **View** and **compare** multiple series of measurements,
4. **Create** and **export** enriched images with measurements, length scale, author's signature, comments, etc.,
5. **Extract** sequences of objects ordered according to various criteria as images,
6. **Store** measurements and user choices.

Piximetre offers two distinct operating modes for collecting measurement series:

1. In **Digital mode**, the user manually enters the **numerical values** of the measurements previously taken on objects. These values are expressed in the units of the measuring device used (for example, the divisions of a ruler). These measurements are converted to length units by *Piximetre*, based on a coefficient provided by the user (typically, the value of each division of the ruler). This mode is a kind of **super calculator**.
2. In **Graphic mode**, the user works with **digital images** representing the objects to be measured. Using the mouse, they directly draw axes on them that correspond to the length and width or thickness of these objects. The measurements of these axes are converted into length units through a preliminary calibration. This is the **ideal operating mode**.

Regardless of the operating mode chosen, the user can:

- **Adjust *Piximetre*'s** operating parameters,
- **Save measurements** to files, from which they can be retrieved later,
- **Copy the dimensional formula and its accessories** (comparator and histogram) to the clipboard for import into personal documents (Word, HTML, XML, etc.).

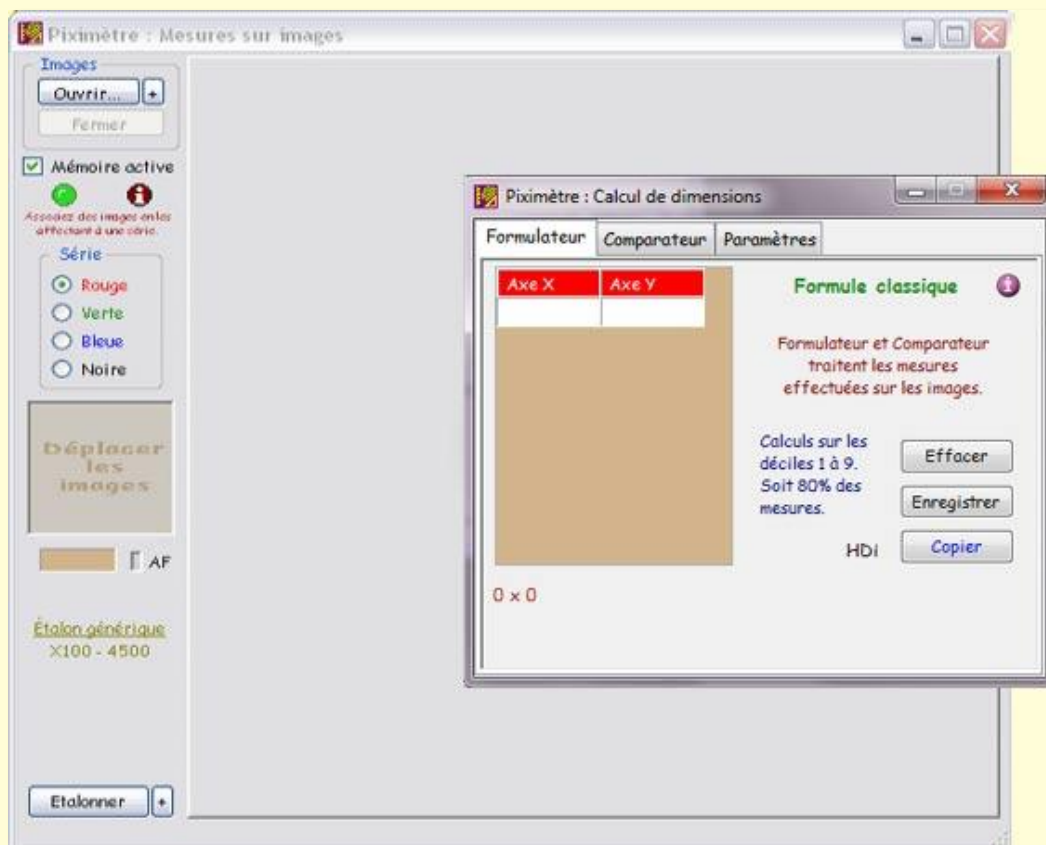
Piximetre's automation is extensive, optimizing repetitive tasks. All parameters, such as digital conversion coefficients, length standards, zoom characteristics of the cameras

used for shooting, the size and position of each window, etc., are stored in memory. *Piximetre* reopens the next time in the same state as when it was closed.

All these features and other operations are detailed below. For now, let's look at the software's presentation.

Overview of Graphics Mode

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



Initial view of *Piximetre*

The smaller one, located at the top, is the main window, also called the "**Calculator**." It has three tabs:

- Formulator,
- Comparator,
- Settings.

The second, larger window, located below and called the "**Measurer**" is only available in **Graphics mode**. It allows you to work with images containing objects to be measured and perform all operations related to their measurement.

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

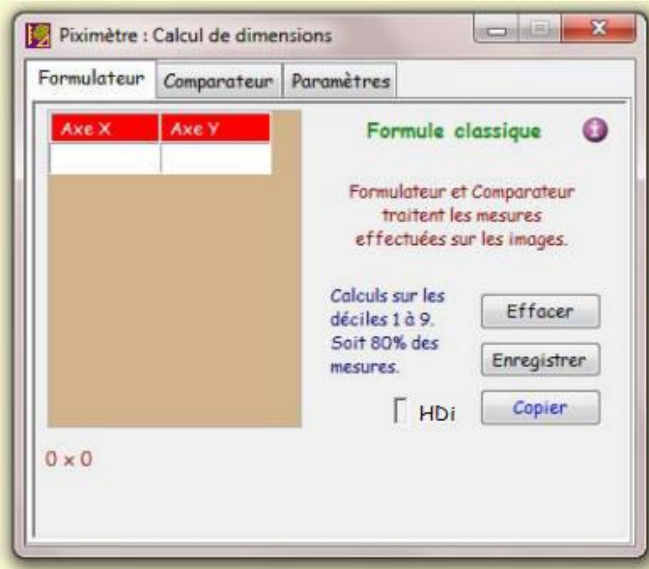
The *Piximetre Formulator* calculates the **dimensional formula** (in the case of mushroom spores, the term "spore formula" is used) in one or two dimensions for a wide variety of objects such as mushroom spores or even galaxies. The calculation is performed based on the numerical or statistical analysis of a representative sample of the population under study. The main features of the Calculator are as follows:

- It is independent of the nature of the objects measured and therefore **independent of the units** used,
- It **directly displays the measurement results** in the form of a dimensional formula,
- It allows you to graphically **visualize the spatial distribution** of measurements and their **statistical interpretation**,
- The dimensional formula and the various available graphs can be copied into electronic documents by simply "**copying and pasting**" them via the clipboard.

Piximetre's two operating modes result in two different presentations of the *Formulator*.

In the *Formulator* in *Graphics* mode, which is initially selected, the first feature is the **input grid** with its two columns: **X-axis** and **Y-axis**. This is used to store measurement pairs (length, width).

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




The calculated **dimensional formula** appears below ("0 x 0" when the grid is empty). It is automatically generated, step by step, based on the data entered into the grid.

This dimensional formula can take two forms: **classical** or **statistical**, which are explained later.

The **Clear** button resets the input grid and formula. The **Save** button saves the grid contents and the dimensional formula to a text file,

while the **Copy** button places them on the clipboard for later pasting into a user's personal document.

The icon  provides access to the **contextual tutorial** (only French).

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](#).

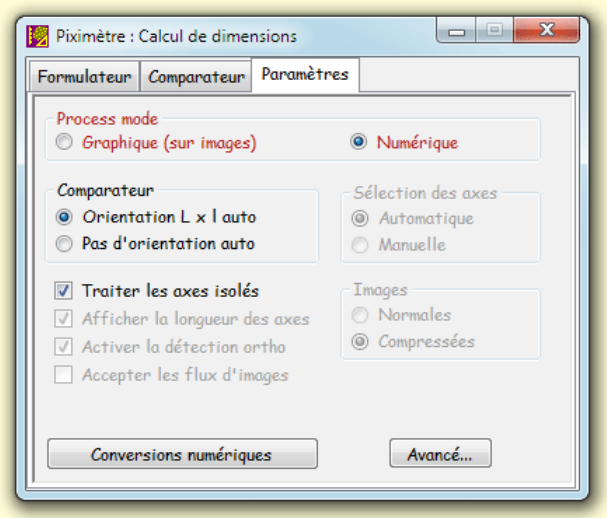
Digital Mode Overview

The operating mode is selected on the "**Settings**" tab, as shown in the following figure.

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

Data is entered manually directly into the Formulator grid. There are three options:

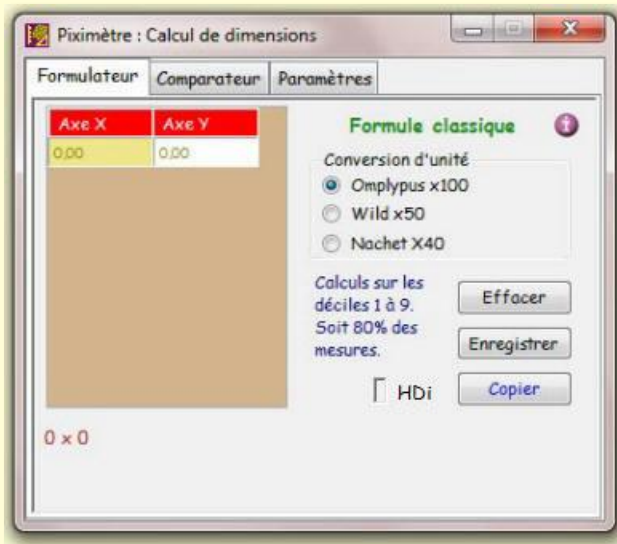
1. The user manually enters the values corresponding to the length and width of the measured objects into the grid. Moving from one cell to the next is done with the "**Tab**" key on the keyboard, not the "**Enter**" key.
2. The user drags one (or more) measurement files onto the grid.
3. The first two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



Switching to Numeric mode disables choices that are no longer relevant and enables the selection of one of the unit conversion coefficients, which can be (re)defined in the "Settings" tab using the "**Numerical Conversions**" button.

Below is the *Calculator* window in Numeric mode. You'll notice the **Unit Conversion** option appears in the *Formulator*. Three conversions have been defined here ([see below](#)).

Unlike in Graph mode, where measurements are expressed directly in units of length through prior calibration (see "[Image Measurements](#)"), here they are expressed in the units of the measuring instrument used (e.g., the divisions of an ocular micrometer).



Each of the three configurable conversions corresponds to a **choice of transforming** this unit into a unit of length. This conversion is **performed automatically** by *Piximetre* to establish and express the dimensional formula.

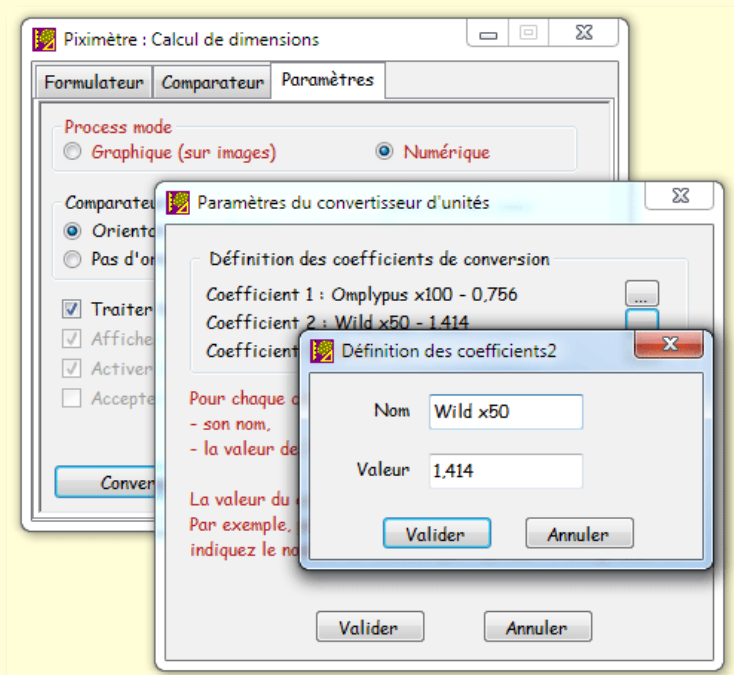
The user must therefore define at **least one** (maximum three) conversion coefficient by assigning each a specific name and value. Note in the figure that only the name appears in the Formulator (example: Olympus x 100).

Define unit conversions in "Numerical" mode

The "Numerical Conversions" button in the *Settings* tab allows you to define the names and values for up to three conversions when Numeric mode is selected, as shown in the opposite figure.

In the middle window titled "Unit Converter Settings," the button to the right of each of the three coefficients allows you to define (or redefine) their parameters.

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



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

Another example: if the dimensions of mushroom spores were measured with a x100 objective where each division of the ocular micrometer is worth 0.8 microns, and cystidia were measured with a x60 objective where each division is worth 1.2 microns, 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 the definition of a **unit of length** for the coefficients.

Graphic or Digital?

Graphics mode offers the convenience of working on a computer screen to perform measurements on objects. It also ensures the graphical and digital memory of all measurements made on each processed image. **This is Piximetre's preferred operating mode.** Its features are described in detail below.

Recommendations for Graphics Mode

Do not compress images from the camera to preserve the metadata they contain. *Piximetre* can work with very large images without any difficulty.

Depending on your image acquisition equipment:

1. If you are using a **camera equipped with a variable focal length lens**, you will need to **initialize Piximetre's autofocus** to automatically compensate for image magnification due to changes in focal length. This will allow you to take measures without changing the standard, regardless of the focal length used.

Then start by **initializing Piximetre's automatic autofocus** corresponding to the camera used and its lens (if you use more than one, you must initialize the autofocus for each of them - *Piximetre* can handle any number), which will allow you to use any focal length when shooting, without any worries about calibrating the measuring tool.

2. If you use a **camera or a 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**, i.e., one that applies to all new images that will be opened by *Piximetre* (which includes a default one, which measures in pixels, but which will generally not correspond to your needs). Standards ensure the conversion of the pixels you measure on the screen by plotting the axes on the images into real dimensions and their units.

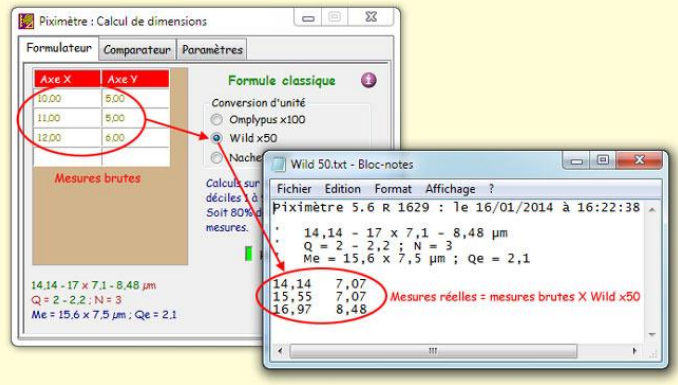
You won't need to make any further adjustments to *Piximetre*; it retains all information from one activation to the next and automatically calibrates itself based on the image source used.

You can now work routinely.

Digital mode provides the same numerical and graphical results as its graphical counterpart. It allows you to work outside of photos, using measurements provided by an

external measuring instrument such as a microscope or telescope. This operating mode is like an **advanced calculator**.

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



grid.

It is also possible to enter measurements by simply **drag and drop** a text file containing them. For example, the one created using the **"Save"** button.

The figure shows the contents of the saved measurement file.

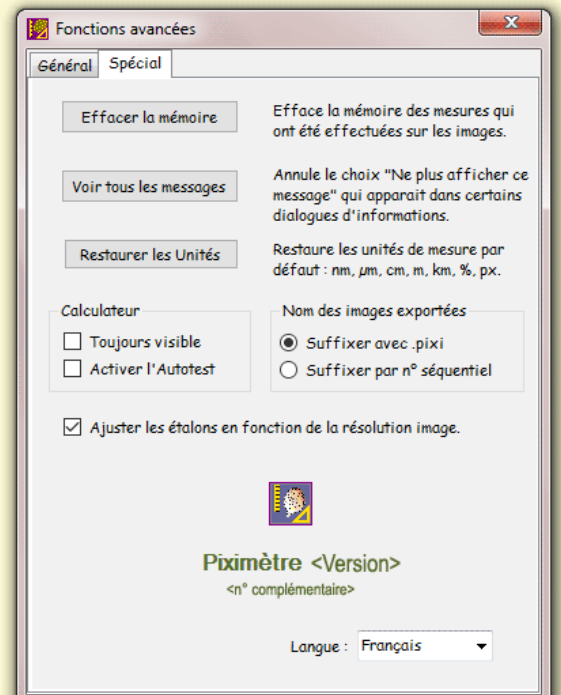
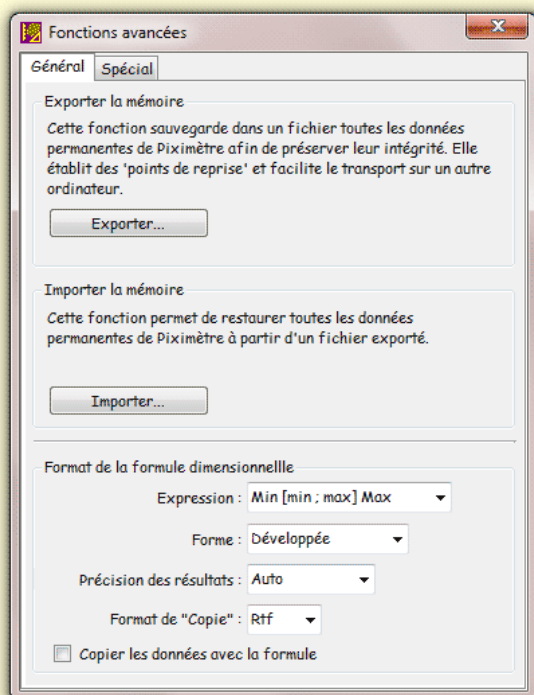
It is important to note that **the recorded values are the real measurements**, in this case the raw measurements entered by the user, assigned the chosen unit conversion factor (in this case, Wild x50).

This allows this file to be used in Graph mode as well. The two operating modes are thus consistent.

Piximetre does not save the conversion factor. When dragging and dropping the file onto the *Formulator* grid, the user must be careful to **select the same factor**. Otherwise, the measurements will be incorrect.

Advanced settings

The "**Advanced...**" button in the *Settings* tab provides access to additional settings organized into two tabs: "**General**" and "**Special**." Here's a description.



Import-Export of the Work Context

The first function allows you to **export** all the data managed by *Piximetre* to a file, specifically **the generic standards** and **the memory** containing all the measurements made on the images. Similarly, a second function allows you to **import** such a file and reload the active memory and other settings.

This pair of functions ensures the transfer of *Piximetre's* working data from one computer to another without loss of information.

In the process, **changing screen resolution is handled**, which preserves the validity of the transferred data and avoids the need to repeat the calibration. *Piximetre* is then immediately operational on the new computer (where it must still be installed to import the data).

These two functions also allow you to perform "**recovery points**", saving the state of *Piximetre*, for example, before changing the calibration, and possibly restoring it afterward. To transfer working 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 *Piximetre*.

Dimensional Formula Formats

This *General* tab allows the user, among other things, to **change the format of the dimensional formula**. It should be noted here that there is another, faster way to change the parameters of this formula: **Click on the formula**. Left clicking on the formula displayed by the *Formulator* allows you to switch between the **statistical formula** and the **classic formula**. Right clicking allows you to change all the display parameters 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 both cases.

1. **Min [min; max] Max** corresponds to the statistical formula.
2. **(Mini) min-max (Maxi)** corresponds to the classic formula. This is *Piximetre's* default formula.

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

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

The "**Info +**" option controls the integration of the standard deviations calculated from the series of measurements taken into the statistical formula. This data, which corresponds to a numerical evaluation of the measurement dispersion, is useful in certain cases.

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

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

Special Functions

On the *Special* tab, "**Clear Memory**" allows you to exceptionally clear all memory settings, particularly all axes plotted on all closed images with **active memory** engaged. This operation is not reversible.

The "**Show All Messages**" button allows you to redisplay messages with the "**Do not show this message again**" option, which may have been checked.

The "**Restore Units**" button allows you to regenerate all default units (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**" checkbox displays a special button on the *Formulator*, which allows the user to generate random series of measurements. These series display different histograms. The "Language" option allows you to define the working language of *Piximetre*. The language can be selected at any time. Data currently being processed is not affected.

The "**Adjust calibration standards based on image resolution**" box allows automatic calibration adjustment when the image and the calibration standard (the image on which it was defined) have different X and Y resolutions but the **same ratio**. This box is checked by default. [Learn more](#).

Image Measurements

The Image Measurements function allows for the direct calculation of the real dimensions of objects present in images.

Its **connection** to the *Comparator* and *Formulator* allows you 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 recalibrating images and regardless of the focal length used during shooting (with fixed focal lengths, autofocus is not necessary).

Principle

The feature is enabled in "**Graphics**" mode ("**Settings**" tab).



Overview.

Since the first versions of the software, measurements have been performed manually by tracing calibrated axes on the images using the mouse. Version 6 introduces **Artificial Intelligence** (AI) to automatically determine the outline of objects. The user simply selects the appropriate ones for their measurements with a single mouse click. This operating mode is called **AutoDetect** and is described [here](#).

The "Open..." button allows you to select from files on disk and open multiple images simultaneously. Images relating to the **same measurement series** (a sample) must be assigned the **same color** (red, green, blue, or black).

The neighboring "+" button allows you to open images while first specifying a series color. It also allows you to enable **Live View** capture using a video camera.

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

Zooming is activated by rolling the mouse wheel over the image. The image's magnification factor appears below the thumbnail (x 1.00 in the Overview example). **Clicking on this value** (two in the case where the image was zoomed in, i.e., has an intrinsic magnification greater than one) resets the zoom to 1.00.

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

Lateral movement of images is done by **moving the mouse over their thumbnail**, with the left button pressed. A **red frame** on the thumbnail represents the part of the image currently displayed on the screen. Movement is only possible when the image is larger than the display area, and the cursor over the thumbnail then takes the form of a **hand**. The movement stops as soon as the mouse is released. Try moving with small successive touches. The **keyboard's arrow keys** (up, down, left, and right) allow you to move the image using smaller keys, making it easier to point **at high magnifications**.

The measuring system must be 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 options. **It is essential to define at least one generic standard** for the measuring system. This generic standard is applied to any new image opened in the Meter. If it is not, the UNITE standard (**in pixels**), which is always present, is applied.

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

[The choice of standard](#) to use for performing measurements on an image is linked to the **state of the optical system** used when the image was taken. **Specific calibration** for each image is also possible. The defined calibration is then applied only to that image.

When an image with active memory is closed, **the image is memorized with its measurement reference**: its calibration becomes specific. This allows all numerical values to be retrieved when the image is "reopened" with active memory. [The complete calibration process is described here](#).

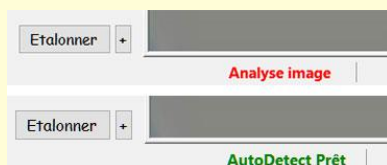
Under certain **initialization** and usage conditions, **autofocus** ensures that dimensional measurements are independent of the focal length used when shooting.

[Autofocus operation](#) is indicated by the "AF" indicator, which lights up green. Clicking on "AF" activates the **autofocus assistant**.



AutoDetect automates object detection

Version 6 of *Piximetre* works identically to the previous standard version 5.10. All your settings and standards remain valid. However, it adds an innovative feature: **AutoDetect**, which automates the **precise detection** of the outline of objects, particularly mushroom spores or similar objects, present in images.



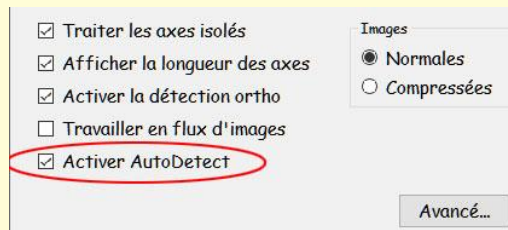
Here, opening images in the Meter triggers the AI, which analyzes them in real time, and just a few seconds later, the **AutoDetect** function is ready. The user can then select the appropriate spores with a simple mouse click on each one. Detection of their measurements is immediate. No more need to meticulously trace their two rectangular axes! **The choice of representative spores remains up to the human.**

Each spore thus selected is **a suggestion** that remains pending user validation. Pressing the **space bar** on the keyboard or clicking the mouse **outside the spore** validates this suggestion. The measurements are then transmitted to the *Formulator*. If the suggestion is incorrect, **clicking the spore again** provides **a new suggestion**. Several successive suggestions are proposed by the AI, displayed under "Axis" on the left panel of the *Measurer*. If none are acceptable, the **Delete key** on the keyboard cancels this selection.

The **Alt key** to the left of the keyboard's space bar allows you to switch from automatic detection mode to standard manual mode and vice versa at any time. This gives the user the ability to **temporarily disable AI** and regain complete control.

To implement this feature, the **AI must be installed** following the instructions provided on the website (Piximetre.fr).

The "Enable **AutoDetect**" option, which is checked by default on the *Piximetre* Settings panel (opposite), allows you to enable or disable this new feature as soon as the AI is installed. *Piximetre* can operate without AI installed, but in this case, the **AutoDetect** feature is disabled.



AI extensions are planned to generalize and further improve detection.

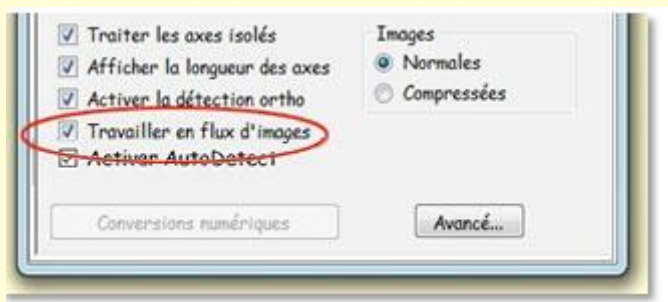
Image Stream

Usually, **several images** are required to perform enough 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 result.

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

Image Stream operation is introduced for this purpose: the measurements performed on the images are retained by the *Calculator* (*Formulator* and *Comparator*), and therefore in the result, when the images are closed after measurements have been performed. Other images can then be opened and measured with the corresponding standard, possibly different from the previous ones. The new measurements are added to those already contained in the *Calculator*. The result is calculated based on all the measurements performed. This feature offers the advantage of being able to mix measurements from images assigned **different standards** (remember that outside of Image Streams, all images in a series must be assigned the same standard).

Image streams are implemented in the *Calculator's Settings* pane, as shown in the figure. It affects all series.



Complete deletion of data, when no longer needed, is done using the "**Clear**" button in the *Formulator* or *Comparator*.

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

Users are cautioned about the **risk of error** due to data mixing that would occur if measurements relating to new objects were introduced into a *Formulator* that still contains **old, obsolete data**. Therefore, caution must be exercised and remember to **delete the Formulator** before undertaking a new series of measurements on objects different from the previous ones.

Validity of Standards

Important: If you use autofocus (for photos with different focal lengths), its initialization **must precede** the definition of generic standards. Otherwise, they may be incorrect.

The photos processed by *Piximetre* come from digital cameras or camcorders on which the operator can adjust, among other things, two parameters that affect 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 $Q = L/W = 4/3$. But other formats also exist, 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 , with the multiplier coefficient $K_T = T'/T$, which defines the homothety between the two images. In other words, K_T can be assigned to each of the two x and y components of the image and therefore to their resultant, the standard.

In the case where Q is different from one image to the other, 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. **Image resolution:** When it differs from one image to another (which is not necessarily the case), the problem can become the same as for size. Adjusting the standard is then only possible if these resolutions R (in x and y) are homothetic between the two images. K_R is this homothety coefficient. But there are also images whose dimensions do not change, although their resolutions differ.

The "**Adjust standards based on image resolution**" setting, on the *Special* tab of the Advanced Settings, controls the processing to be performed. When selected, and if it is **homothetic**, the transformation is calculated and performed. When deselected, no transformation is performed and $K_R = 1$. This setting is selected by default. Regardless

of this setting, the lack of homothety between resolutions systematically produces a warning and turns the **general indicator red**.

When a generic standard is applied to an image, analyzing the **R** and **T** parameters (resolution and size) determines whether or not there is a homothety between this image and the one that defined the standard and, if so, calculates the overall magnification factor $K = KT \times KR$ to be applied to the standard.

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

- **Green** indicates that the selected image is **compatible** with the chosen generic standard. The homothety factor has been calculated and measurements can be validly performed.
- **Red** indicates that the selected image is **not compatible** with the chosen generic standard. This image must be calibrated before taking measurements.

A mouse click on this indicator displays an information bubble, in the same color as the indicator, which explains the case if necessary.

In the example opposite, at the top, **the bubble is green like the indicator light**. This means that the **selected image is compatible** with the chosen generic standard. Measurements can be validly performed. 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 light**. 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. Furthermore, the image's resolution $res = (0 \times 0)$ means that the metadata is missing (this can be confirmed by observing the status bar below the image; if this is the case, it contains blank fields). The image has probably been over compressed. Its calibration is necessary to perform valid measurements.



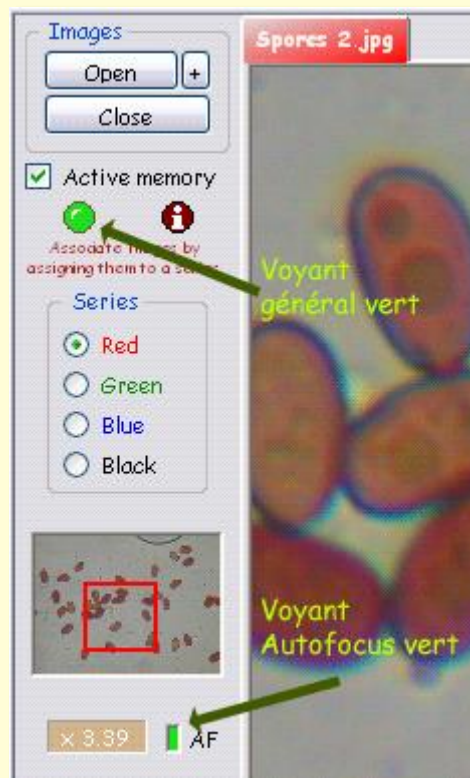
When this happens, **the red information bubble** only appears spontaneously once to attract the user's attention. Then, a mouse click on the indicator light is required to make it appear.

Regardless of the image selection made in the Settings tab (**Normal** or **Compressed**), as long as the image opened in the *Measurer* contains metadata and the autofocus is correctly initialized, the measurement system's consistency will be indicated by the general indicator and the autofocus indicator both lighting up green (figure opposite).

In the case of a compressed image or one **without metadata** opened in the Meter:

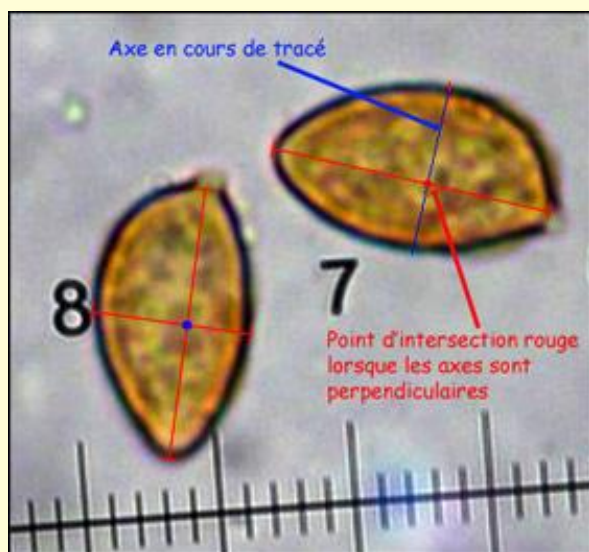
- Selecting **Normal Images** in the Settings tab will signal an error. The general indicator will be light red.
- Selecting **Compressed Images** in the Settings tab will signal no error. The general indicator will be light green.

In the figure, the measurement system is ready.



Coupling the axes

A **first mouse click** on an image places the first point of a new axis. **Release** the button and **move the mouse** to the position of the second point. The axis has materialized during this operation and follows the mouse movement. 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 is adjustable via [the context menu](#).



While moving the mouse to find the second point, it is possible to **vary the image zoom** and **lateral positioning** by clicking on its thumbnail. This mechanism ensures high pointing accuracy and allows, where necessary, the **drawing of long axes** (for example, during calibration, where longer axes offer greater precision, as long as the image remains within the linearity zone). Very small axes, on the order of a few pixels, are not accepted. You **must zoom** in to plot very small objects.

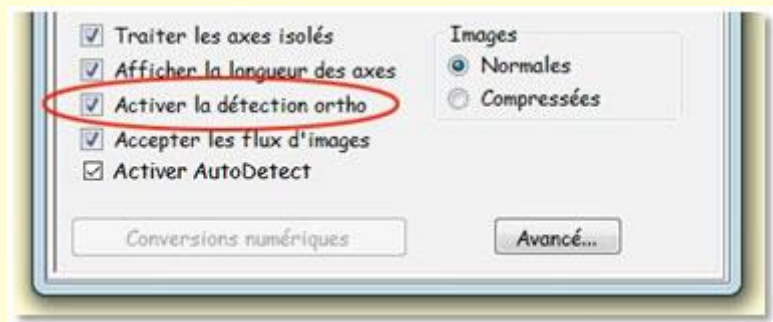
When an object to be measured is **viewed perfectly in profile**, meaning there is no ambiguity about its outline, its two axes can be plotted, measuring its length and width. Once plotted, their connection is indicated by a **blue dot** drawn on the image, as shown in the figure above. The two dimensions, correctly evaluated, appear in the X and Y columns of the *Formulator's* input grid.

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

Drawing Orthogonal Axes

When the axis being drawn intersects a first axis **perpendicularly**, their intersection point appears in **red** during drawing (figure above). Therefore, you must systematically search for this red point to ensure the perpendicularity of the axes measuring two dimensions of the same object. **Axis coupling is performed automatically.**

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



The mouse cursor becomes captive and can no longer move from **the imposed orthogonal path**.

However, it is possible to **temporarily disable** this automatic function by **holding down the Ctrl key** on the keyboard. Similarly, when the function is disabled, the Ctrl key activates it as long as it remains pressed. Remember to **release Ctrl** before marking the point, otherwise a new segment will be linked.

Ctrl is a "magic key" with several [other functions](#).

Axis Specification

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

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

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

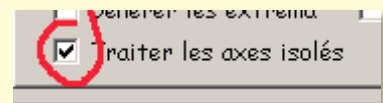
When an axis has been specified in this way, the corresponding **context menu item is checked**. Clicking on this menu item again **cancels the axis specification**.

It is also possible to plot **isolated axes**, i.e., uncoupled axes, as is the case in the Overview above. They improve the accuracy of length or width calculations by increasing the number of measurements. However, these isolated axes are not counted in the calculation of the ratio $Q = \text{length} / \text{width}$, which is **only performed on coupled axes**, i.e., axes belonging to the same object.

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

Isolated Axis Processing

Isolated axes processing is enabled by the user on the *Setting* tab. When the box is unchecked, isolated axes are not processed by the Formulator.



When an object does not present on a perfect profile, it may be impossible to accurately evaluate its actual contour, and in the least difficult cases, only one dimension may be measured. **An isolated axis** is then plotted on the image, as also shown in the overview (above).

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

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

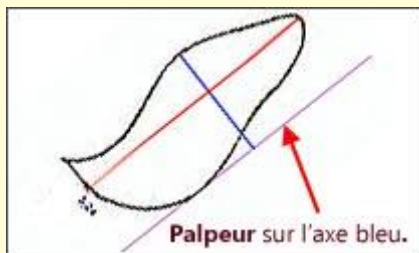
10,50	21,00
10,00	20,00
11,00	0,00
10,50	0,00

When isolated axes processing is enabled by the user, **all non-zero axes participate in the calculation** of each dimension (figure opposite, below, the sample includes four measurements for one dimension and only two for the other). Let's reiterate that in this second case, **only the pairs contribute to the calculation of the dimension ratio $Q=L/W$** .

Note that this principle also applies to *Piximetre's* operation in "Digital" mode.

The **automatic placement** of axes, as length or width, is performed based on the distance of their length from the average of the lengths in each X or Y column. For example, [in the overview](#), the isolated axes of lengths 4.45, 4.52, and 4.70 are automatically placed in the Y column, where they are closer to the average than to the average of the X column.

The Feeler



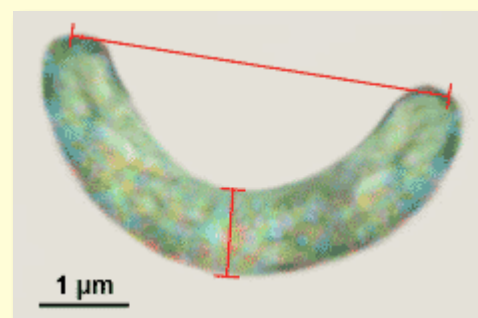
In some cases, such as the virguliform spore example on the left, the geometry of the object to be measured does not allow for the two rectangular axes surrounding it to be directly traced. **The Piximetre feeler** is then a valuable aid, allowing you to approximate the actual dimensions as closely as possible.

The feeler 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 display the feeler, hold down the **Shift key** on the keyboard while tracing the second point of an axis (in the figure, it appears at the end of the blue axis).

Moving Axes on the Image

Piximetre automatically couples intersecting axes, as explained above. However, it is not always possible to trace two intersecting axes on the object to be measured. This is the case, for example, with objects such as the one shown opposite.

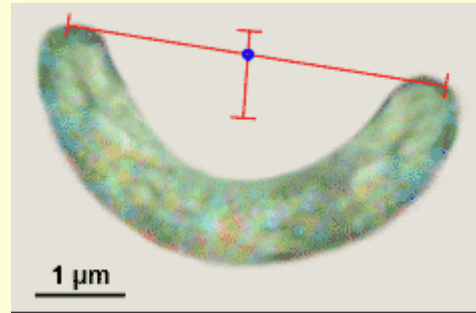


Here, the *Calculator* receives measurements in the form of two isolated axes (one large and one small), as shown in the table. It therefore cannot validly calculate their ratio $Q = X / Y$. For this calculation to be possible, the two axes must be coupled.

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

Piximetre offers the ability to **move axes on their image**, **without changing their length**, of course!

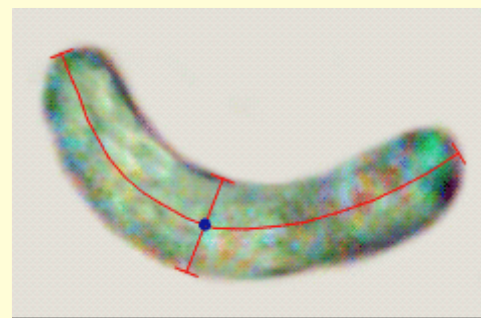
1. Hold down the **Ctrl** key on your keyboard while moving the mouse cursor toward the chosen axis. When it gets close enough, its shape changes. It transforms into a hand that allows you to grab the axis and move it elsewhere.
2. Grab the axis with the **left mouse click** and drag it while holding down the mouse button. When the axis intersects another axis, the intersection point automatically appears.
3. Release the mouse when the two axes are coupled. And the Calculator's response comes, allowing the calculation of the desired ratio.



Axe X	Axe Y
4,27	0,97

Nonlinear Axes

Piximetre allows you to plot **nonlinear axes** as shown in the figure opposite. The major **curvilinear axis** of the figure is obtained by a succession of small linear segments drawn while **holding down the Ctrl key**. The plotting ends when this key is released.



In the figure below, **an axis has been plotted on a map** representing a route. The plotting is carried out as a curvilinear axis. Here, the image has been calibrated using the 300 m scale.




Nonlinear axes are a generalization of the axes managed by Piximetre. They are processed in the same way as the others, both numerically and graphically.

The **context menu**, activated by a right mouse click, allows you, if necessary, **to delete an end segment** (the first or last) as soon as the cursor gets close enough to one of them. Placed elsewhere on the axis, it allows you to **completely delete** it, like a classic linear axis. Measure with a curvilinear axis.

This ease of action on end segments is especially useful when **the last segment drawn is a supernumerary one**.

Axis Correction

It may happen that an axis isn't drawn exactly where it should be, for example, due to a pointing error. As a result, it doesn't have the correct length, thus introducing an error in the measurements. In this case, there are two solutions: **erase the axis** (right click on its label or near the axis and select the "Erase Axis" menu) and then redraw it, or **resume drawing it** and correct it directly, without erasing it.

To correct an axis, simply move the cursor near one of its ends while **holding down the Ctrl key**. The cursor changes shape to the one  that allows you to capture the end in question.

At this precise moment, **without releasing the Ctrl key**, press the left mouse button. The cursor changes again and returns to its initial shape. This enters the standard axis drawing mode. This turns blue and follows the mouse movements as long as you keep the left mouse button pressed.

Two actions are then possible:

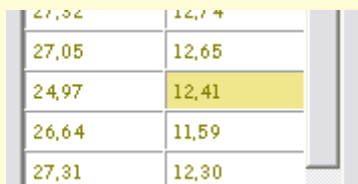
- **Release the Ctrl key** then moves the cursor to its correct position on the image. Release the left mouse button. The axis stabilizes in its new position. Its drawing is complete.
- **Hold down the Ctrl key**. You are then in [non-linear axis creation](#) mode as explained above. As soon as you release the left mouse button, *Piximetre* stabilizes the drawn axis and creates a new segment after it, linked to the previous one. To draw the last segment and not continue with a new one, **release the Ctrl key**.

All axes drawn on the images can thus be adjusted at any time, whether linear or not.

In the same way that you redraw the endpoint of a linear axis, you can modify the junction point of two segments of a non-linear axis. Move the cursor near the junction point and proceed as above. The two continuous segments are then redrawn.

Marking Axes on the Formulator's Measurement Grid

When the mouse cursor gets close enough to an axis or a non-linear axis segment with the **Ctrl key pressed**, the mouse cursor changes to a hand shape, as described above.



27,02	12,7 *
27,05	12,65
24,97	12,41
26,64	11,59
27,31	12,30

At this point, **holding down the left mouse button** displays the axis with a yellow background in the *Formulator's grid*, as shown in the figure above.

In this example, the user is pointing at the axis with a length of 12.41. It intersects with another axis with a length of 24.97. If it intersects

with multiple axes, it would be displayed multiple times. This link between physical axis and numeric value allows for **easy verification**, improving data entry.

Ctrl and Shift: Magic keys

Actions	Comment faire
Locate an axis in the Formulator grid.	<p>Ctrl + mouse over the axis until the cursor changes shape to a hand. With the left mouse button pressed, the axis appears with a yellow background in the Formulator grid, which automatically positions itself for viewing.</p> <p>Détails</p>
Draw a nonlinear axis.	<p>Place the first point on the image. Hold down the Ctrl key and successively place the other points. Release the Ctrl key to place the last point.</p> <p>Détails</p>
Move an axis on the image.	<p>Ctrl + mouse near the axis until the cursor changes shape to a hand. Left click and move the axis to the desired position. Release.</p> <p>Détails</p>
Place a measurement file in the Formulator grid.	<p>From Windows Explorer, select and drag the measurement file onto the Formulator grid. If the Ctrl key is pressed, the file replaces any other files already dragged onto the grid. Otherwise, the file is added to the others.</p>
Modify an axis already drawn on the image.	<p>Ctrl + mouse over the end of the axis to modify. The cursor changes to a selection box. Left-click to grab this end and move it to the desired location. Release.</p> <p>It is also possible to modify a multi-segment axis by pointing at one of its connection points.</p>
Locally invert the automatic orthogonal axes plotting function.	<p>When the function is enabled (<i>Formulator Settings</i> tab), pressing Ctrl deactivates it at the intersection of the axes being plotted. Otherwise, pressing Ctrl activates the function. Release Ctrl before marking the point or a new segment will be linked to the sequence.</p>
Display the Feeler while drawing an axis on the image.	<p>Draw the first point of the axis normally, then hold down Shift while moving the mouse to the second desired point. The Feeler appears under the cursor. Left-click to mark the point.</p>
Move the image in the Measurer.	<p>Shift + move the mouse over the image, then left-click.</p>

Also on the keyboard, using the arrow keys (right-left-up-down), with or without Ctrl pressed.

Length Scales

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

Since the objects are homogeneous, all images in the same series are automatically **assigned to the same scale**.

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

- **Display or hide the scale** on the images in the series,
- **Set its unit**. Several symbols are available for this purpose,
- **Set the scale value to be represented** on the image.
- **Automatically adjust** this value based on the image magnification using manual zoom. If the value is fixed, the length it represents, which changes with the zoom, can become large. Otherwise, the value represented automatically changes to maintain a suitable length scale on the image.
- **Change the scale color**.



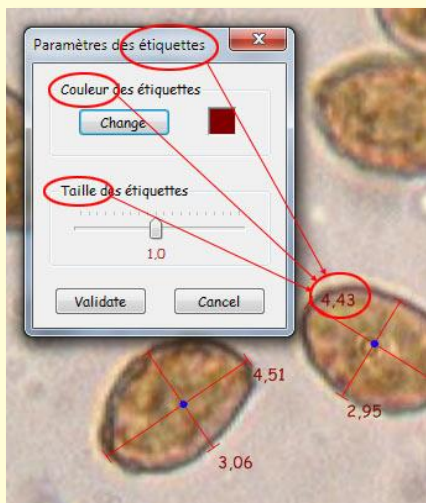
All these settings are applied to all images in the series.

Axis Labels

Axis labels are the dimensions indicated near the axes plotted on the images. They can be displayed or hidden (Settings tab: "**Show axis length**").

The label also allows you to **easily act on the axis** to move it (press Ctrl) or delete it (right click).

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



The color of the labels is reflected in all images opened in the *Measurer*.

The **Label size** slider allows you to vary the font size, up or down, around the central value of 1.0.

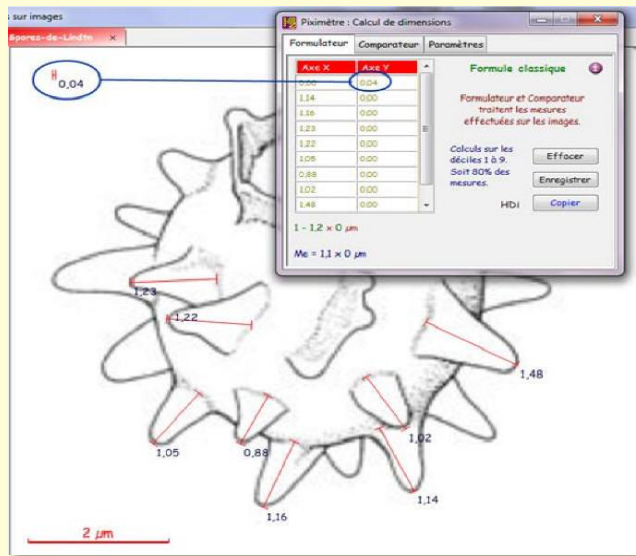
The real size of the characters displayed on **exported images** also depends on their size: when their width is between 2048 and 4096 pixels, the font size is multiplied by 2. Beyond that, it is multiplied by 2.4.

1, 2, 3 Dimensions

Basically, *Piximetre* measures **pairs of axes** corresponding to only two spatial dimensions (length x width or length x thickness, for example). As we just [saw above](#), it automatically breaks down measurements into one or the other dimension based on their value or the user's specifications. While it is not possible to measure all three dimensions of an object simultaneously, it is **easy to measure a single dimension** (the third, for example) by forcing all measurements into a single column of the *Calculator's* input grid. You have previously checked the "[Process isolated axes](#)" option on the *Calculator's Settings* tab.

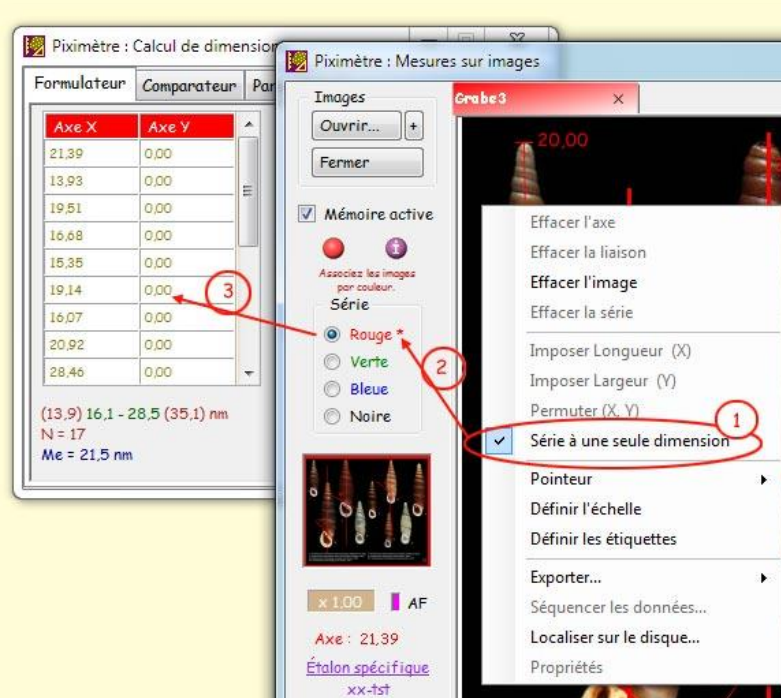
There are **two ways to do this**.

The first method, the oldest, consists of plotting any axis (only one per series of images) whose length is either very far from the values to be measured, either much greater than the maximum, or much less than the minimum. All subsequent measured values will then automatically fall into a single column. The opposite figure shows such an operation, where the goal is to measure the height of the spore spines: the left column contains the valid measurements, while the right column has been forced by plotting a very small axis of length 0.04, much smaller than the values to be measured. Zero values (in particular, here, the 0.00 opposite the 0.04) are obviously not considered for the calculation. A similar result would have been obtained by plotting a very large axis on the image, but in this case, the X and Y columns would have been reversed. To plot a very large or small axis, 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 Piximetre introduced a new, more direct method, via the context menu, for measuring only a single dimension (figure below):



Switching to one-dimensional mode via the context menu.

The **context menu** opened on a *Measurer* image (right-click) allows you to specify that the current series, the red one in this example, is to be treated as a **one-dimensional series**. This means that all axes plotted on all 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 zeros, which are not significant. The dimensional formula has only one dimension, the result of the X calculations.

One-dimensional mode provides a **classic formula** as well as a **statistical formula**, just like in two-dimensional mode.

Note in point 1 of the image **the check mark that appears in the context menu**, which indicates that the series is in one-dimensional mode. Also note in point 2 of the image that **the series name in the Measurer is marked with an asterisk**.

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

Clicking the menu again switches the series back to two-dimensional mode.

Series are automatically in **two-dimensional mode** when *Piximetre* starts.

The multi-series

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

It can be useful to work with and compare multiple measurement series. To do this, assign **a different color** to the images in each series. For example, a red series (all its images will be red) and a green series (all its images will be green).

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

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

When multiple series are present in the *Measurer*, an arrow appears as shown in the figure opposite. Clicking on this arrow allows you, if necessary, **to circularly swap the series colors**. Green becomes red, blue becomes green, and so on. The *Formulator* and *Comparator* reflect this swap.



Opening images

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



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

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

1. Frame an image for **the first time using** the mouse wheel (or the right-left buttons on the PAD). In the example opposite, the view (red frame) entirely covers the image, which is therefore fully visible in the Meter window; the zoom here is 0.73x.
2. **Right-click on the zoom** to bring up the context menu and select **Set as Default**.

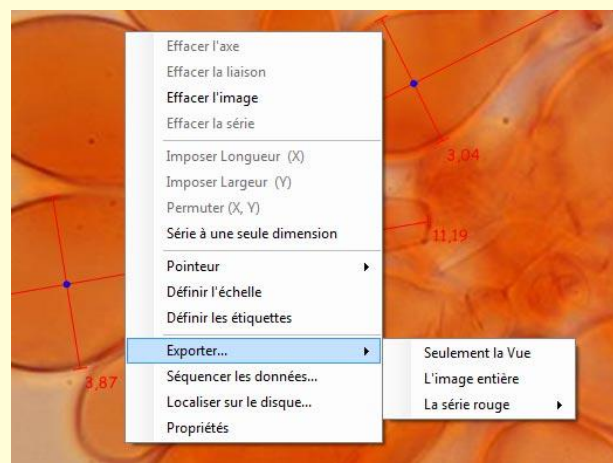
The **zoom value is saved** and will be applied when opening any new image, as well as when reopening an old one with Active Memory unselected.

Note, however, that the image presentation will only be consistent if they have the **same dimensions** as the image that set the default zoom.

The context menu

To finish with the image measurement function, let's return to the **context menu**, activated by right-clicking on an image, which allows you to:

- **Delete any axis** as soon as the cursor pointer gets close enough to it,
- **Delete two intersecting axes** by clicking on the materialized coupling point,



- **Delete or reestablish a connection** between two axes by clicking on their intersection,
- **Clear the image** (all axes plotted on the image - be careful, there is no going back),
- **Clear the series** (all images in the series, when several of them contain axes),
- Force the pointed axis **to be recorded as an object length**, i.e., in the X column of the Formulator,
- Force the pointed axis **to be recorded as an object width**, i.e., in the Y column of the Formulator,
- **Swap the two axes** in the *Formulator*,
- Change the measurement mode of the series in question **from two-dimensional to one-dimensional** (see [1, 2, 3 Dimensions](#)),
- **Change the pointer shape**,
- **Set the scale properties** of the current series,
- **Set 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](#)),
- **Sequence** the data ([see here](#)),
- **Locate** the image on disk,
- **Display the image properties** in a dedicated window. This window can also remain open. It then permanently displays the properties of the selected image (if they are present in the image, i.e., if the image is not overly compressed).

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

Data mixing

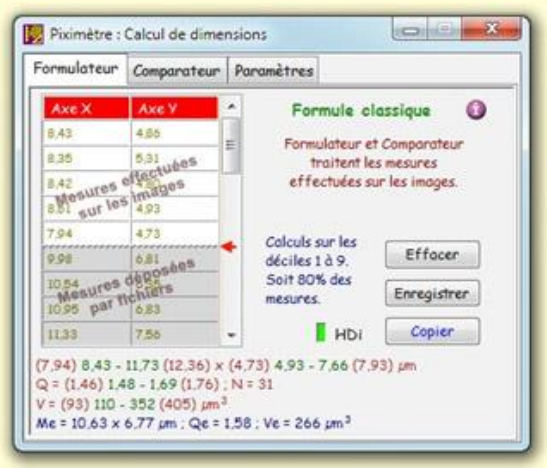
Data mixing is the ability to add new measurements to existing data. During a previous session, the user saved results to a file using the "Save" button in the *Formulator*. To improve these results, they now want to add new measurements because they have new data. The process used to do this is called **Data Mixing**.

Methods

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

1. When multiple measurement sessions have been performed and each saved in a separate file, simply **drag and drop** each of them onto the initially empty *Formulator* grid. All the files thus placed in the *Formulator* are summed, and the dimensional formula is calculated **for all the data**.
2. The second method involves using **two data streams simultaneously**. One consists of data entered as described above, by dragging and dropping files into the *Formulator*, and the other consists of data directly measured on images opened in the *Measurer*. The streams are added together in the *Formulator*, and the dimensional formula is calculated **for all the data**.
 - Drag the measurement file(s) onto the initially empty *Formulator* grid. 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 perform the measurements. These are automatically added to the grid (this time **on a white background**), and the overall dimensional formula appears below (remember 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:



- A dragged-and-dropped file containing previously established measurements (**gray area at the bottom of the grid**),
- 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 streams. A new file dropped onto the grid will be automatically added to the existing data in the **grayed-out area**. New measurements from the images will be added to the **white area above**.

Remember that data dragged and dropped onto the grid, intended for the grayed-out area, **can be manually modified by the user**, who can correct, add, or delete data as needed.

To delete data in the grayed-out area, simply **replace it with 0** (0 are not counted in the results). The row is deleted from the grid when its two cells have the value of 0. To manually add new data in the grayed-out area, simply enter its value in an empty cell at the bottom of the grid. You can 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. First, simply open all the images that generated it. If and only if they were initially closed with **Active Memory** selected, their measurements are retrieved and loaded into the *Calculator*. The new images are then added to the series; the measurements taken from them will be added to the originals.

Mixing Conventions

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

In practice, data can be entered into the *Formulator* at any time and in any order: via the *Measurer* or via uploaded files, and even manually in the gray area.

Furthermore, when both columns of the grid are used (the general case of measuring two dimensions), *Piximetre* always processes the data under the assumption that column X represents the length of the measured objects and column Y their width. Therefore, **X > Y** is always the case. The dimensional formula is oriented in this direction. However, it is also possible to organize dragged and dropped files in the reverse order: **X < Y**. This is particularly the case for manually created files.

When all the dropped files are organized according to this second convention, *Piximetre* preserves it and displays the results in that order. When both conventions are present, *Piximetre* does not mix the data but automatically aligns them according to its **preferred convention: X > Y**.

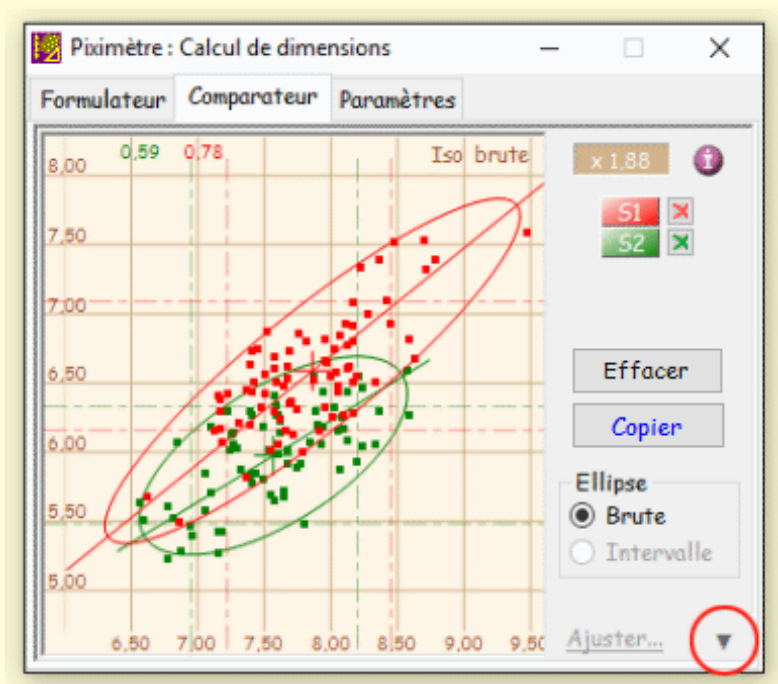
Data mixing is fully **compatible with [Image Stream](#)** mode.

The Measurement Comparator

The *Comparator* allows you to **visualize and compare** the spatial distribution of multiple measurement series. The *Comparator* image can be copied into a document (e.g., Office) by simply **copying and pasting** it from the clipboard.

Principle

The *Comparator* can **visualize all series simultaneously** (red, green, blue, and black). Each series is represented by a scatter plot corresponding to the raw data of its well-formed (x, y) pairs (both components of which are present). Also displayed are their trendy ellipses, major axes, slope values, etc. The figure below shows a red 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 (X, Y) plane is **normalized**, and the axis origins are automatically adjusted based on the values presented. It can be **translatable** by moving the mouse over it and left clicking.

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

The combined action of these two tools allows you to freely frame the series displayed in the *Comparator* map.

All elements of each series are plotted in the color of the series. 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) 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 clear the series, which, in *Graphics* mode, closes all open images of that series. The **Copy** button places the *Comparator* image on the clipboard. It can then be pasted (Ctrl + V) into a working document, such as an Office document.

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

Ellipse Parameters

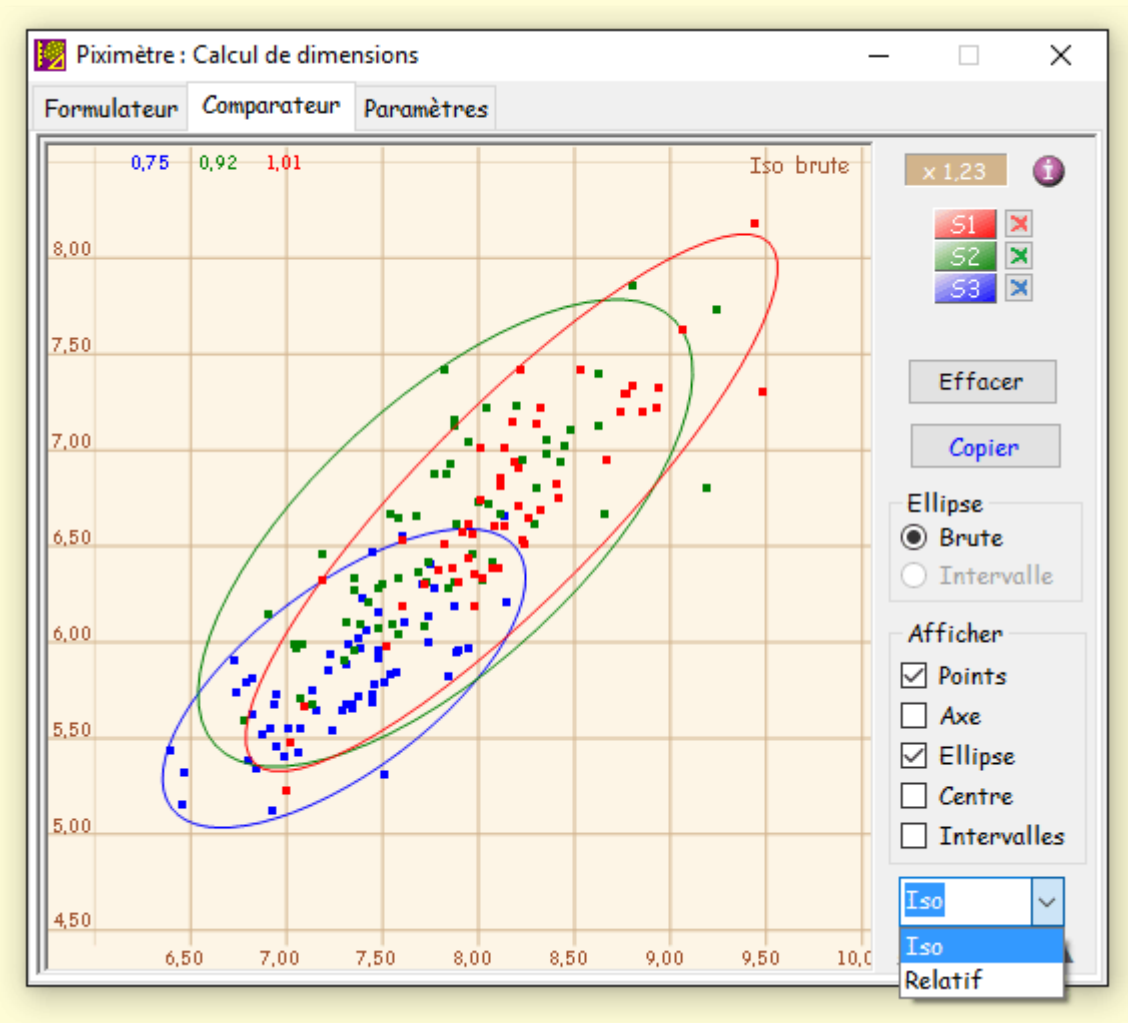
The **trend ellipse** represents raw data. It has no mathematical meaning. Its characteristics are as follows:

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

Comparator Settings

Two *Comparator* views are available: the **reduced view** and the **expanded view**. Access them by clicking on the **small triangle** in the bottom right corner (see figure above).

The expanded view, in addition to enlarging the *Comparator* map, provides access to various display settings, as shown in the following figure.



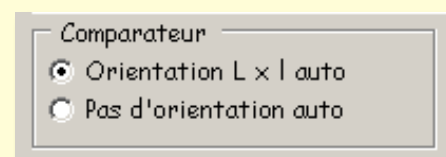
The *Comparator* in "expanded" view.

Note at the bottom the choice of two grid views: **Iso**, which is the default view and shown here, or **Relative**. In this second case, the axes are not numbered, and the series are centered on the center of gravity of the set (designated by G).

The grayed-out options are not active. They are reserved for future development.

Measurement Orientation

At the user's discretion, measurements can be noted and recorded in the form $L \times W$ or $W \times L$ (with $L > W$). This corresponds to two different series orientations. Therefore, comparing series with different orientations poses a problem.



To solve this problem, *Piximetre* has an automatic measurement orientation feature. When this feature is enabled (above, *Settings* tab), the series entered into the *Comparator* are automatically oriented like the red series, which is the **reference series**.

Importing Series into the Comparator

The user can directly enter a series contained in a measurement file by simply **drag and drop** the file (from Windows Explorer) onto the *Comparator* window.

The feasibility of this operation is indicated by the mouse cursor changing to a significant shape. To be accepted by the *Comparator*, the data must have been previously saved using the **Save** button in the *Formulator* or must correspond to the syntax indicated 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 in the *Formulator*).

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

The Dimensional Formula, the Result of Measurements

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

For a quantity being studied (length, width, or their quotient), it is useful to know **the range** within which its values fall as well as **their average**. An essential solution, when these values become very numerous, is to take them from **a sample deemed representative** of the total population, and traditionally, for example, we write:

$$8.1 - \underline{8.8} - 9.7$$

However, statistics and probability allow for **greater precision** by limiting the uncertainty associated with the choice of a sample.

Piximetre offers the user the option of obtaining results using either traditional (also called **classical** here) or **statistical** methods. Switching between the two is done in the *Formulator*, by **clicking on the dimensional formula**, or in the Advanced properties of the *Settings* tab. Starting with version 5.9, Piximetre offers the ability to add the calculation of [spore volume](#).

The classic formula and its calculation

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

1. The measurements 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 reduced by its two extreme groups,
4. The exceptional values are the extreme values of the entire sample,
5. The interval chosen for the quantity corresponds to the extreme values of the reduced sample; deciles 1 and 9.

The classic formula has the general form: **(Mini) min - max (Max)** where the values in parentheses are the **exceptional values measured** and min - max are the limits of the interval corresponding to **80% of the measurements taken** (deciles 1 and 9). Exceptional values are only shown if they are different from the range limits.

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

In practice, Piximetre expresses the **classical formula** in one of the following forms. Feel free to click (right or left) on the dimensional formula displayed in the *Formulator*.

- The expanded form, as in this example:

$$(4.1) \ 4.15 - 5.06 \ (5.1) \times (2.8) \ 3 - 3.8 \ (4.2) \ \mu\text{m}$$

$$Q = (1) \ 1.1 - 1.5 \ (1.6) ; N = 30$$

$$Me = 4.62 \times 3.45 \ \mu\text{m} ; Qe = 1.35$$

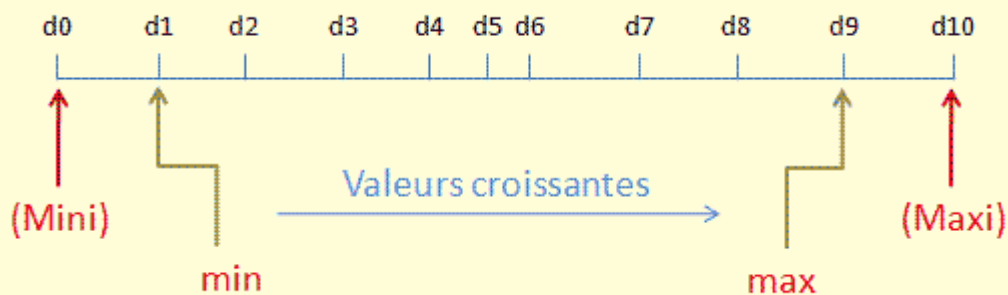
Me and Qe (e for sample) are the average values of the measurements.

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

$$(4.1) \ 4.15 - 4.62 - 5.06 \ (5.1) \times (2.8) \ 3 - 3.45 - 3.8 \ (4.2) \ \mu\text{m}$$

$$Q = (1) \ 1.1 - 1.35 - 1.5 \ (1.6) ; N = 30$$

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 dividing the sample into deciles.

The statistical formula and its calculation

For a **sample** of size n , we calculate the **mean** and **standard deviation** m_e and σ_e .

For the **total population**, we can estimate the meaning **m** by m_e and the standard deviation σ by $\sigma_e \sqrt{n/(n-1)}$

In Excel, σ_e and σ are calculated by STDEV and STDEVP, respectively.

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

Unfortunately, the **assumption of a Gaussian distribution does not always seem relevant**, especially for small samples, because when grouping the sample values into classes, we see that the histogram is most often **not homogeneous** (Hentic: 176). On the other hand, the situation is quite different for the average values: if we take sufficiently

large samples (for example, of size $n > 30$), the average of the sample values follows approximately a normal distribution, with parameters m and σ/\sqrt{n} (Verlant: 124). We then access a confidence interval at the threshold $c\%$ for the population average.

Presentation of the results

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

$$7.9 [8.7; 9.1] 9.9$$

Which translates to:

- There is a 95% chance that the length of a mushroom spore is in the interval [7.9; 9.9]. And
- There is a 95% chance that the interval [8.7; 9.1] contains the average length of the mushroom spores.

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

Where:

[Min; Max] is an interval centered on the **sample mean**. At a threshold of $c\%$, it has a radius $k(c) \sigma_e \sqrt{n/(n-1)}$ and the values **Min** and **Max** are its bounds. This indicates that there is a $c\%$ chance that the value of the quantity studied lies between **Min** and **Max**.

[m; M] is the confidence interval on the **population mean**. It is centered on the sample mean and, at a threshold of $c\%$, it has a radius $k(c) \sigma_e/\sqrt{n-1}$. The values **m** and **M** are its bounds. This indicates that there is a $c\%$ chance that this interval contains the average of the values of the quantity studied over the entire population.

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

In practice, Piximetre expresses the **statistical formula** in one of the following forms.

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

$$11.4 [14.6 ; 15.4] 18.6 \times 11.4 [14.6 ; 15.3] 18.6$$

$$Q = 0.9 [1] 1.1 ; N = 100; C = 95\%$$

$$Me = 15 \times 15; Qe = 1$$

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

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

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

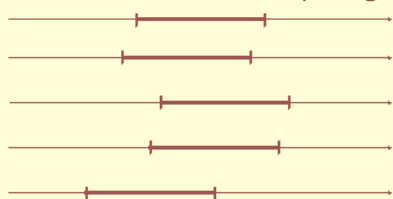
Here, the population mean interval bounds (in green) only appear if they are different from the mean value. The expression for Q in this example is typical and replaces the form [1 -1- 1]. Increasing the number of decimal places in the result can change this expression.

Note

With other samples of the same size, we would obtain new confidence intervals for this mean **m**, with the same confidence coefficient. All would have the same amplitude:
 $2 \times 1.96 \sigma/\sqrt{n}$

Here are some examples:

If we were to take a very large number of such samples, approximately 95% of them would contain the unknown population mean **m**. In fact, we only take one sample, and we cannot know for sure whether or not it contains the number **m**, but the method used allows us to obtain a "good" interval in 95% of cases. A "good" interval contains **m**.



Mathematical formulas

1. Sample

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

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

classic formulas:

2. Total population

- **Point estimate**

$$\text{Moyenne } m = \frac{1}{n} \sum_{i=1}^n x_i \text{ et écart-type } \sigma = \sqrt{\frac{1}{n} \sum_{i=1}^n (x_i - m)^2} = \sigma_e \sqrt{\frac{n}{n-1}}$$

- **Interval estimate**

$k(c) = 1,96$ au seuil de 95 % et $k(c) = 1$ au seuil de 68 %.

- Confidence interval for the magnitude values:

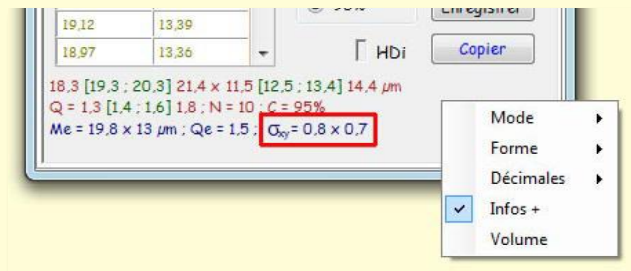
$$m_e \pm k(c)\sigma = m_e \pm 1,96 \sigma_e \sqrt{\frac{n}{n-1}}$$

- Confidence interval for the meaning of the magnitude values:

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

Accessing statistical parameters

Piximetre also provides the **standard deviation value** calculated for each sample. This information is added to the dimensional formula as shown, outlined in red, in the opposite figure.



This information is only available in the statistical formula, by **right-clicking on the formula and selecting "Info +"**. It is copied with the formula.

Spore volume

Regarding the study of fungi, some recent publications refer to other parameters, including **spore volume**. Jean-Louis JALLA, an experienced mycologist, drew our attention to the importance of adding it to the dimensional formula calculated by *Piximetre*. This data is certainly very significant and varies greatly between closely related species.

The formula for calculating spore volume currently used in mycology tends to equate spores with **ellipsoids of revolution**, which is, of course, only a theoretical view. This volume will be all the more accurate as the shape of the spores approximates this perfect structure as closely as possible. For real shapes, the calculated volume will therefore be **affected by a significant error**, depending on their distance from the ellipsoid. Nevertheless, it can still serve as a **reference**, provided, of course, that the limitations of the method are always kept in mind.

The formula for calculating this volume is $\frac{4}{3} \pi \times [(l/2)^2 \times L/2]$ where *l* and *L* represent the small and large dimensions of the measured objects, respectively.

Be careful, however, as a measurement error in dimensions has significant repercussions on the volume. For example, for a spore measuring $8.5 \times 10 \mu\text{m}$, the calculated volume is $378 \mu\text{m}^3$, which becomes $445 \mu\text{m}^3$ for a $0.5 \mu\text{m}$ excess error in both dimensions. The benefit 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 (e.g., *Piximetre* calibration error) always exists, hence the importance of the most rigorous calibration possible.

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

Volume *V* is added, here in classic form, with the average volume *V_e* calculated for the sample.

The **context menu** (right-click on the formula) allows you to display or hide the volume, as shown in the image.

All information displayed by the Formulator is copied or saved using the corresponding buttons, like other data.

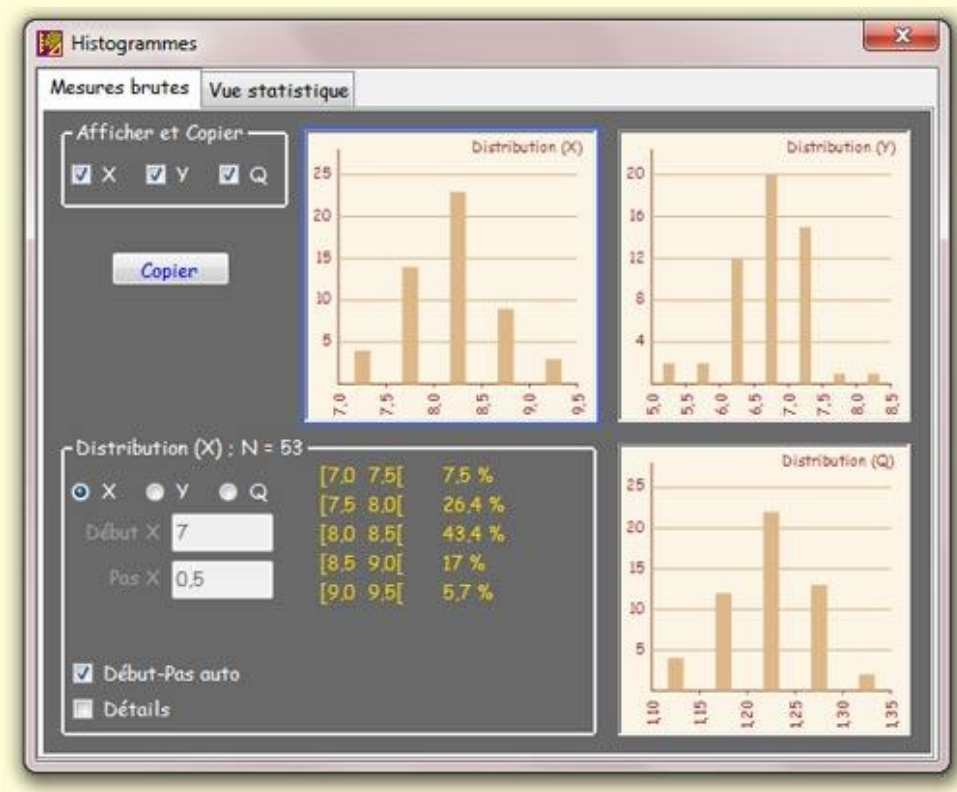


Histograms of the measurements

The floating window below is activated by clicking "HDi" with the mouse. It provides additional data on the measurements taken:

- Graphical 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 the X, Y, and Q axes. By default, all three histograms are displayed, but it is possible to select only some of them. The "Display and Copy" box is reserved for this purpose.

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

When the "Auto Start-Step" box is checked, Piximetre automatically chooses the data range to explore and the resolution step. When unchecked, the user can modify these settings.

The selected intervals and their population are shown in yellow.

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

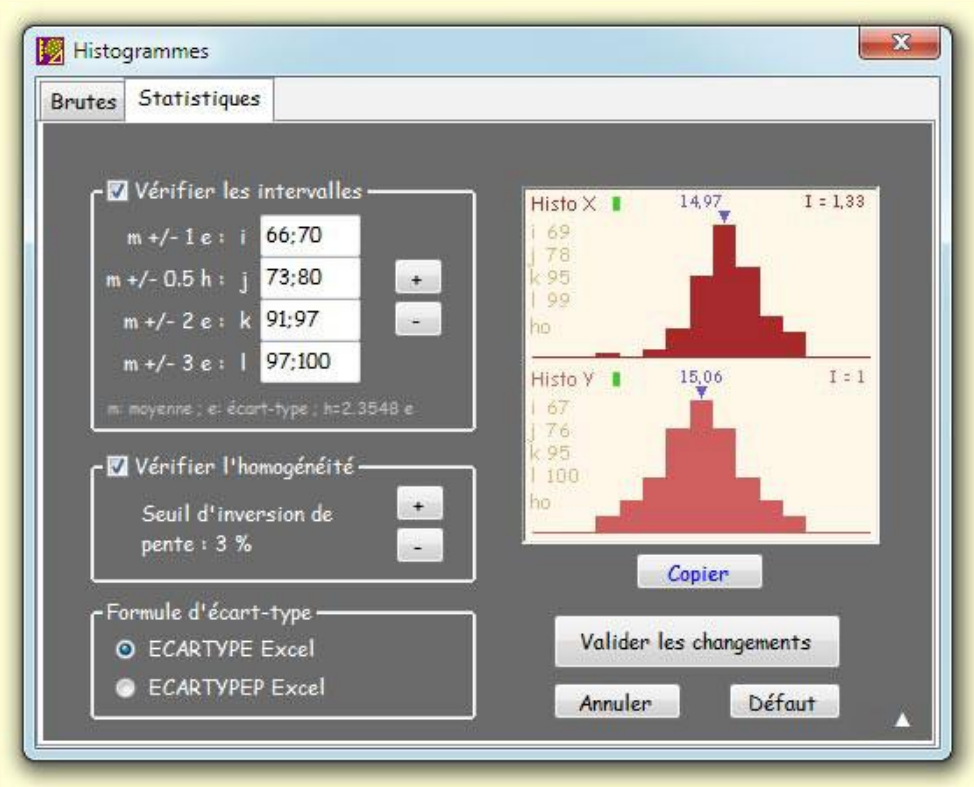
Finally, the "Copy" button places the selected histograms and numerical data on the clipboard. The data can then be pasted into a Word document, for example. Below is an example of a document after a three-column layout in Word.

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 then be pasted into a Word document, for example. Below is an example of a document after a three-column layout in Word.

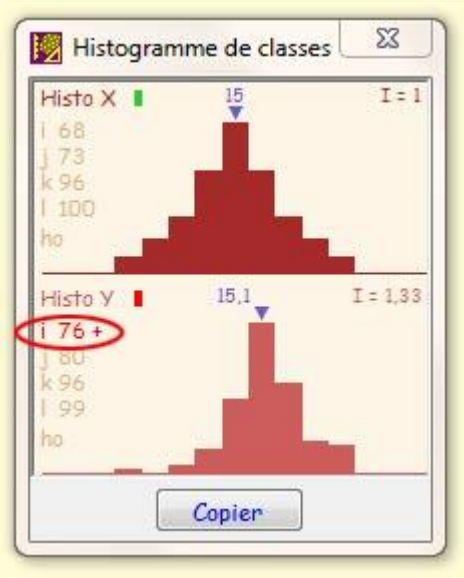
Statistical Results

Expanding the **Statistics** tab (▼) provides access to several elements that control histogram conformity tests.



Statistics and normality tests.

In the following figure, the histogram is **normal** in X but **not** in Y.



Experience shows that in mycology, when measuring mushroom spores, it is not uncommon for many measurements (100-200, or even more) to result in the visualization of the **famous bell curve of the normal distribution**, which further validates the results of the statistical formulation. *Piximetre* can handle a very large number of measurements without difficulty.

The samples obtained are divided into **classes** (typically nine classes) represented 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]** is valid regardless of this distribution.

Piximetre verifies the normality of the sample distribution law and gives the *Formulator* the **green light** 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:
 - 68% of the measurements in the interval $m \pm \sigma$
 - 76% of the measurements in the interval $m \pm 1.1774 \sigma$,
 - 95% of the measurements in the interval $m \pm 2 \sigma$,
 - 99% of the measurements in the interval $m \pm 3 \sigma$,

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

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

2. **Verification of the homogeneity** of the histograms. This check ensures that the content of the classes increases regularly, then decreases beyond the median class. Here too, *Piximetre* allows a certain tolerance: a reversal of growth (or decrease) is only considered beyond a certain threshold, corresponding to a

defined percentage of the sample (by default, 5%). Clearly, a slope inversion of less than 5% (in this example) is not counted as such.

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

The Formulator's general indicator light is green only when samples X and Y are both NORMAL. It is orange if only one of the two is normal; otherwise, it remains off.

In the Y histogram in the figure above, i **76 +** (in red) means that the interval i ($m \pm \sigma$) of the sample Y contains **76% of the sample values**, which is more (+) than defined (by default [64%, 75%]). The corresponding distribution low is not normal, although the histogram is homogeneous (ho is not red). As a result, **the histogram light turns red**, which causes the *Formulator's* general light to turn orange, not green (in our example, since X is green).

Note: **The interval provided in red by Piximetre**, in its statistical dimensional formula, is only valid within the framework of a normal population attested by a normal sample. It is understood, however, that **the interval on the mean is always valid** since there is a normal distribution for means.

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

Interested users can refer to detailed information on [Wikipedia](#).

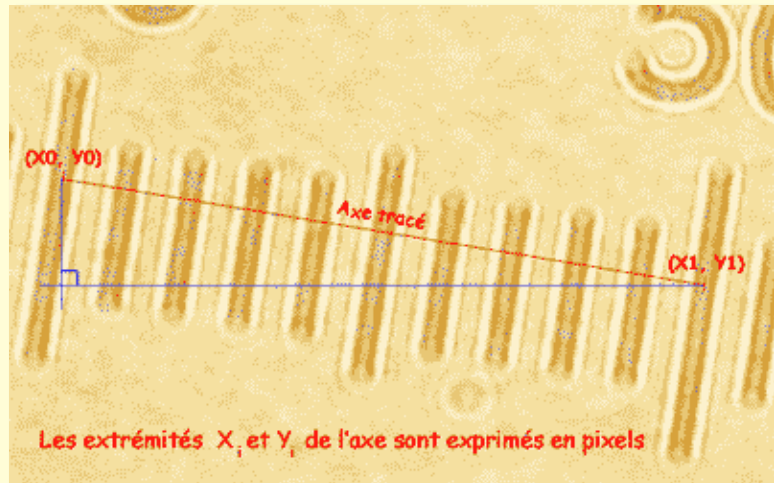
It is also possible to choose the standard deviation calculation formula used. *Piximetre* uses the Excel formulas: STDEV and STDEVP [explained above](#), respectively, when analyzing a sample of the total population, which is generally the case in mycology, or the entire population in other cases. By default, **STDEV** is used.

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

Piximetre calibration

Converting pixels to length unit

The calculation of the length of an axis plotted on a screen image is first done in pixels. The two ends point of this axis are determined by their coordinates (x, y) relative to the screen (relative to the window that displays the image, but this is only a matter of origin since the unit remains the same: the pixel). The axis is therefore **the hypotenuse of the right triangle**. Its length is calculated by a square root (Pythagore theorem).



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

Introducing a **magnifying glass**, which allows you to expand or contract the scale, corresponds to modifying this relationship by applying the magnifier's multiplier coefficient. **Very precise length measurements**, commonly with less than 2% error (often only 0.1 to 0.2%), can be performed with *Piximetre*, provided that a precise calibration has been performed beforehand.

When images are captured using variable focal length (zoom) cameras, **two independent operations** are required to fully perform this calibration:

1. **Initializing the automatic autofocus** for each camera used. This operation is intended to calculate the image magnification for each zoom position used when shooting.
2. **The length calibration itself**, which aims to establish the pixel-to-unit length relationship.

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

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

The *Settings* tab of the *Piximetre* calculator allows you to specify the type of images used: **Normal** (with metadata) or **Compressed** otherwise.

It is strongly recommended that you read [this note](#) regarding optics and measurement errors.

Initializing Automatic Autofocus

Routine measurements, as well as length calibration, are performed on photos taken with one or more digital cameras. The **intrinsic magnification** of the image depends on the zoom position used when shooting, all other things being equal regarding shooting conditions.

The purpose of initializing *Piximetre's* automatic autofocus is to precisely establish the **mathematical function** that allows the calculation of this magnification ([see this operation here](#)).

Piximetre uses the metadata contained in the photos (but invisible to the human eye) to determine the focal length used when shooting. Therefore, care should be taken to **never compress the images** intended for it. This operation can delete the metadata they contain if the compression rate is high. Furthermore, *Piximetre* easily handles very large images (the only limitation is the memory capacity of the computer used).

Since this autofocus initialization operation is performed once and for all, for each camera used, all photos can now be correctly interpreted, **regardless of the focal length used** to capture them.

Piximetre's autofocus supports **any number of different cameras**. Therefore, to work with images from several of them, it will be necessary to perform this initialization for each of them first.

Piximetre also offers the ability to **directly import the mathematical functions** in question, calculated and exported elsewhere ([see here](#)). This feature is useful when a user wants to work on images taken by others, with a camera they don't have.

Remember also that *Piximetre* visually indicates how its autofocus is working on the images it processes ([see here](#)).

Length Calibration

Which **reference object** should you choose and how should you measure it to calibrate *Piximetre*? The answer to the first part of the question is simple: **any object** visible through the optical system used (e.g., a microscope) and **whose actual length is perfectly**

known! This reference object should be chosen so that **its length is in scale** with the subsequent objects to be measured. For example, if you want to measure mushroom spores with a size of around ten microns, you will choose a ruler or an object of this size as your reference standard rather than a two-decimeter ruler.

As for the second part of the question, simply photograph this object **for a defined state of the optical system**. *Piximetre* calibration is then performed:

1. By **tracing an axis** on the image of this object of known length (figure above), and,
2. By manually **indicating the exact length** represented by this axis.

This operation is performed using *Piximetre's* **calibration wizard**. Note that if you have previously initialized autofocus for the camera in question, **you can use any zoom lens** for the shot. The correction will be performed automatically. **You can also use the magnifying glass and lateral image movement**, primarily to enlarge and position the image on the screen and thus increase pointing accuracy.

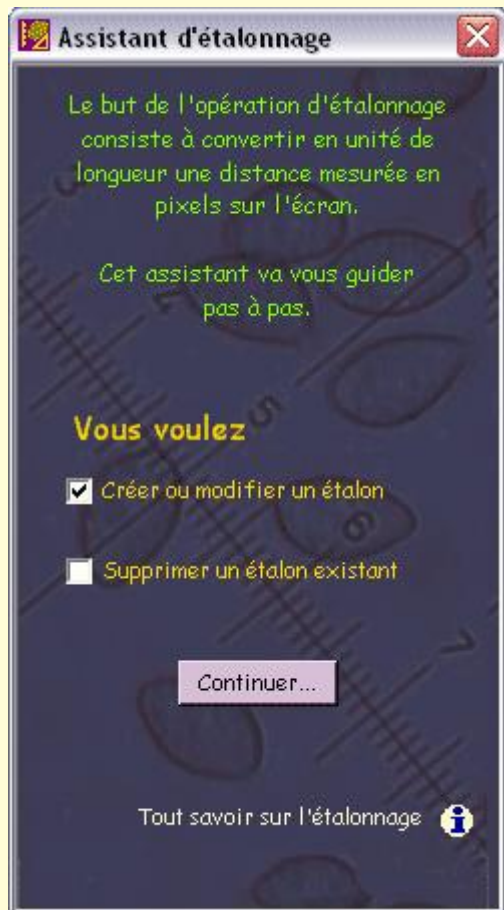
Consider, for example, the case of a microscope:

1. You are photographing through a calibrated micrometer eyepiece, meaning **you know the precise value of each division of its ruler** for the microscope objective being used. The image of this ruler appears in the photo, and *Piximetre* is calibrated by tracing an axis along all or part of this ruler (figure above) and then indicating the exact length it represents.
2. You are photographing through a microscope **without an eyepiece** or through a non-micrometer eyepiece, meaning you don't have a graduated scale on the image. *Piximetre* must then be calibrated independently of these images, using a reference graduated ruler **placed directly under the microscope objective**. This ruler is different from the previous one. It is called **an object micrometer**. It generally consists of a series of divisions **spaced 10 μm** apart and is traditionally used to calibrate optical microscopes. Here too, the interval between graduations is precise and perfectly known. We therefore take a photo of this ruler and proceed as in the case above.

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

Piximetre offers the ability 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 measurement window or by the "+" button to its right. This "+" button initiates the first step of the process. The "Calibrate" button is a shortcut that leads directly to the second step. The first step (figure opposite) initiated by the "+" button specifies the operations to be performed:

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 to facilitate their use and to modify them if necessary.

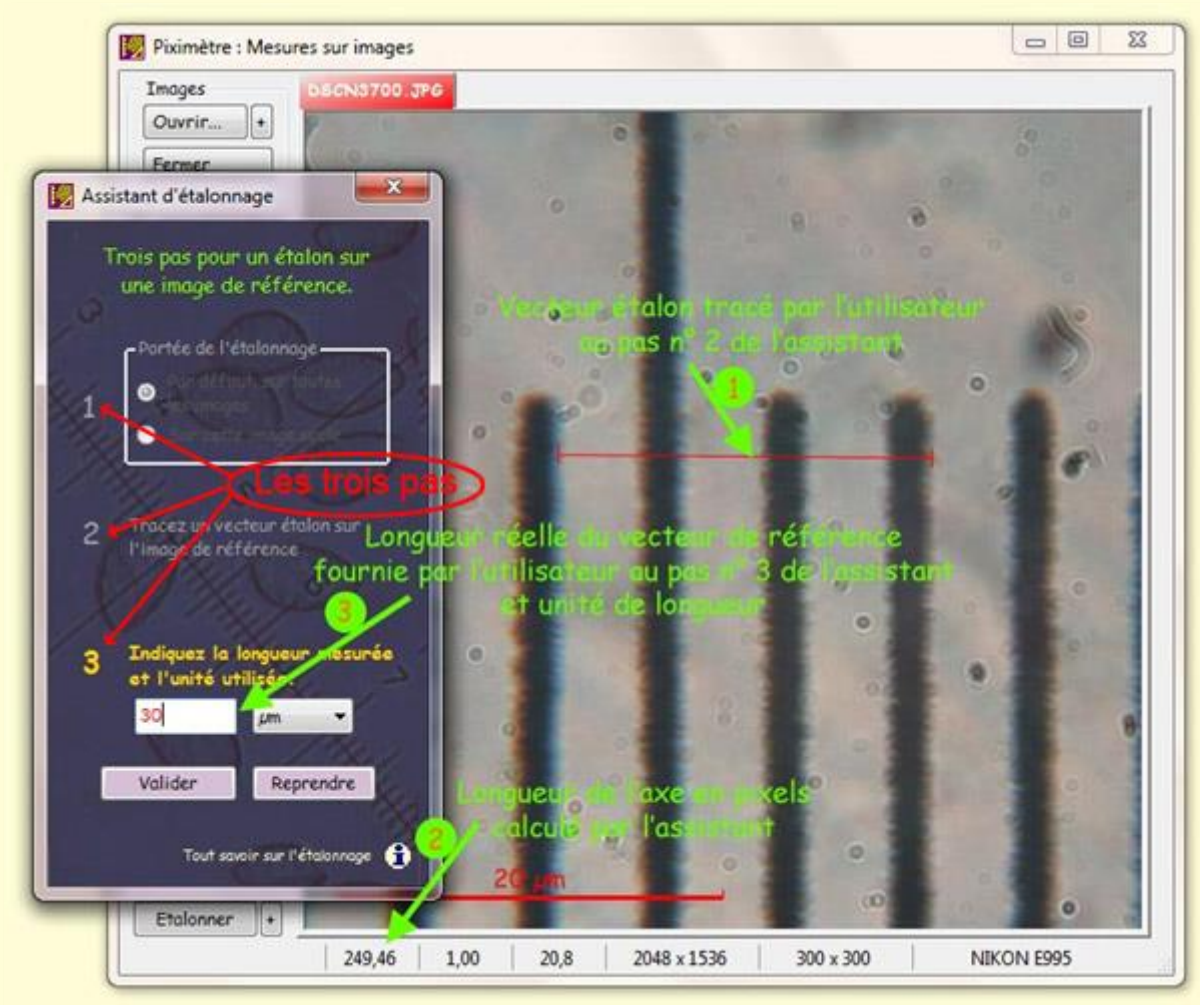
Delete an existing standard.

The icon  activates the calibration tutorial.

The second step depends on the choices made. If creation or modification is selected, the wizard helps you define a standard. If deletion alone is selected, it takes you directly to that operation. If both options are selected, the wizard first helps you create (or modify) a

reference standard and then delete another.

Let's look at the calibration process in three steps.



The calibration process at step 3.

The calibration process consists of 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, if necessary, resume the process from step 1 ("Resume" button).

1. In step 1, you choose the scope of the calibration. You can decide whether the standard will be specific to this image only or, conversely, generic, i.e., applied by default to **all images, open or future**, that do not already have a specific standard (see below). This is where the two types of standards appear: **specific** or **generic**.
2. In step 2, you draw an axis on the ruler or object of known length, present in the image. Note the display of the length of this axis in pixels (249.46 in the

figure). Also note that greater accuracy will be achieved by choosing a **longer axis** (reducing the relative error inherent in the measurement), provided, however, that the image is perfectly linear. See [here](#) for more on this. You can also **use the magnifying glass**, which improves pointing accuracy as well as **the lateral movement of the image on the screen** between each of the two points.

3. Finally, in step 3, **you indicate the actual length** of this axis in the appropriate unit (30 microns in this example, where the object micrometer is 10 μm per division).

The calibration wizard, by default, offers **different length units**, but you can define new ones here if the proposed ones are not suitable. The "- Edit -" option available in the drop-down list provides access to this feature.

Validate completes this process when it concerns a specific calibration, **on this image only**. 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 to all images**, the "Validate" button presents a new wizard step asking you to **name this standard** (figure opposite).

Managing **multiple generic standards** requires naming them, 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 are user-defined. Selecting an existing standard allows it to be redefined.

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

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

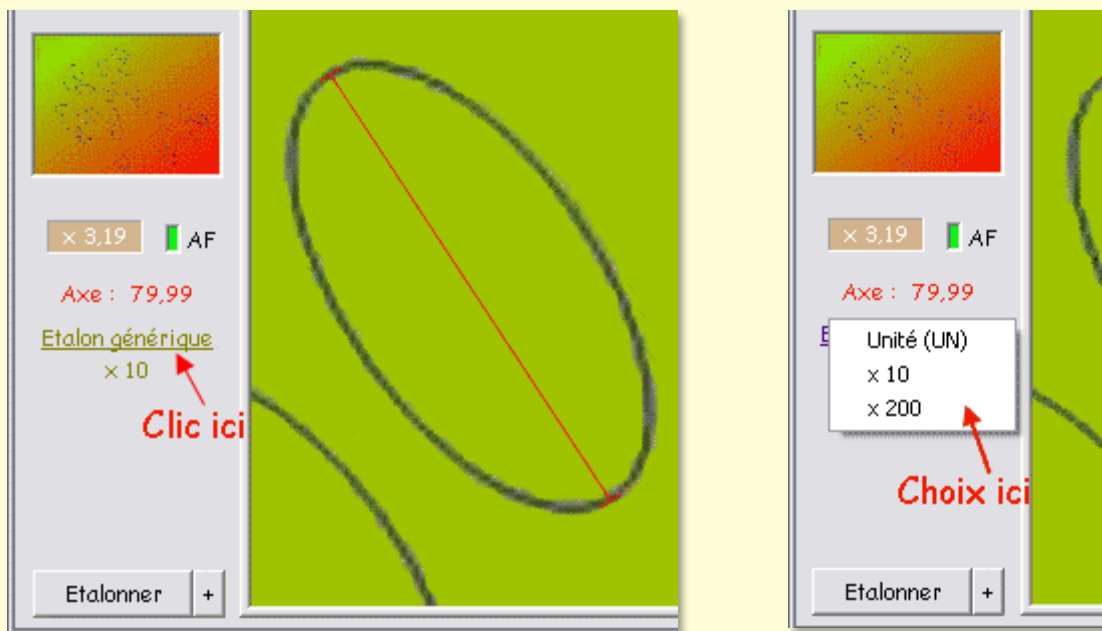
The "Delete" button allows you to delete a selected standard from the list.



Choosing a Generic Standard

Standards are created as described above.

In the **Image Measurements window** (figure below), the user chooses the generic standard that will be applied to all currently opened or future images that do not already reference a specific standard. Remember that this standard should be **chosen based on the state of the optical system used** when the image was taken; in other words, in the case of a microscope, the objective used (one for the 40x and another for the 100x, not to mention the eyepiece or camera used).



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

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

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

Generic Standard, Specific Standard

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

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

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

Upon subsequent reopening, 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 that enabled the axes calculation 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 *Piximetre*, **an image is assigned to a generic standard by default**, which the user can keep or, if necessary, change. However, upon subsequent reopening, this standard, whatever it was, **becomes specific to the image** and can no longer be affected by any possible modification of the generic standards; its name is retained. This principle ensures that **the measurements made on the images are preserved** when they are subsequently reopened.

If the user **deletes or redefines a generic standard** that he previously created and used, all the images in the 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 in this state. When it is reopened with active memory activated, **the image regains the value of its initial standard**, but the name of the latter is then prefixed by "ex".

The Live View Video Wizard

The **Live View Video Wizard** allows *Piximetre* to connect directly to **cameras installed** on the computer, view the images they produce, perform key adjustments for lighting, contrast, etc., and finally, capture images for the desired measurements.

Images captured by the wizard are **automatically opened in the *Measurer***, which performs all standard metrology operations, which are identical to those performed on images from other media, such as cameras.

A **generic standard** must be established for each optical system used - for example, for each lens used on the microscope and for each video camera with a given aspect ratio (image width/height ratio). It should be noted here that, to be valid, **the generic standard must be created and used under identical conditions** (same lens, same axial camera position, same image aspect ratio, etc.).

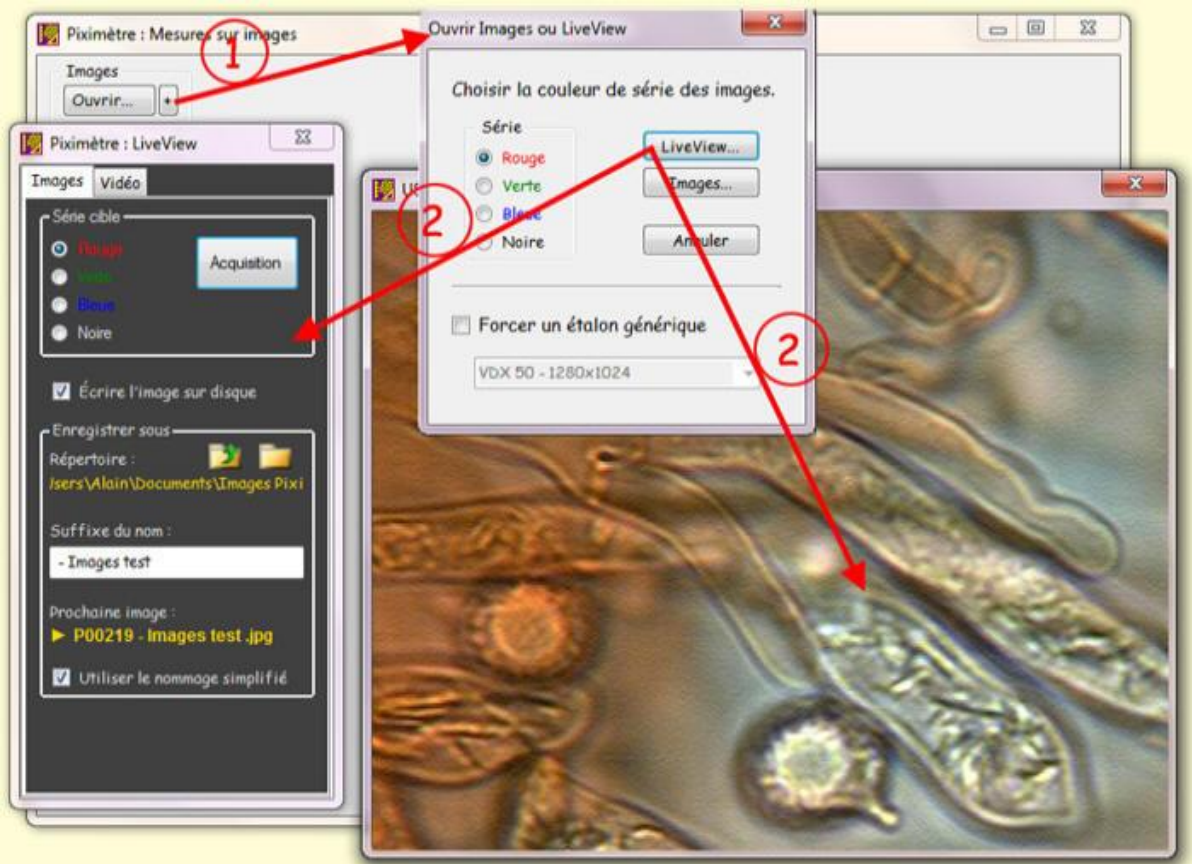
The main advantage, and not the least, of the Live View video assistant lies in the **simplification of image acquisition**: there's no need to manually transfer images from the camera's memory card or use separate video capture software. *Piximetre* provides a **fully integrated image capture service**.

The Live View video assistant supports cameras installed on the computer and **opened at third-party applications**. Their installation must, of course, have been completed using the specific software provided by their manufacturer. Furthermore, to be usable by *Piximetre*, cameras must meet **recent standards**, particularly regarding their Windows integration driver. A camera that is too old or does not meet these standards will not be usable. *Piximetre* and its Live View 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 cameras generally do not contain the metadata specifying their acquisition conditions. However, for them to be processed by the *Measurer*, *Piximetre*, starting with version 5.3, **adds the necessary metadata to the images**: camera name, image dimensions, and fixed focal length.

The advantage of this camera identification lies in **the consistency check** performed by *Piximetre* between measured images and the reference standards used. Images from different sources cannot be accidentally measured by the same reference standard without generating a warning message. Reducing sources of error always benefits the user.

Implementation



Opening the Live View Video Wizard from the *Measurer*.

The Live View Video Wizard is opened using the Meter's "**Open +**" button, 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 to be captured. Otherwise, the images will be sent to the *Measurer's* active series. It also allows you to **choose a generic standard** other than the one currently selected.

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

The wizard has **two windows** that remain in the foreground: The smaller (black), fixed-size window is the control window that allows you to specify the capture parameters (see below). The second, variable-size window, **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, usually located at the top right edge of one or other of the two windows.

The **wizard's control window** has two tabs, one for selecting the camera and the other for acquiring images. All settings are retained from one activation to the next.



The **"Video" tab**, shown opposite, allows you to **select the desired camera** from among those connected to the computer and recognized by the wizard. If the camera is not connected currently, simply close the wizard, connect it, and reopen it.

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

It should also be noted here that **the wizard displays exactly the image provided by the camera**, regardless of the size of the video window. The aspect ratio is preserved.


The **"Adjust Settings"** button provides access to the camera's available settings: light, contrast, gamma, exposure, etc.


The **"Images" tab** below allows you to **acquire images**, name them, and save them to disk. Images are captured using the **"Acquisition"** button. They are **transmitted to the Piximetre's Measurer** in the specified target series (Red, Green, etc.)

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


Note that a checkbox specifies whether the captured images should be **saved to disk** by the Live View video assistant before being transmitted to the *Measurer*. If the images are saved to disk, they can be reopened later by *Piximetre* and, as with all images processed by it, will instantly retrieve the measurements made on them. Otherwise, images not written to disk here will be presented to the user before being closed by the *Measurer* to allow for a final backup.

The "Save As" box is used to name the images and their destination directory (using folders).

 Opens the directory selector from the **current position**: "...Alain\Documents\Images Pixi" in the example shown in the opposite figure (or "My Documents" if no directory is defined). The user can create new subdirectories in the usual way or navigate through the accessible directory structure of their 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 enabled by checking the "Use simplified naming" box (see opposite).

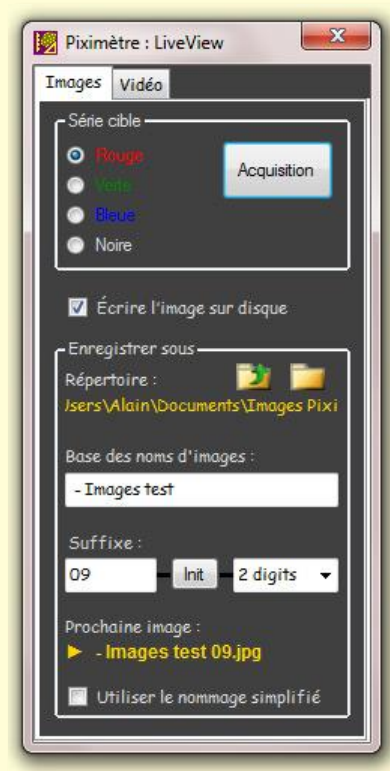
The tab displays, behind the symbol , the name of the next captured image, "- Images test 09.jpg" in our example, which will be saved in the selected directory "...\Alain\Documents\Images Pixi".

Standard naming

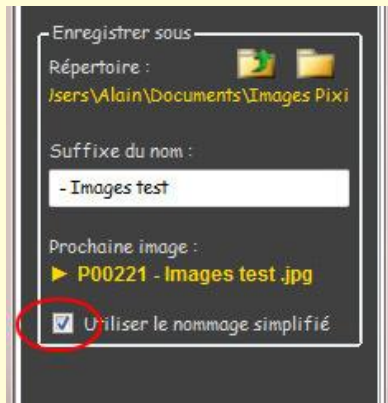
The name of the created image consists of a typically alphabetical base followed by a numeric suffix, as shown in the Wizard figure above. These two parameters can be modified by replacing them with new values.

The number of digits in the suffix is adjustable. It cannot be zero. The "Init" button resets the suffix to 1.

This naming method is also found in the [Image Export Wizard](#), which allows you to save enhanced images to disk. Both wizards refer to the **same selected directory**, ensuring complete consistency.



Simplified Naming



The figure opposite shows the layout of the "Save As" box in simplified naming mode.

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

This simple mechanism ensures both name uniqueness and precise image identification.

When operating in this naming mode, the wizard, upon opening, offers the user the choice of continuing to save images in the same directory as before, or changing it. This mechanism is very useful for saving images in a convenient location on disk.

Tip

To make it easier to capture images while operating an external device, such as a microscope, telescope, or other device, the wizard also captures images by simply pressing any key on the keyboard, instead of the "Acquire" button.

Live View Standard Compatibility

Version 5.3 of *Piximetre* marked a significant change in Live View image processing: the cameras used are now identified by **EXIF metadata** that *Piximetre* inserts into the captured images, just as cameras do. This allows it to perform a **consistency check** between the measured images and the reference standards used.

All Live View images captured prior to this version, and particularly those used to create a reference standard, did not contain this metadata since the cameras do not output it. *Piximetre* was therefore unable to perform the necessary consistency checks.

Starting with version 5.3, *Piximetre* introduces **EXIF metadata into the images**, the cameras are identified, and it then becomes necessary to **redefine their reference standards** based on this new type of image. This is **done automatically by Piximetre** when the Live View images are captured. A message requests the user's approval.

If the user refuses to update the relevant standards, their use remains valid, but consistency checks will not be possible.

For your convenience, here's a quick reminder on how to create a standard using a microscope and a camera installed on it.

Creating a standard in Live View

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

A generic standard can be applied to all images with the same ratio as the image that defined it.

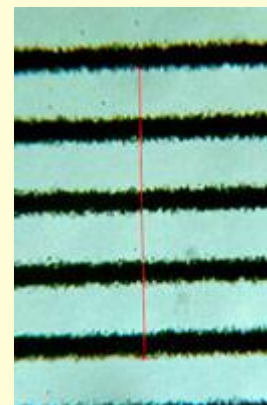
The first step is to **obtain an image of this standard** for each state of the optical system (i.e., one image for each objective used, in the case of a microscope).

When acquiring images with the camera, **two guidelines** must be strictly followed:

- **The axial position** of the camera on the optical system (i.e., its height) must remain strictly constant for all images.
- **The aspect ratio** of the images provided by the camera must remain constant (generally, several sizes are available), or a generic standard must be defined for each of them.

Proceed with calibration

1. Place an **object micrometer** on the microscope stage and adjust it "by eye."
2. Open *Piximetre* in **Graphics mode** and select "Normal Images" (Formulator Settings tab).
3. Activate the **Video Wizard**, refine the sharp settings if necessary, and capture an image of the object micrometer. The image appears in the *Measurer*.
4. Close the Video Wizard,
5. Activate the *Piximetre Calibration Wizard* ("Calibrate" button):
 - **Step 1:** Choose "On all images",
 - **Step 2:** Precisely draw a **reference axis** on the standard ruler visible in the image (figure opposite),
 - **Step 3:** Indicate its **actual length** (which you must know - on an object micrometer, each division corresponds to 10 microns), and **confirm**,
 - The Wizard then asks you **to name this standard**: give a name, for example, "test", or better for mycologists, a name related to the camera, the microscope objective being used, and the image size, for example, "**CX40 1280-1024 Q=1.25**" and finish.
6. Close the image.



You have just defined a **generic standard** that will apply by default to **all images you subsequently open** and that are taken in the same optical system state (the same objective 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** that may be used, for example:

- **CX10 1280-1024 Q=1.25** for objective 10, with Q=1.25 image width/height ratio.
- **CX40 1280-1024 Q=1.25** for the 40mm lens
- **CX100 1280-1024 Q=1.25** for the 100mm lens
- **CX100 1280-960 Q=1.33** for the 100mm lens on a 1.33 aspect ratio

Note for MOTICAM cameras

The Moticam camera manufacturer's driver is exclusively 32-bit. On some 64-bit PCs, *Piximetre*, which then runs in 64-bit mode, does not detect it, and connected cameras are therefore not recognized.

To resolve this 32-64-bit compatibility issue, download the special 64-bit version of the driver (available on the *Piximetre* website) and manually copy the file to the **Windows/System32** system directory, **without changing its name** (MUCam32.dll). **Administrator's rights are required**. Unfortunately, this operation cannot be performed automatically by *Piximetre*, for security reasons.

Exporting images

The purpose of exporting is to save images (or parts of images) to disk identical to the original images but **enriched with additional information**:

- the plot of the measurements taken 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 image name,
- any comments.

Principle

When at least one image is open in *Piximetre*, the [context menu](#) (right-click on the image) provides access to the "Export..." function:

1. **View only**: the view is the part of the image visible at a given time in the window's *Measurer*,
2. **The entire image**,
3. **The entire series of images** (all images in the series will then be exported automatically).

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

- **Comment phase**: The measurements are already entered on the image, but here the user adds the desired text information. Some are available directly at the touch of a button (image name, dimensional formula, author's signature - once saved as such). Others are free text. Numerous options are offered for defining text styles (choice of font, size, color, shadow, opacity, etc.). **Adjusting the scale bar** allows it to be adjusted to the actual dimensions of the images.
- **Compression phase**: The JPEG compression process allows you to set **the desired level** as well as **the physical dimensions** of the image.
- **Save phase**: The enhanced image is saved to disk under a specific name.

An **Export Wizard** is responsible for executing these three successive phases. It is activated directly when exporting a **single image or its view**. A second wizard, called the **Batch Export Wizard**, supports exporting multiple images. It calls upon the first.

The initial images are not affected by their exports.

Images and Views

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

The view in the Measurer window is moved by dragging **the red frame** on the pad with the mouse (hold down the left mouse button while dragging).

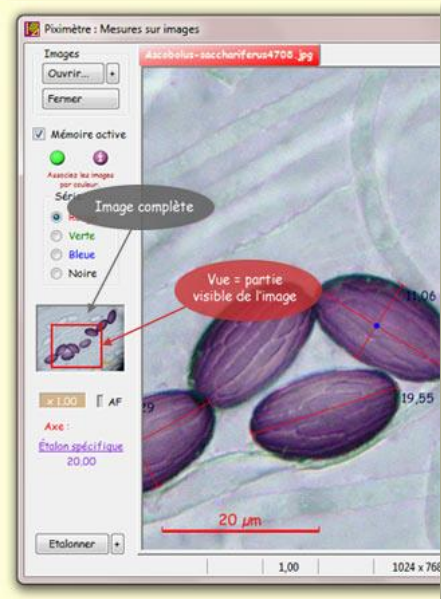
The magnifying glass allows you to enlarge or reduce this frame. It works either with the mouse wheel or by right-clicking or left-clicking on the red frame.

The scale displayed in the bottom left corner of the window is automatic (20 μm in the figure). It is not integrated into the image, as it would not always be visible on the screen, but is **superimposed on the window**, i.e., the image view.

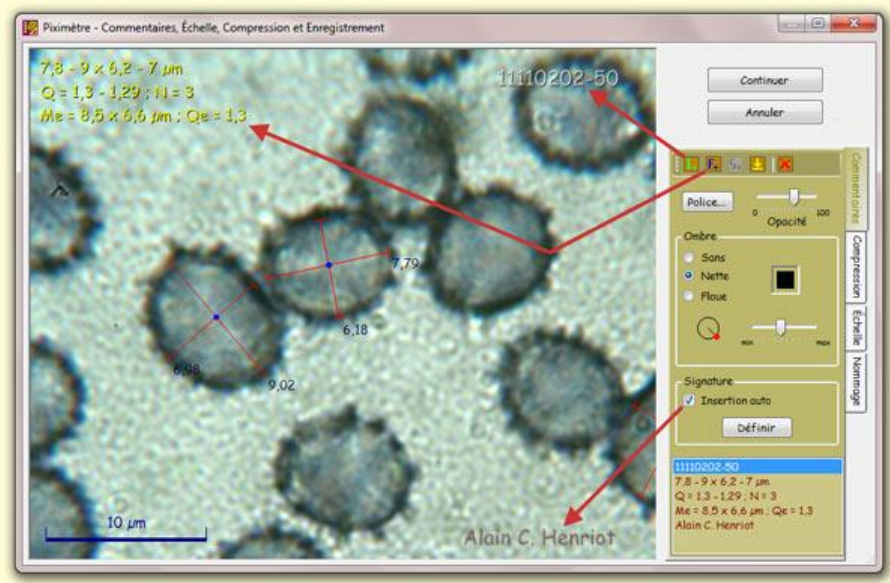
The combination of the magnifying glass and the view acts as a crop of the image and makes it easy to isolate visual objects, which can be saved via exporting the view.

The elements that will enrich the exported image must have **been previously defined**, meaning their location on the image must have been specified, as well as their format (font, size, color, shading, transparency, etc.). This is the purpose of the so-called **comments phase**, performed by the Export Wizard.

We will successively examine the Export Wizard (for a single image) and the Batch Export Wizard (for multiple images).



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 the right side, there are several tabs: **Comments** and **Compression**, which handle the phases of the same name; **Scale**, which allows you to [adjust the scale](#) of the image and finally, **Naming**, which allows you to define the naming mode for [exported images](#). You can move from the Comments phase to the Compression phase by **clicking the Continue** button or by directly selecting the **Compression** tab.

You can abort and close the wizard by clicking the **Cancel** button or by clicking the red cross in the upper right corner of the wizard.

When the image is larger than the wizard's working area, you can move it by **holding the left mouse button and dragging the mouse**.

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

The user is now ready to enter text comments as shown below, or to proceed directly to image compression by pressing "**Continue**."

Adding Comments to the Exported Image

Left clicking on the image (without moving) opens a new text editor that accepts all characters typed on the keyboard. Any text can also be entered from the clipboard using **Ctrl+V**. Any **special characters** not available on the keyboard will be placed in the clipboard from a specialized word processing application (e.g., Word) and then entered here using **Ctrl+V**.

The "**Enter**" key terminates the current text, as will clicking outside the text box. Each text can therefore only contain a single line. It is perfectly possible to **create multiple 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 "**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 that allows, among other things, the selection of any text in the image.

Left clicking on a text selects it. Moving the mouse while holding the left mouse button allows you to **move the text** to the desired location in the image. A left-click on a text, released without moving it, **opens it for editing**.



Exported images do not all have the same dimensions, so the **position of text** is anchored in two ways, depending on its **proximity to the image edges**:




- **fixed to the image edges**, which is indicated by a double line that appears when dragged, or
- **relative to the image dimensions**, which is indicated by a single line that appears when dragged.

This mechanism ensures correct text placement, regardless of image size. For example, text placed on one **view** will remain correctly positioned across the **entire** exported image, which is normally larger.


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



-  Automatically inserts the **image name** by creating a new text.
-  Inserts the **dimensional formula** as three texts corresponding to its three lines.

-  Inserts **the user's signature**. This tool is only active if a signature has been defined and its automatic insertion has not been selected.
-  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.
-  **Deletes** the selected text.

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

In the figure, **the image name** has been inserted using 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 type of shading to apply,
- its color,
- its orientation,
- its distance from the text.

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

Text created on one image **remains applicable to all subsequent images**.

Examples of text creation

The "Comments" tab of the Wizard is selected.

Creating a free text


- **Mouse clicks on the image**. A blank text box appears, prompting the user to enter characters using 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, size, and color.
- **Add a drop shadow** to the text: sharp, blurred, or none; its color, distance from the text, and position.
- **Adjust the overall transparency**.

- Use the mouse to **move this text** to the precise location in the image where it will be inserted.
- **That's it.**



Creating the Author Signature

- Proceed as for free text to create and place the signature text.
- Use the @ button in the Signature frame to insert **the Copyright character**, if desired.
- **Select the text** (left click) and use the "Define" button in the Signature frame **to declare this text as the signature**. The signature is now saved.
- Optionally, check the "Auto Insert" box so that the signature is automatically inserted on each exported image.
- **That's it.**

Inserting the Image Name

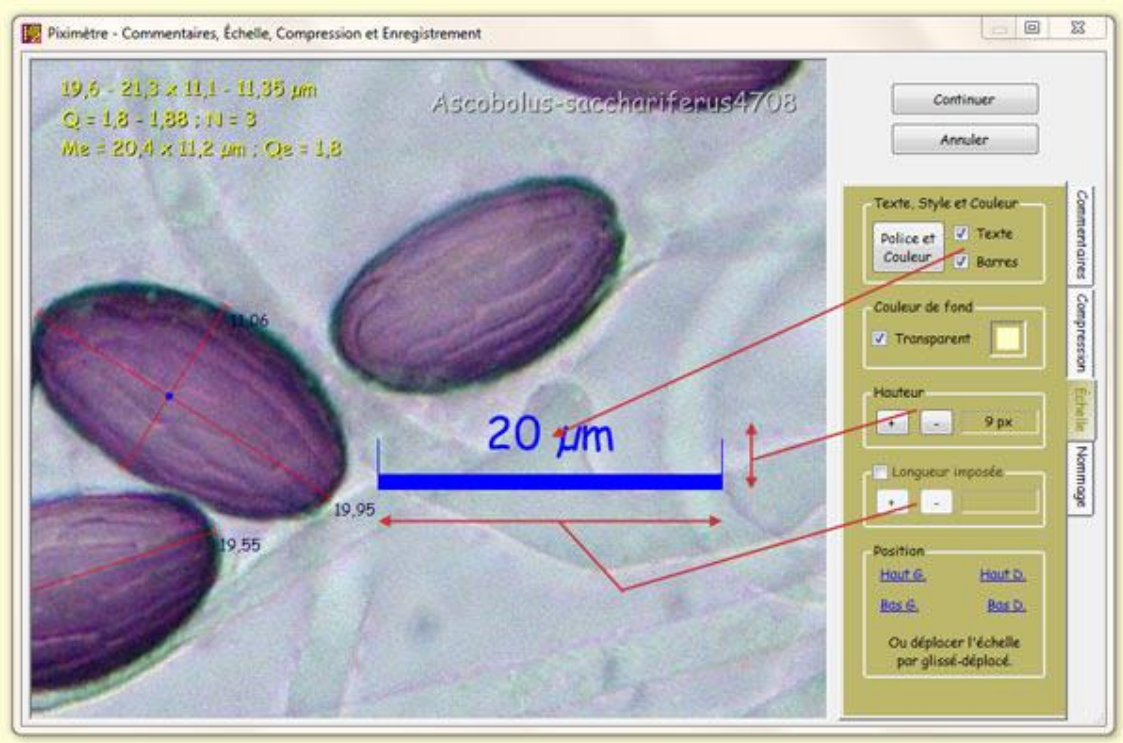
- Mouse click on the image to open a blank text box.
- Press  from the toolbox: **the image name is entered at the cursor location**.
- Format as described above for free text.
- Using the mouse, **move the text to the desired position** on the image.
- **That's it.**

Inserting the Dimensional Formula

- Mouse click on the image to open a blank text box,
- Press  from the toolbox: **the three lines of the formula appear**, one below the other, at the cursor location,
- **Click on a line** of the formula to select it,
- **Format it** as described above for free text,
- **Simply single-click** (not double-click) on another line of the formula and press  from the toolbox. **This 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,
- **That's it.**

Positioning of texts and scale on images

Adjusting the Scale to Image Dimensions



As mentioned above, **the scale visible** on the initial image in the *Measurer* window is adapted to a permanent view in that window, i.e., adapted to the image view, not 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 performed on the "**Scale**" tab of the Export Wizard (figure above), and only on this tab. The scale cannot be modified in any other way.

Here, the scale can be resized in height and length so that it is **clearly visible and correctly positioned on the exported image**. These adjustments obviously maintain its accuracy. The figure shows the buttons to use for this purpose.

The scale length is only adjustable if the "**Fixed Length**" option is checked. Otherwise, it is not editable and retains 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**" option is not checked. The "**Bars**" option draws or hides 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 (shown in yellow in the figure).

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

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

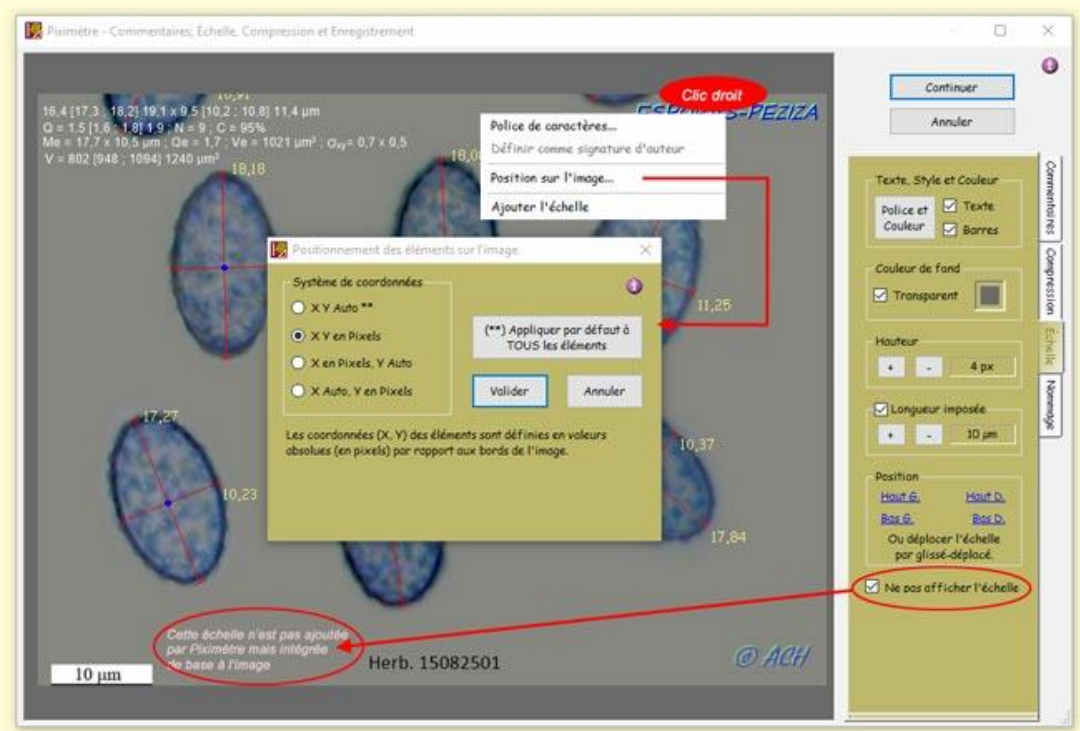
1. **Fixed to the image edges**, which is indicated by a double line that appears when dragged, or
2. **Relative to the image dimensions**, which is indicated by a single line that appears when dragged.

As with text, the scale thus remains correctly positioned on the images, regardless of their dimensions.

Advanced Features

Version 5.9 of *Piximetre* introduces several new features:

1. On the **Comments** tab, the scale added to the image by *Piximetre* can be **dragged directly** onto the image with the mouse, without going through the Scale tab.
2. On the **Scale** tab, a new option is available: "**Do not add scale**" to the image (as the original image may already have one). By default, this option 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, allowing you to change certain settings detailed below.



Positioning Elements on the Image

Processed and exported images have highly variable dimensions (height, width). The positioning of text and scale, added automatically, must be **independent of their dimensions**. The context menu provides access to the coordinate systems that define this positioning.

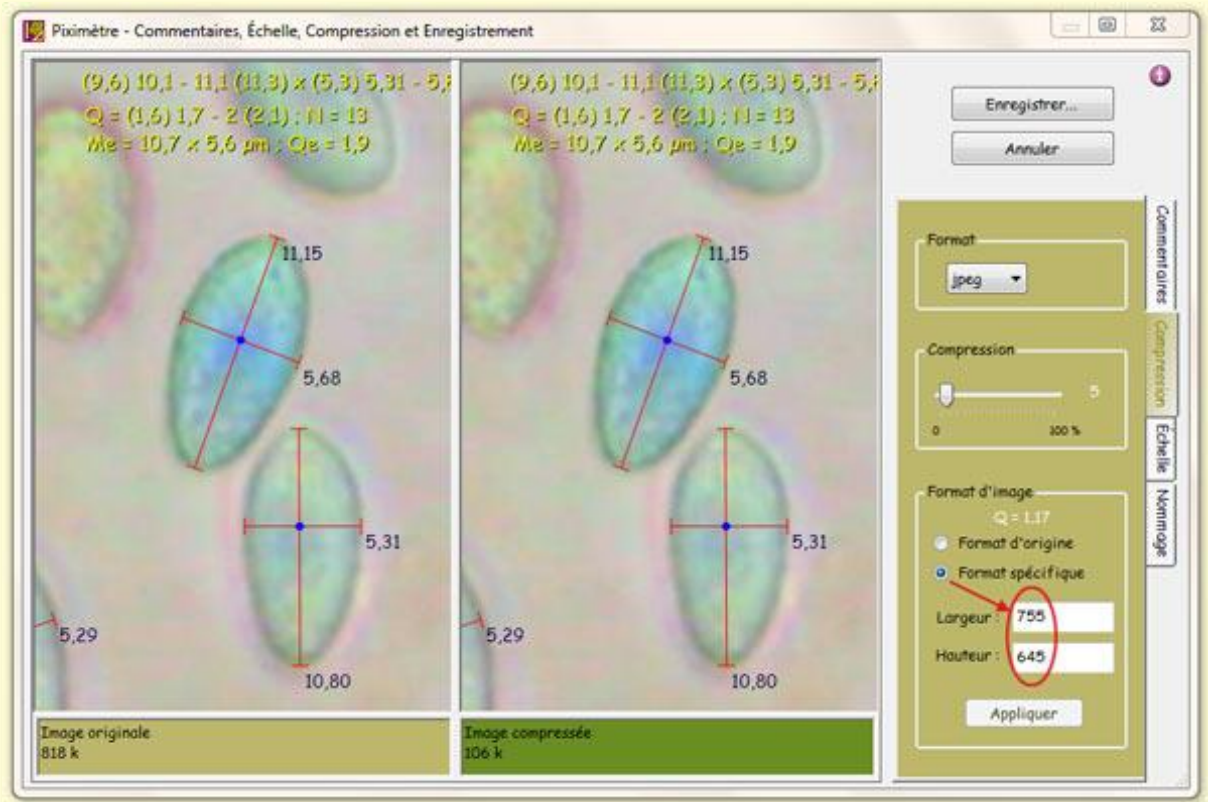
There are **four different coordinate systems**. Each element added to the image (text and scale) can have its own coordinate system:

1. **XY Auto**: The X and Y coordinates of the element are calculated in pixels when positioned in a corner of the image and in relative values relative to the image size when positioned elsewhere. The adopted positioning is displayed on the image when the element is moved: by two parallel lines for pixel positioning, a single line for relative positioning.
2. **XY in Pixels**: Elements can no longer be positioned outside the corners of the image. A check is performed to ensure that they never move outside the image, regardless of their dimensions.
3. **X in Pixels Y Auto** or **X Auto Y in Pixels**: These are a combination of the two previous options.

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 the figure above).

Compress the exported image



From the Comments phase, pressing the **Continue** button gives access to the next phase, **Image Compression** (figure above). This button then turns into a **Save...** button, which gives access to the next phase, **Saving to Disk**.

The Wizard presents **two versions of the image**: on the left, the uncompressed image, and on the right, the compressed image with the compressor settings.

Images can be dragged into their frames by clicking left and dragging the mouse.

Only the **JPEG** compression format is available.

The user can adjust **the compression level** and change **the dimensions of the resulting image**. Note that in this case, the aspect ratio is preserved. **The Wizard does not modify the geometry of the images**. The figure above explains how to change the size of the resulting images. Changing one value automatically changes the other.

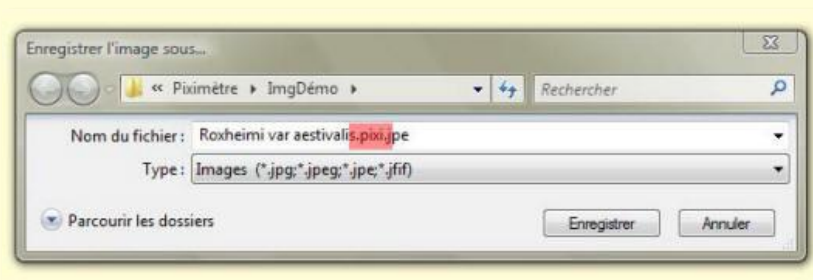
Finally, it is possible to return to the **Comments phase** by selecting the corresponding tab.

Save the exported image

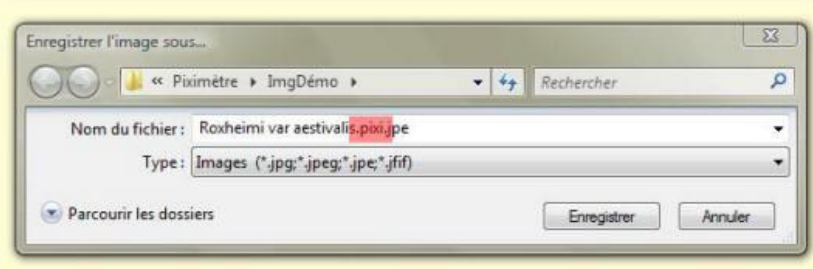
It is essential to understand that the image, or only its view, exported in this way **should not be confused with the original image**. Indeed, the original image contains no axes, intersection points, scale, or comments; these are only plotted on the screen. Conversely, the exported image contains all these elements. It is *Piximetre's active memory* that allows these elements to be retrieved and plotted on the original image when it is reopened.

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

1. **Adding 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."

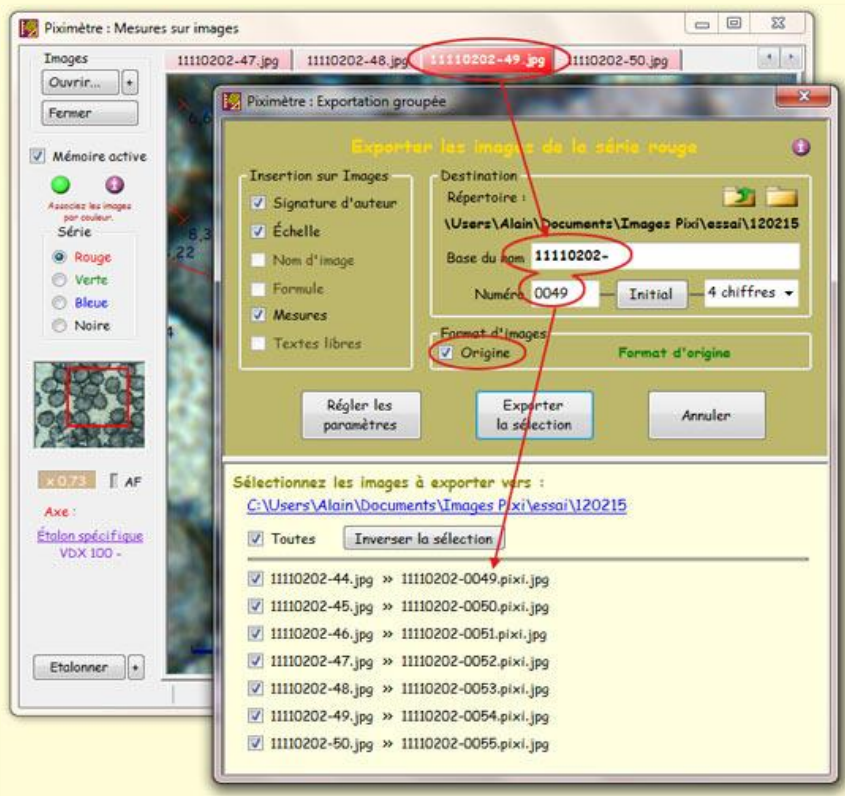


2. **Add a sequential numeric suffix** before the extension. For example, if the last image exported was named "Ma belle image 0001.jpeg," the Wizard would suggest naming the next one "Ma belle image 0002.jpeg" (figure below). For this process to work, **you must have specified a name ending with a number at least once**. *Piximetre* retains the number of digits in the number.



You can select one or the other method on the **Naming tab of the Wizard** and on the **Formulator Settings tab, Advanced... option, Special pane**.

The Batch Export Wizard





The **Batch Export Wizard** automates the export of multiple images from the series selected in the Meter. For example, all images in the red series.


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

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

All images in the red series have been automatically included in the **Wizard's** list (11110202-44.jpg, -45.jpg, ..., -50.jpg). A checkbox allows the user to **choose which ones will be exported**.

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

The  and  buttons allow you to choose the **destination directory**.

 Opens the directory selector from the current position: "...\\Pixi Images\\essai\\120215" in the example shown in the figure (or "My Documents" if no directory is defined).



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

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. These two parameters can be modified by replacing them with new values. The "Initial" button resets the suffix to one.

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

If the user chooses "None" 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 ".pixi" suffix is added to differentiate these images from the original images.

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

This **Destination** frame works in [the Live View Video Wizard](#), which allows you to save video images. Both Wizards reference the same directory, ensuring complete consistency.

Also note the **Image Format** selected here: **Origin**. This format is always proposed by default. It means that the images will be exported without changing their dimensions; each image can have its own dimensions. However, when exporting frames rather than entire images, all images will have the same dimensions, those of the frame.

When a specific format has been defined, the "Origin" option is enabled, allowing the user to select this format.

The "Insert into images" box offers the automatic insertion of various objects available at that time. In the figure above, "Author's signature, Scale" and "Measurements" are available and can therefore be selected by the user. The other objects are not yet defined and are therefore not selectable for integration into images.

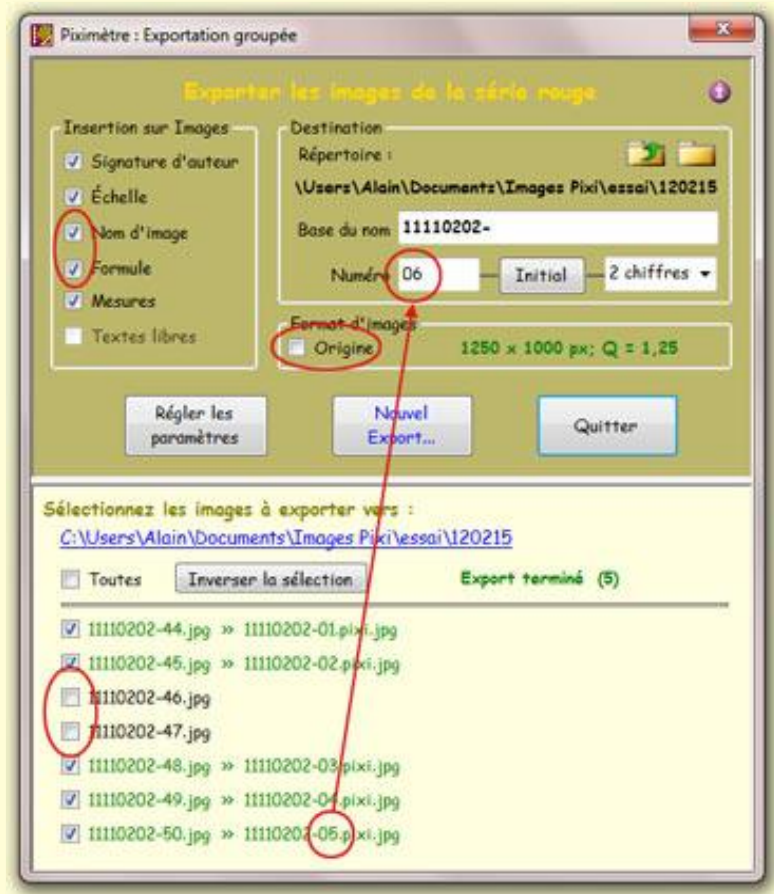
The image name and sizing formula are already known at this point. For example, the name of the first exported image is "11110202-44.jpg." However, the Wizard doesn't yet know where to place this name on the image it will create, nor does it know the font to use, its color, etc. Therefore, for the image name, sizing formula, author's signature, and free text, it is necessary to first define their shape and position on the images before being able to select them.

These shape and position definitions, as well as the image format, are made once and for all, but can be modified at any time using the "Set parameters" button, which activates the first Wizard.

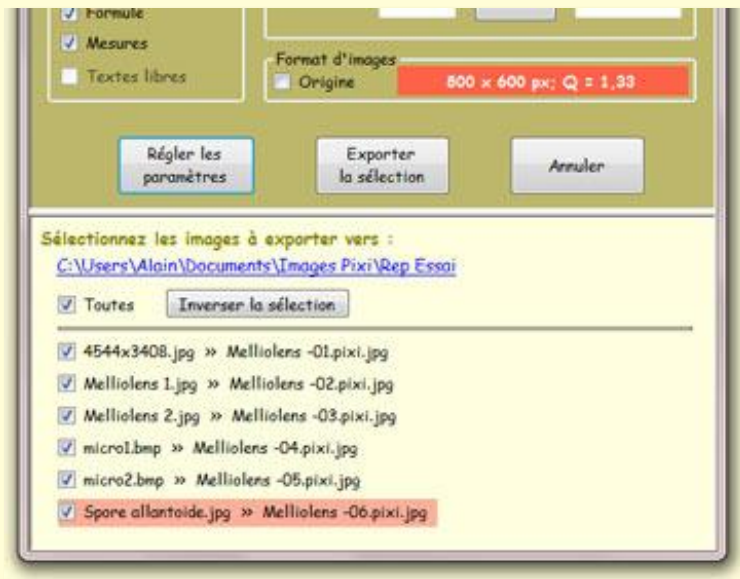
The figure opposite shows the Batch Export Wizard after:

- Defining the objects to insert on the images (except for free text),
- Defining a new image format (1250 x 1000),
- Deselecting two images in the list,
- Changing 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 exporting, the "Export Selection" button changes to "New Export...". Pressing this button rennumbers the selected images, which are then ready to be exported. The "Export Selection" button then reappears.



Geometry of the exported images



The Wizard does not modify the geometry of exported images.

It systematically checks the compatibility of their format with the specific format selected.

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

To export this image, the operator must define a new, compatible format ("Set parameters" button) or keep the original format.

The Graphic Object Sequencer

Piximetre performs dimensional measurements on various "**graphic objects**" present in images (from mushroom spores to galaxies in the Universe; it's simply their size that changes). Their orientation and distribution in the images are generally arbitrary, sometimes making visual comparison difficult.

The *Sequencer's* goal is to **facilitate the visual comparison of objects**. It **extracts** all or part of these objects from images, **normalizes** them to respect their sizes and proportions, **orients** them in space at the same angle, **classifies** them according to various criteria, **presents** them on the screen, and finally, **saves** them as images independent of the source images from which they originate.

The *Sequencer* is a new *Piximetre* assistant that performs these tasks automatically with just a few mouse clicks. And there you have it!

Principle

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

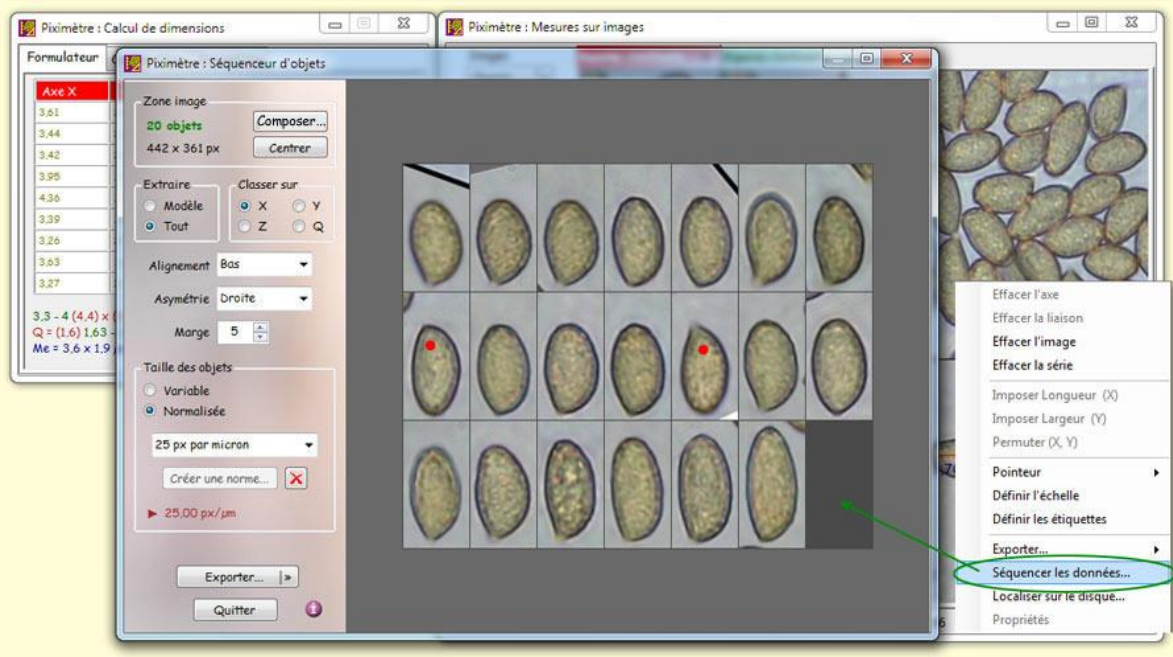
Taking measurements with the *Measurer*:

1. **Open** the image series containing the objects to be measured. Assign it the appropriate length standard.
2. **Draw** the two rectangular axes that delimit the objects as usual. The *Formulator* calculates and displays the dimensional formula.

The *Sequencer* then intervenes:

1. In the *Measurer*, **right-click** on either image in the series to display the context menu and select "**Sequence Data...**". The *Sequencer* opens (figure below).
2. If they aren't already, **adjust its parameters**, and possibly drag the edges of the window to change its shape. The sequenced objects appear in the **image area** (dark gray in the figure).
3. Press the "**Export...**" button to save the image area as a JPEG file.
4. That's it, exit the *Sequencer*.

We'll see later that recording, which is **exporting images**, may require additional settings.



Open the Sequencer using the **context menu**.

How it works

The figure above shows the *Sequencer* opened on a series of images of mushroom spores on which measurements were taken. It has an adjustment panel on the left side and an image panel, of variable size, on the 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 in the adjustment panel (here set to 5) allows this size to be increased or decreased.

The geometry of the image zone (there three lines, the last of which is incomplete) can take several forms defined with the "**Compose...**" button located at the top of the adjustment panel, in the "**Image Zone**" frame. How it works 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 the window. The "**Image Area**" frame on the settings panel displays 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. **Sorted in ascending order** of one or the other of their parameters defined in the "**Sort on**" frame, 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 in which the measurements were entered (the coupling points of the two axes).

2. **Aligned vertically** relative to their support frame. The position is set by the "Alignment" option, here to the **Bottom** value. Other possible values are **Center** and **Top**.
3. **Oriented from right to left**: In this example, all spores (except two highlighted in red in the figure) have their apices at the bottom left. The right-left orientation is set by the "Asymmetry" option, here set to **Right**. The other possible values are **Auto** and **Left**.

Note: We will see later that the vertical presentation of objects (by default) can be replaced by 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 the icon  provides direct access to this tutorial embedded in *Piximetre*.

Object orientation

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

In the example in the figure above, the two spores highlighted in red were intentionally 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

Piximetre also measures complex objects, such as curved objects, by drawing non-linear axes consisting of successive contiguous segments that follow their shape ([see here](#)). However, **these non-linear axes** do not currently allow the complete circumscription of the objects they measure. Therefore, the *Sequencer* can only process objects defined by two rectangular linear axes. So how do you sequence objects measured by non-linear axes?

Spanish mycologist *Miguel Angel Ribes Ripoll* suggests a workaround: **fictitious measurements**. The method consists of replacing the non-linear axes that precisely measure the objects with the two linear axes that allow them to be circumscribed.

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

Choosing the sequenced objects

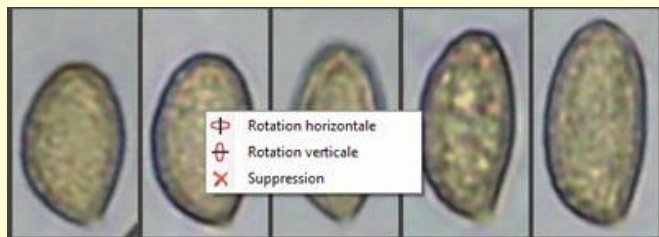
The "Extract" box offers two options: **Model** or **All**.

"All," as its name suggests, means that all objects measured in the image series will be introduced into the sequence.

The "**Model**" option, however, requires some commentary: which model is it? In fact, all 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 magnitude of the data. Each dimension therefore has a specific number of classes. This numerical data and its graphical representation are accessible via the "[HDI](#)" button in the *Formulator*. The model created by the *Sequencer* includes **one representative image per class**.

Context Menu

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



- Rotate the object around the vertical axis,
- Rotate it around the horizontal axis,
- Delete the object altogether.

Normalization

One of the important features of the *Sequencer* is its ability to **respect object scale**. For example, the image of an object measuring 10 μm will be half that of one measuring 20 μm . **Normalization** is the operation that allows you to set and maintain the scale of the various objects in the *Sequencer*.

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

The "**Object Size**" section of the adjustment panel offers two operating modes: **Normalized** or **Variable**.

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

The "Variable" size provides access to the "**Create 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 serves as a **reference** for calculating the size of the objects that will be sequenced subsequently.

Standardization ensures that objects are represented with a size consistent with their measurement.

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

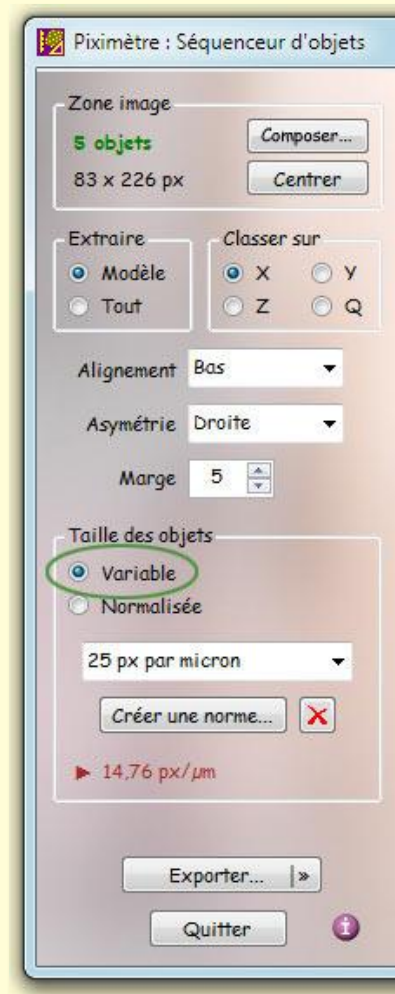
To sequence objects of very different sizes, it is possible to **create multiple standards**, each adapted to the subject.

It is also possible to **redefine a standard** at any time by switching back to "**Variable**" size mode, then changing the image size (mouse wheel), and finally pressing the "**Create Standard**" button again. However, doing so 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 saved standards.

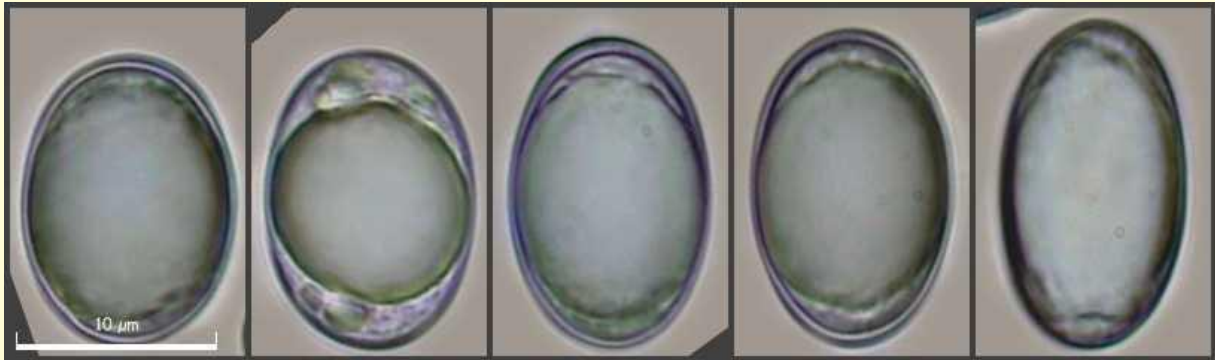
Also note that the ► field indicates the current size of the objects (here 14.76 px / μm).

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





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

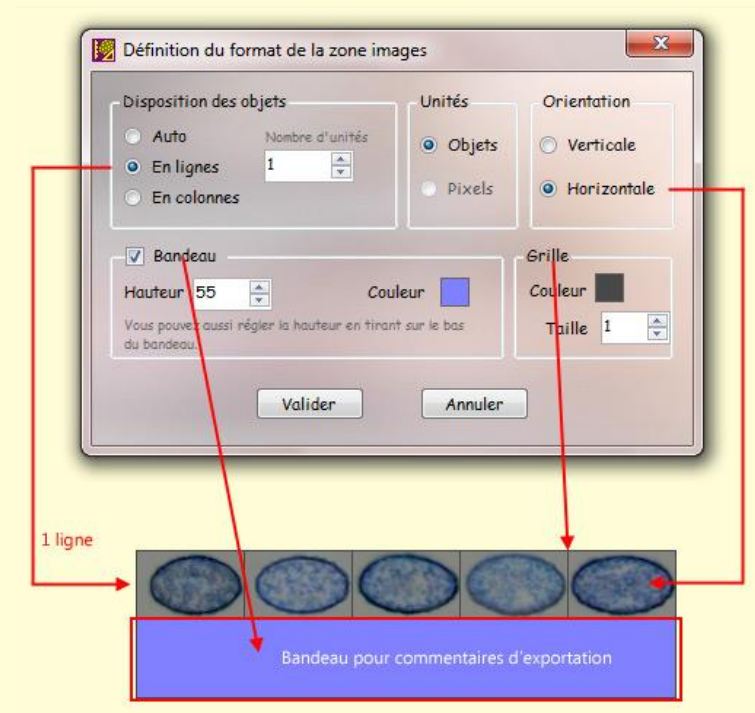


(14.8) 15 - 16.4 (16.7) × (9.2) 9.6 - 10.7 (11) μm
 Q = (1.4) 1.42 - 1.7 (1.8) ; N = 11 ; Me = 15.8 × 10.2 μm ; Qe = 1.6

Image area format

As mentioned above, the "Compose..." button in the **Image Area frame** allows you to specify the presentation of objects in the **image area**.

The available options are presented below.



The **arrangement of objects** can be done either automatically, based on the geometry of the image area, or in rows or columns. In the latter two cases, you will specify the desired number of rows or columns.

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

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

The **Banner frame** allows you to specify the presence, height, and color of a horizontal banner added to 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 information is added to the banner **during the export process**.

Finally, the **Grid frame** allows you to adjust the size and color of the border surrounding the image and separating objects from one another.

Saving

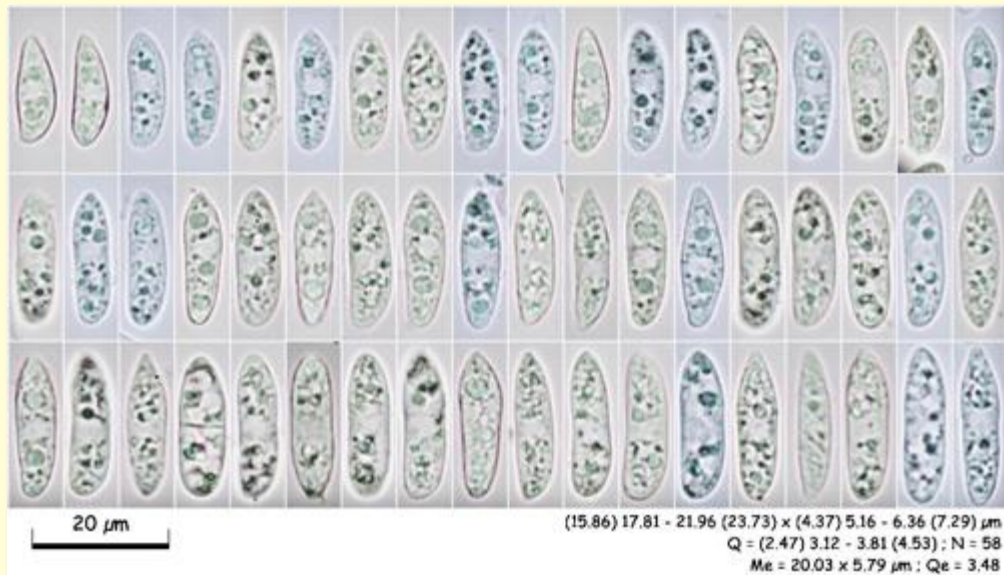
The "**Export...**" button allows you to save the displayed sequence to disk. Either as a **single image** represented by the image area, or as a **series of images**, with each object generating a different image.

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

Images are saved using the **Export Wizard**, which offers the option to enhance the images (scale, text, author's signature, etc.). 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 sorted in ascending order of size. It was exported with comments that are automatically entered on the banner. The other is a sequence of paraphyses obtained using several photos, in different reagents and mounting fluids.



How autofocus works

In the absence of autofocus, using **generic calibration** implies that photos are taken with a constant focal length and all other things being equal regarding shooting conditions. Otherwise, **specific calibration** is required.

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

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

Principle

Piximetre's autofocus is combined with the graphical image measurement function. It supports the **intrinsic magnification** of photos taken with varying focal lengths (zoom).

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

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

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

The **automatic calculation** of this intrinsic magnification, specific to each camera, is indicated by the Measurer's "AF" indicator, which is lit green. If the indicator is off or a different color, this calculation could not be performed. In this case, manual calibration must be performed before taking measurements.



Autofocus requires a **prior initialization** to implement the mathematical function, **specific to each camera and its lens**, which allows for the precise calculation of the intrinsic image magnification for any focal length. This initialization is performed using a **wizard** activated by clicking on "AF."

Initializing the autofocus also sets the camera's **reference focal length**, for which the intrinsic image magnification factor is **set to ONE**.

The **overall magnification** of the image on the screen, shown in the box to the left of the autofocus (x3.00 in the figure above), is the product of the intrinsic image magnification and the *Piximetre's* manual zoom, implemented using the mouse wheel.

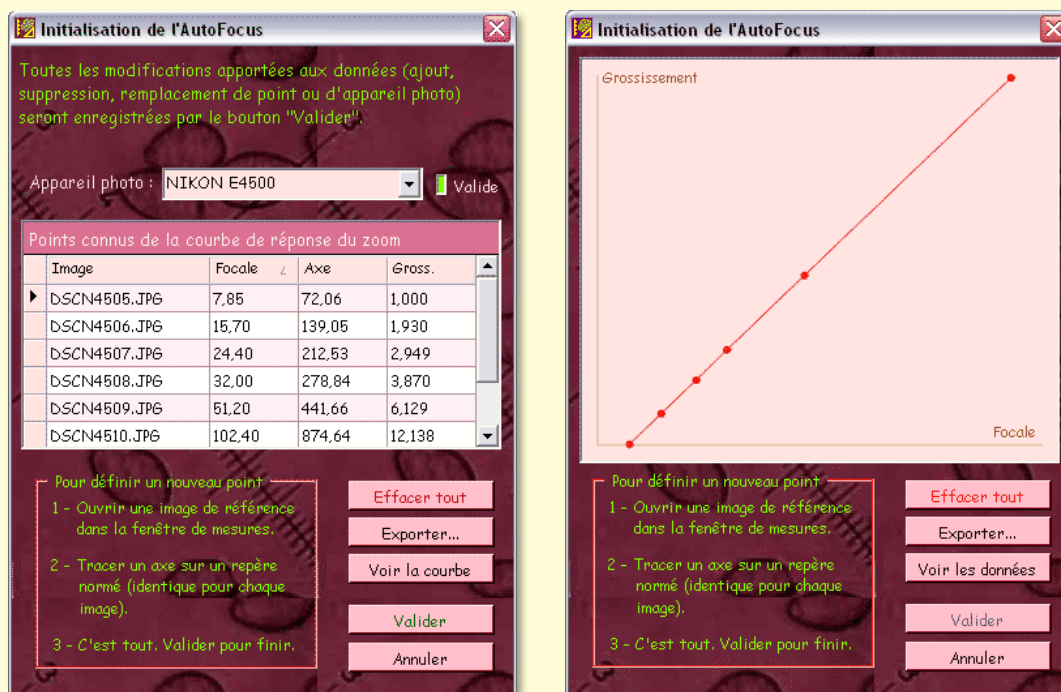
Initializing the autofocus

Autofocus initialization must be performed for each camera and variable focal length lens that may be used.

The **Autofocus wizard**, activated by clicking on "AF," assists with this operation. It has two operating modes:

1. **Viewing** saved autofocus data: All operations with the assistant are possible in this mode, but changes made cannot be validated. The validation button is absent. Measurements in progress in the Meter are not affected.
2. **Creating-Modifying** of autofocus data: To ensure that the image measurements required for this operation are performed **under identical conditions**, the assistant:
 - **Closes any images** that may be open in the Meter,
 - **Forces the generic standard** to "Unit (UN)",
 - **Deactivates its memory.**

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



The "View curve" or "View data" button allows you to switch between views of the wizard.

The curve shown on the right corresponds to the points determined on the left. It provides the intrinsic magnification of the image as a function of the focal length used by the camera. The camera used in this example is a Nikon E4500.

Setting up the autofocus curve

The method to use to define the points of this curve is simple, but first, [see this note](#) on optics and measurement errors:

1. First, choose **a length standard** to photograph, i.e., a **specific, unique object** (for example, the interval between divisions 50 and 60 of a ruler).
2. Take **a series of photos** (for example, 10) **by varying the camera's focal length**.
3. **Open the Autofocus wizard** in "Edit-Create" mode.
4. **Open the series of photos** in the Measurer.
5. On each photo, **draw an axis that overlaps the chosen length standard** (as if measuring its length). The assistant **reads the focal length used** in the image metadata and **associates it with the apparent length of the standard represented by the traced axis**. It records a new point on the curve.
6. **Confirm** when all the images in the series have been processed; you're done.

The wizard selects the smallest focal length used in the photo series as **the reference focal length**. It also **checks the consistency of the measurements**, which is reflected by the monotony of the curve: the length of the axes must increase, like the focal length. The "Valid" indicator demonstrates this consistency: **green** means the measurements are valid, **red** indicates a probable error. Such an error can occur when the measured object (**the standard**) differs from one photo to the next.

During this initialization phase, the points can be defined in **any order**; that is, the photos can be opened in any order in the Meter. The wizard takes care of placing the focal lengths in ascending order. **New points can also be added later**.

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

The "**Validate**" button saves the data and closes the wizard. The "**Cancel**" button closes the table without saving the data, but the user is prompted for confirmation if the data has been modified. The "**Clear All**" button erases the points from the table, allowing a new one to be created. Changes are only final after validation.

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

Finally, the "**Export...**" button allows you to save the curve points to a file that can, for example, be sent to other users and **integrated into Piximetre** by the wizard, simply by dragging and dropping this file onto the table of values or onto the curve.

Photographing the Length Standard

One of the following two methods will be used to obtain the necessary **sequence of photos**:

1. Mount the camera on the microscope, choose a lens (it doesn't matter which one), and take several photos with the eyepiece micrometer or object micrometer mounted on the microscope (or telescope).
2. Mount the camera on a tripod and photograph a double decimeter.

Vary the focal length (zoom) between each photo, and in Method 2, take care not to vary the distance between the camera and the ruler.

Important Note

The accuracy of calculating the intrinsic magnification of images depends on 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 a nonlinear camera response.

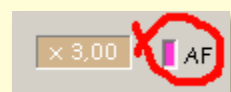
Also note that the focal length used when taking the photo is provided by the camera to **two decimal** places. Measurement accuracy, when using an intermediate focal length, cannot exceed 1%.

Using autofocus

In routine measurements, **autofocus is automatic** as long as the camera used is described in the [Piximetre database](#). Otherwise, autofocus is disabled.

The **AF indicator** indicates autofocus operation on **the selected image** in the *Measurer* window. Clicking on this indicator provides **information on its status**:

1. **An off indicator** means that (in several cases):
 - The image comes from the *Piximetre Live View*. The sensor is normally fixed focal length; autofocus is disabled,
 - In Compressed Image mode, the image is **highly compressed** and no longer contains the information necessary for its identification (metadata),
 - Still in Compressed Image mode, the image contains metadata, but **autofocus is not initialized** or is invalid for the camera used,
 - **No image** is open in the *Measurer*.
2. **A purple light** means that:
 - In "Normal Images" mode, the image has been **heavily compressed** and no longer contains the information necessary for its identification (metadata), so a less compressed image must be used.



3. A red light means that:

- In "Normal Images" mode, autofocus initialization is **invalid** for the camera used. The camera is unknown, or its characteristic curve is not monotonous but has a cusp. **Initialization must be restarted** using the AF wizard.



When the light is **off**, **purple** or **red**, autofocus is **inactive** on this image; its intrinsic magnification is set to **ONE**. It may then be necessary to perform specific image calibration based on the zoom.

4. A green light means that autofocus is active on this image:

- The camera used to take the image is **correctly described** and
- The image metadata has been used to determine its **intrinsic magnification**.

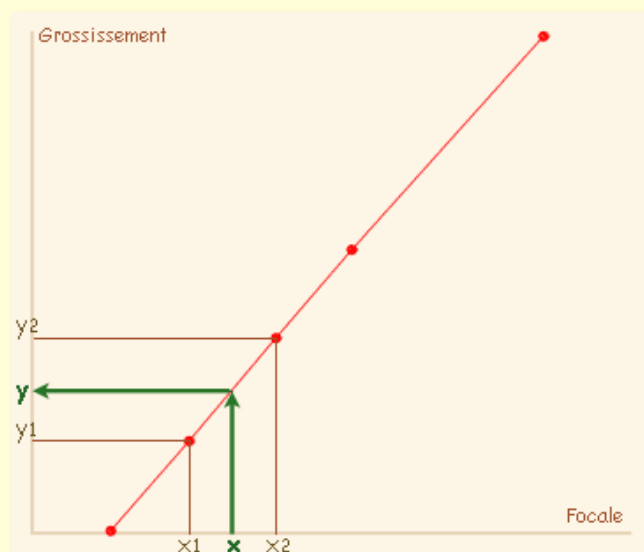


When the indicator is green, the intrinsic magnification y of the image, corresponding to the focal length x used, is calculated by linear extrapolation of the curve between the known points x_1 and x_2 that frame the value x .

If the focal length x is less than the first known point or greater than the last (which can occur if the initialization did not include these extreme points), the calculation is performed by extrapolating the first or last segment of the curve, respectively.

This calculation method, combined with the multiplicity of points, allows for a very good approximation of the **actual magnification** of images, even with a non-linear curve.

Object dimensions are then measured normally.



Optics and measurement errors

Optics and Image Distortion

Performing valid length measurements on objects present in an image presupposes **perfect linearity**. Unfortunately, images produced by optical systems are generally not free from distortion. This phenomenon, well known to photographers, is due to **defects in the lenses used**, which are indeed not perfect.

When it comes to photographs taken with a microscope, at least two lenses are involved: **the microscope objective** and **the camera objective**.

Geometric defects in images produced by cameras vary greatly depending on the quality of their lenses and the focal length used. They **generally increase at short focal lengths** and are greatest at wide-angle lenses (which should therefore be avoided here).

Microscope objectives, on the other hand, vary greatly in quality, and therefore in cost. Geometric corrections are achieved with **planar lenses**. Chromatic aberrations, which depend on the wavelength of the incident light, must also be corrected. **Plan apochromatic** objectives are thus corrected for three characteristic wavelengths. They produce excellent results but are obviously very expensive.

As a result, the images provided by a camera mounted on a microscope contain **significant geometric distortions**. They can be spherical or pincushion-shaped or even take on more complex shapes. These distortions are generally more significant toward the periphery of the images. The central area can be considered linear, i.e., free of distortion.

Performing measurements using a **linear scale** across the entire surface of such images therefore exposes oneself to errors inherent in the geometric distortions of the images. Two ways exist to reduce these errors:

- **Correct the geometry of the images**. However, currently no measurement software, including *Piximetre*, performs this correction.
- Taking measurements **toward the center of the images** only, avoiding their edges as much as possible is the easiest way to achieve this.

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

1. Place an object micrometer under the microscope lens and focus it,
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 (the standard for object micrometers),

3. Enter this image into *Piximetre* or a quality graphics software program, such as Photoshop, for example,
4. Draw an axis superimposing two divisions in the center of the image,
5. Then move this axis toward the divisions located at the periphery of the image and note that it no longer perfectly superimposes two divisions, their spacing being altered by the image distortion.
6. Evaluate the error (length of the axis measuring the difference).

Using a video camera, in addition to allowing direct viewing and capturing of images, **largely eliminates distortion**. Generally, these cameras do not have a lens, but rather a simple protective glass for the sensor. Furthermore, their field of view is reduced and centered on that of the microscope objective. This eliminates the two sources of error mentioned above.

Piximetre connects to **many video cameras installed on the computer** (with some caveats, however).

Calibration and autofocus

In mycology, *Piximetre* calibration is performed on an image of the **object micrometer** (usually a 200 μm ruler made up of 20 divisions spaced 10 μm apart). A photograph of this micrometer will be taken with each lens likely to be used.

Given the above, and without any certainty of perfect linear images, the interval limited to only **two or three divisions located in the center of the image** will be taken as a reference, avoiding choosing an interval that is too large, which could be altered by geometric aberrations.

Furthermore, the camera's autofocus curve must be carefully established. 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. As a **length standard**, we will use an interval located at the approximate center of the image, such that it is **not excessively 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 solely on the known elements of a **representative subset** called a sample (a series in *Piximetre*).

The least that can be said 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 available to the mycologist is very limited. On the other hand, **the methods used**

by authors vary greatly and are generally not indicated in publications. These two reasons lead to variable results.

Due to their generally very small size (20 to 30) compared to the total population (several 10×10^6 to 100×10^6), spore samples are not always sufficiently homogeneous for the laws of statistics and probability to apply with all the necessary rigors. *Piximetre* introduces a new measurement that is perfectly valid with respect to these laws. This new measurement consists of a confidence interval that relates to the average value of each dimension (length, width, thickness, and their ratio) for the entire population.

Regarding the sample selected for measurements, users should be aware of the need to consider only **representative spores** of the total population and not all those that fall within the target range. Here, as elsewhere, the quality of the result depends primarily on the quality of the operator, which is reflected here in the choice of sample. We will eliminate those that are **abnormal**, that is, clearly too large, too small, or immature, and we will take care to measure only spores seen in profile, exactly as specialist mycologists do.

In the case of **multimodal populations**, that is, comprising several distinct subsets of spores (usually two; beyond that, they become difficult to discern), it will be necessary to make as many series as possible of measurements to account for this aspect.

Ultimately, **the choice of spores measured** in the images must be identical to that made for spores traditionally measured visually, using the microscope eyepiece.

Furthermore, to increase the number of measurements, and therefore the accuracy of the result, *Piximetre* allows us 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 plotting of axes that measure the dimensions of objects is assisted to facilitate the operation: when the axis being plotted intersects a first axis **perpendicularly**, their **intersection point appears in red**. It is therefore necessary to systematically **look for the red point** when plotting the second intersecting axis or to use automatic axis orthogonality detection.

Measurement software in general, and *Piximetre* in particular, does not provide an answer to the relevance of sample selection. They do nothing more than **facilitate measurements**, which is already no small feat compared to the "eyeball" work traditionally performed with a microscope eyepiece. The choice of objects to be measured and the interpretation of the results remain, fortunately, within **the domain of user expertise**.

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