# WHEN SIZE MATTERS…

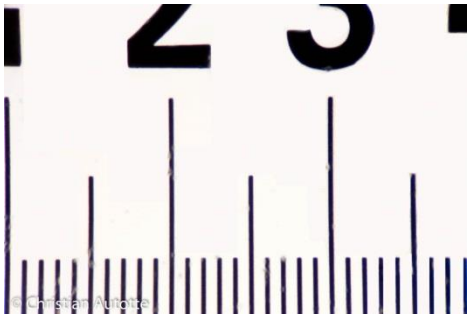
Most of my microscopic adventures turn around taking pictures of whatever I find out there. And most of the time the exact size of what I see doesn't really matter. I do record what microscope was used, along with the magnification, but that's about it. But every now and then I need to identify a new critter I just found. I then turn to some of my books, which more often than not include the size of microorganisms. And that's when size matters… For instance, *Colpidium* measure between 50 and 70 µm, while similar looking (for a beginner) *Paramecium* can reach 330 µm.

There are several ways to estimate the size of what you are looking at. The first one is through the use of a specialized eyepiece equipped with a micrometric scale. I don't remember where I found mine; could be on EBay or with one of my former suppliers. The principle behind such a scale is easy: simply use it as you would a ruler. Of course, it's easier with things that don't move; trying to measure an overactive ciliate quickly becomes a challenge!

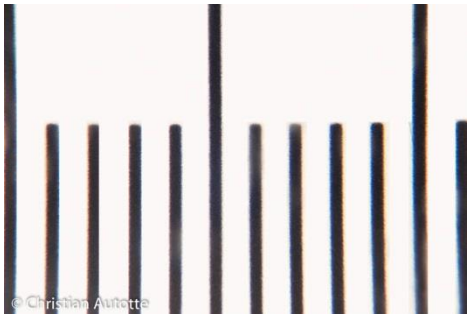

Another way to estimate size is with the field of view. First, we must estimate the field of view at each magnification. When we look though the eyepiece visually, the image is obviously round, so the field of view measurements are for its diameter. Switch to a camera and the field of view becomes rectangular. The values shown in the table below reflect the longest side of the rectangle from a full-frame camera.

| MAGNIFICATION | VISIBLE FIELD | LENGTH OF IMAGE FULL FRAME |
|---|---|---|
| 40X | 4.5mm (4500 µm) | 2.8 mm, 2800 µm |
| 100X | 2mm (2000 µm) | 1.12 mm, 1120 µm |
| 200X | 1mm (1000 µm) | 0.56mm, 560 µm |
| 400X | 0.5mm (500 µm) | 0.28 mm, 280 µm |

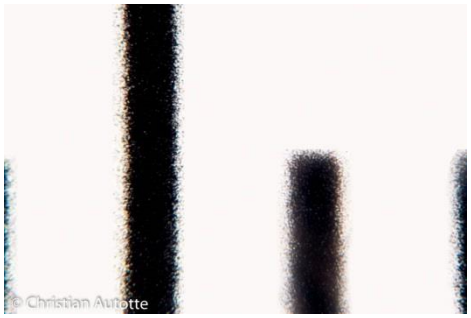
To get those measurements I simply used a transparent micrometric scale placed on a microscope slide. I first made visual estimates, and then took pictures with all my cameras and adapters.




40x




100x

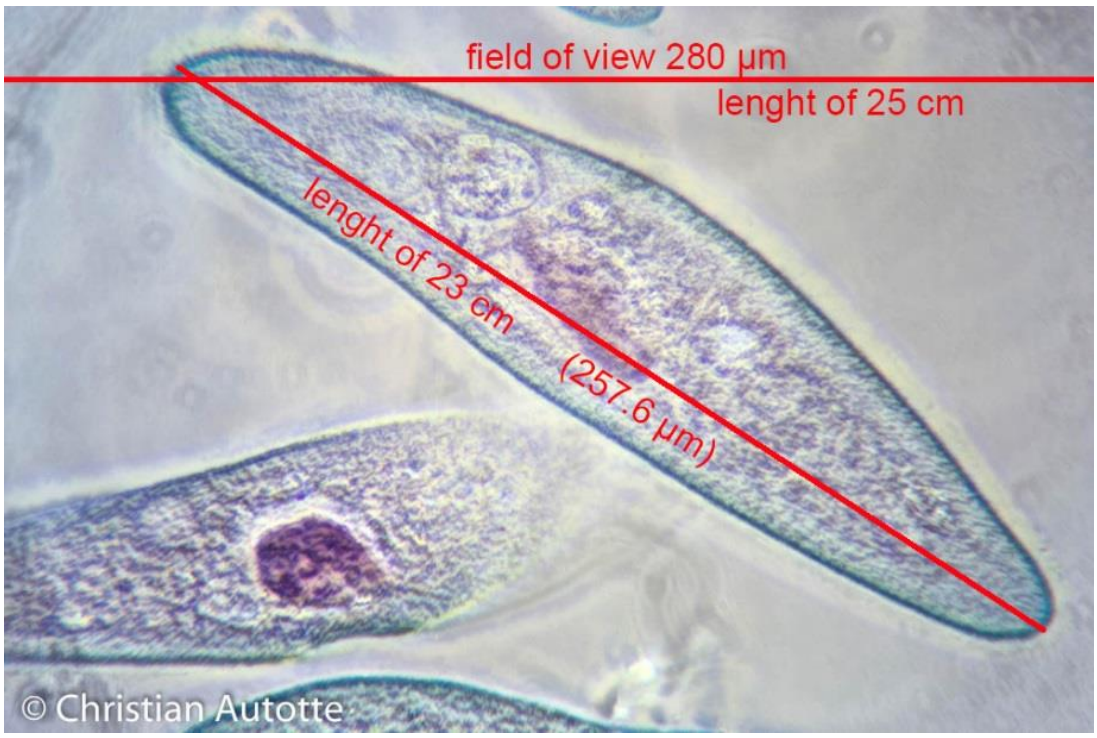



400x

This scale shows ten lines per millimetre. In the image at 40x, we count 2.8 mm or 2800 µm. At 100x, we have about 1.12 mm or 1200 µm (both from calculation and visual measurements). The field of view is reduced to 0.28 mm (or 280 µm) at 400x.

From there, size estimates are simple arithmetic. To measure some paramecium viewed at 400x, I take a picture and view it on the computer screen. I know that the field of view is 280 µm. The size of the picture is then measured with a ruler; here I get 25 cm. To know the image size of each micron I simply divide the field of view by the image size: 280/25=11.2 microns per cm. Next, I measure the size of the subject. Here we get 23 cm; so my calculations give 11.2x23=257.6 µm, which is a good size for a *Paramecium*.

A quick estimate could be made visually. For instance, knowing that the field of view at 400x is 500 µm, we could visually estimate that the *Paramecium* occupies about half the field of view and so measures about 250 µm. But for more precise measurements there is nothing that can replace a picture and a ruler.



Back in 2002, the late Walter Dioni did an article on the subject:

(http://www.microscopy-uk.org.uk/mag/indexmag.html?http://www.microscopy-uk.org.uk/mag/artapr02/wdmeasure.html)

His values were slightly different from mine visually, but very different with the sizes calculated for his photographs.

| MAGNIFICATION | VISIBLE FIELD | IMAGE 640x480 |
|---|---|---|
| 40X | 4450 µm | 3400 x 2550 µm |
| 100X | 1780 µm | 1333 x 1000 µm |
| 400X | 445 µm | 340 x 255 µm |

That is explained by the differences of both his microscope and his camera: his was a webcam, mine is a full-frame DSLR. On top of it, we didn't use the same camera adapter, which can also influence the final results of the pictures. In other words, photographers should do their own tests and measurements with their own equipment to get precise results. That said, the values given here are close enough to give you a starting point in your own calculations.

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