

A Short Manual for On-Line Diagnosis with COCCIMORPH

Introduction

COCCIMORPH is an experimental real-time diagnosis system accessible through a web interface. It allows users to upload an image, detect the contour interactively and obtain a real-time classification. The system was originally designed for the diagnosis of the seven *Eimeria* species that infect the domestic fowl using an image processing analysis. We intend to incorporate other parasites to the system in a near future.

An example data set of images is provided for testing purposes. You can download it from the address below:

<http://puma.icb.usp.br/coccimorph/tutorials/SevenSpecies.zip>

To get familiarized with the system, try to use these sample pictures before utilizing your own images.

Online diagnosis procedure

1. The online diagnosis system is located at <http://puma.icb.usp.br/coccimorph>. Click on the option On-Line Diagnosis. It can also be directly reached using the following URL: <http://puma.icb.usp.br/uploadoocyst/uploadimg.php>. Figure 1 shows a screenshot of the remote diagnosis page.

The different components of COCCIMORPH's user interface can be identified (Fig. 1), as follows:

- A. The `Scale` box, where the user fills in the pixels/micrometer value of his/her capture system for scale normalization.
- B. Segmentation threshold section. This value defines the threshold for the system to determine the object contour. The lateral buttons (`<<`, `>`, `>`, `>>`) permit to change the value in one- or five-unit increments, respectively. To monitor the effect of the new segmentation value, the user must press the `Segment image` button.
- C. The `Select image file` box. The full path of the image file can be specified here. Alternatively, the user can browse the disk and select the file by clicking on the `Browse...` button.
- D. Once the image file has been selected, it can be uploaded to the server by pressing the `Upload image` button.
- E. The image classification process is executed by clicking the `Classify` button.
- F. A copy of the results is sent by e-mail if the user provides a valid e-mail address in the fill-in-the-blank box.

Welcome to COCCIMORPH's discrimination page.

Please read the [instructions](#) before using the program.

Scale: Pixels/micrometer (Blank for original size)

Segmentation threshold (0-255) :

<< < segment image > >>

Select image file: Browse...

Upload image

Classify | If you want to receive classification results in your e-mail, please fill in your address :

Figure 1 – The home page of COCCIMORPH diagnosis system

2. To upload an image, click on the `Browse...` button and select the image file. Only one single file can be uploaded at a time. If you are using our example data set, it is not necessary to fill in the `Scale` box (default value is 11.2). Click on the `Upload image` button to upload the file to COCCIMORPH server.
3. Once the image file has been uploaded, you should see a display, side by side, of the picture and its corresponding binarized version (Fig. 2).
4. The next step is the oocyst segmentation. By segmentation one means the identification of objects above background noise using image-processing methods. In COCCIMORPH, segmentation is achieved by detecting the object (oocyst) boundaries through a contour-orientated segmentation.

The correct threshold value for segmentation must be chosen. A default value of 140 is provided, but it is not guaranteed to work for all images. An adequate value will result in a white line defining the whole contour of the oocyst (Figure 2). If the value is too low, only part of the contour is defined (Figure 3). Too high threshold values yield no visible contour lines (Figure 4). The segmentation threshold can be changed by entering a value in the `Segmentation threshold (0-255)` box, or by using the arrow buttons. The two-arrow button changes the threshold value in 5 units, whereas the one-arrow button permits fine tuning by changing it one unit at a time. Press the `Segment image` button to monitor the effect of changing the threshold value.

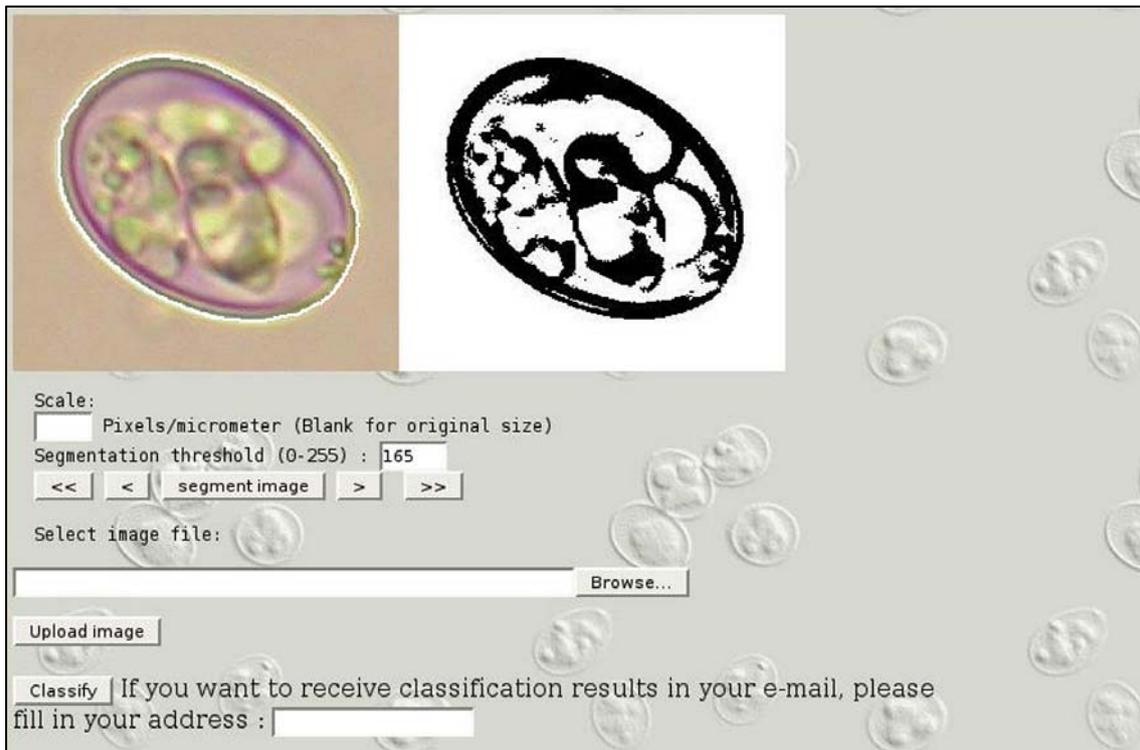


Figure 2 – An adequate segmentation value yields a well defined white contour line surrounding the oocyst image (picture on the left).

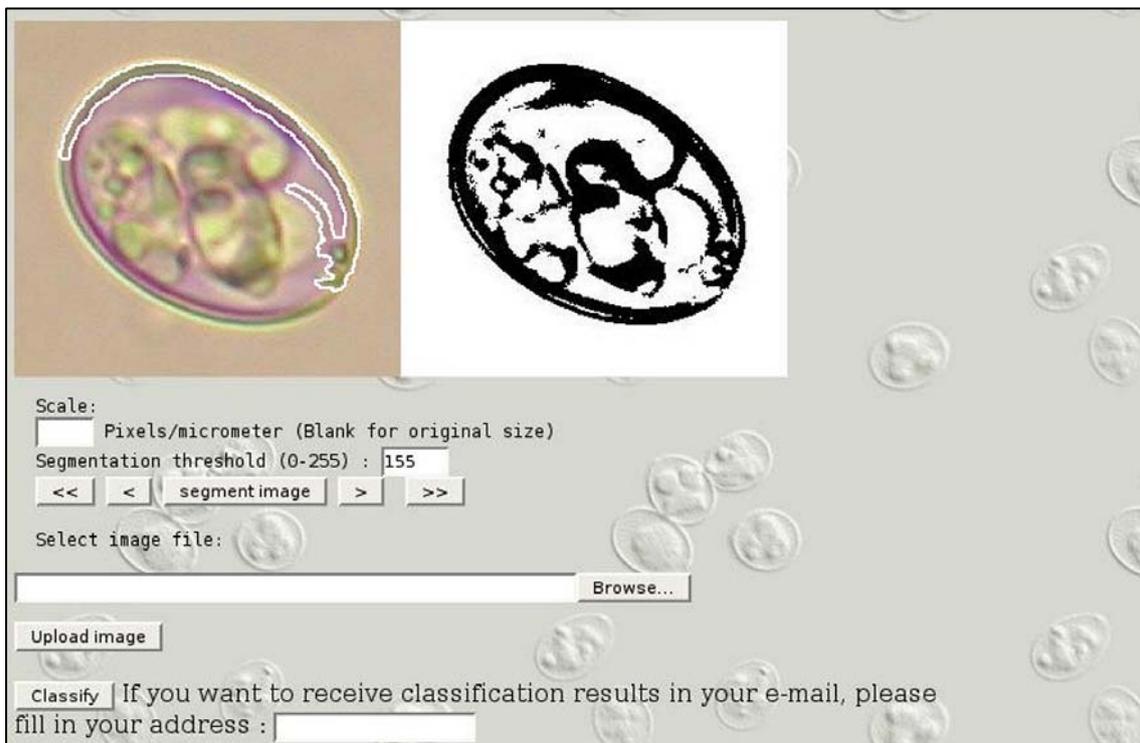


Figure 3 – Too low segmentation values generate a poorly defined white line that does not follow the entire oocyst contour (picture on the left).

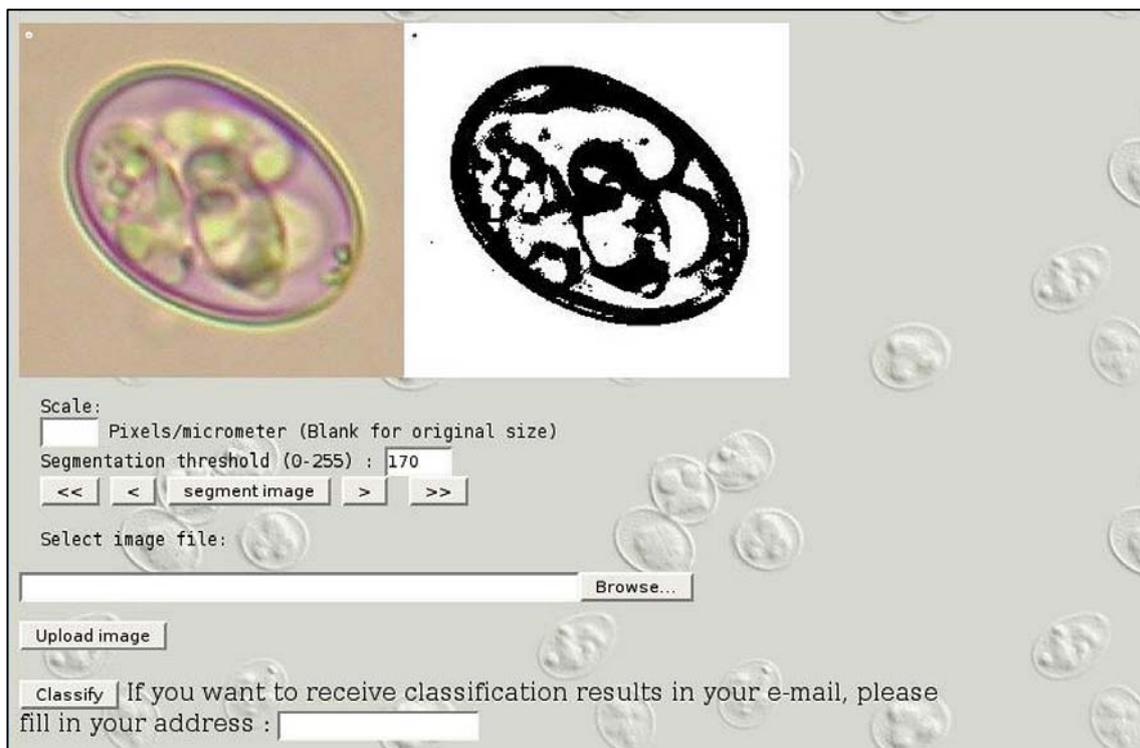


Figure 4 – Too high segmentation values result in no visible line of contour. In fact, the delimited area can be seen as a small circle on the upper left corner of the picture on the left.

5. After segmenting the image, click on the `Classify` button to start the classification process. A typical result is displayed in Figure 5. The program outputs a series of feature measures. The larger and smaller diameters are given in pixels, and can be easily converted into μm by dividing the values by the scale of the picture. The last line of the output displays the classification results. Results are displayed as a percentage of feature similarity between the query image and prototype values, calculated from a training set, for each one of the seven *Eimeria* species. If the feature values found in the query image are similar to more than one single species, the program will list the other similar species and their corresponding similarity values.

NOTE 1 - The similarity values **do not** represent probabilities and hence, they do not sum 100%. If the program reports a similarity of 88.54% to *E. acervulina* and 53.88% to *E. necatrix* (see Fig. 4), the interpretation is that the feature values of the query oocyst image were 88.54% similar to the prototype values calculated from the training set of *E. acervulina*. However, the relatively high similarity to *E. necatrix* values suggests that there is also some chance that the image corresponds to this latter species.

NOTE 2 - Not all images permit the program to infer the species. If not enough similarity is found, no classification results are presented.

NOTE 3 - Be aware that the system is still experimental and that the overall correct species assignment is around 85%, varying from 70% to 98%, depending on the species. Thus, some false results are expected. Also, the accuracy depends on several factors such as the purity of sample, physical integrity the oocyst, quality of the microscope, resolution of the camera, absence of debris, etc.

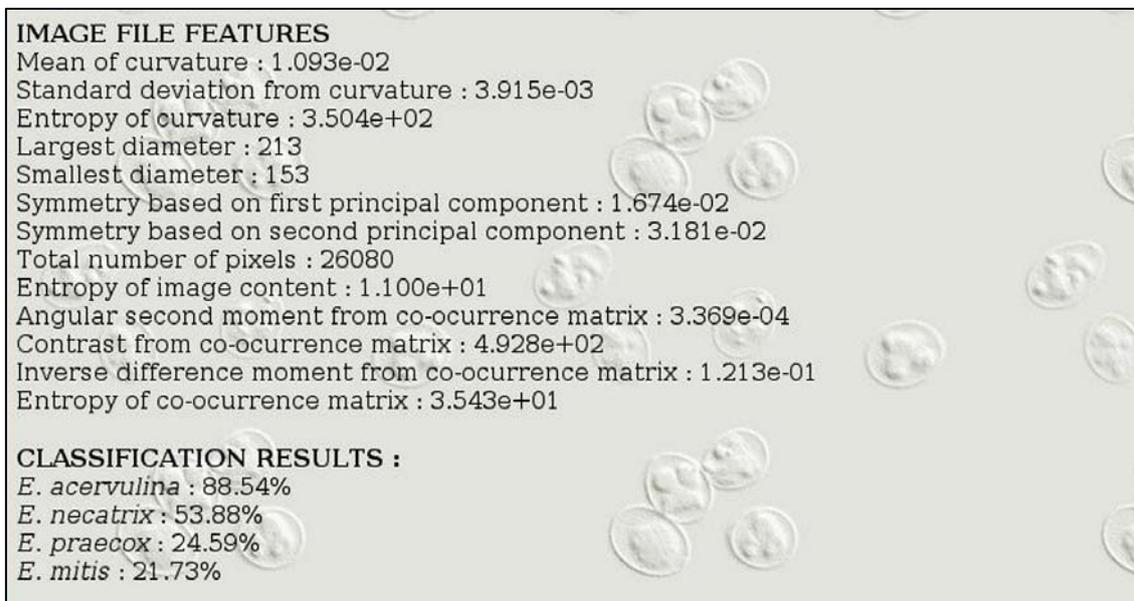


Figure 5 – Screenshot of the classification result page. A list of image features and the corresponding measures is displayed. The similarity values in respect to prototype feature values of each species are presented.

Capturing your own images

Once you get familiarized with the interface, you can also capture your own oocyst images and submit them to COCCIMORPH. Here are some recommendations:

1. Try to capture oocyst images at the highest resolution you can. We regularly use a 4-megapixel camera with a 40x magnification objective. The lower resolution you use, the less information you provide for COCCIMORPH. In our experience, images with less than 2 megapixels can be hardly diagnosed.
2. Crop out single oocyst images to be used in the program. Do not try to use multiple-oocyst images. In the current implementation, COCCIMORPH does not handle such images.
3. Always use well-defined oocysts images, especially well focused images.
4. Upload your image file. You can use TIF, JPEG, BMP or PNG file formats. Do not use GIF format, as it results in a severe loss of information, thus decreasing the accuracy of COCCIMORPH.

5. Use the scale value you have determined for your capture equipment (see the section “Scale Normalization for COCCIMORPH”) and fill in the `Scale` box in COCCIMORPH's interface. DO NOT use the default value (11.2), as it was determined for our system and should not work for your capture device.
6. Click on the `segment_image` button and, using the arrow buttons, find the most adequate threshold value for segmentation (see Figures 2 to 4).
7. Click on the `Classify` button and observe the results.

System calibration for COCCIMORPH

The scale determination process

Because different cameras and microscopes present distinct magnifications and resolutions, it is essential to calibrate your system in respect to the scale of the image. In simpler words, this means that one needs to determine how many pixels are comprised in one micrometer of the captured image using a given camera system, under a set of image capture parameters.

The number of pixels per micrometer changes every time we alter the following parameters: magnification objective of the microscope, zoom (focal distance of the camera lens), and resolution of the camera sensor.

The effect of magnification

The easiest way to increase magnification in a microscope is to change the objective (e.g. using a 40x objective instead of a 20x one). A higher magnification implies that any image object will be represented by a higher number of pixels and vice-versa. Therefore, if we want to use more than a single objective, we need to determine the pixels/ μm value of the corresponding digital image for each one.

The effect of zoom

A zoom lens is a system comprised by two or more optical parts that are moved with respect to each other to obtain a continuously variable focal length and hence magnification. Thus, if we change the zoom of the camera lens, the magnification is consequently changed as well. Therefore, the pixels/ μm value must be determined for a defined zoom.

The effect of the resolution of the camera sensor

Digital camera sensors are electronic devices that allow one to capture images in different resolutions. By resolution we mean the total number of picture forming elements (pixels) that are captured and that will compose the final digital image. Given an image taken under a chosen magnification and zoom, the resolution employed will define how many pixels will represent any image object. The resolution can be modified when we

change the resolution parameter in the camera or, alternatively, when we replace the camera itself by another one with a different resolution. Concluding, the pixels/ μm value must be determined for each combination of camera/resolution employed.

Procedure for scale determination

Once you choose a given combination of microscope objective, camera/resolution, and zoom, you are now ready to determine the scale value of your system. Provided that the same conditions will be used from now to capture oocyst images, this calculation needs to be done only once.

Calculating the scale value

Figures 6 and 7 show images of a microscope slide scale and a hemocytometer, respectively. Both images were captured in a resolution of 2272x1704 pixels (3,9Mpixels). In Fig. 6, each interval is defined by short vertical bars corresponding to 10 micrometers, whereas the larger vertical bars define distance intervals of 100 μm . In Fig. 7, the counting chamber reticule defines 50-micrometer squares.

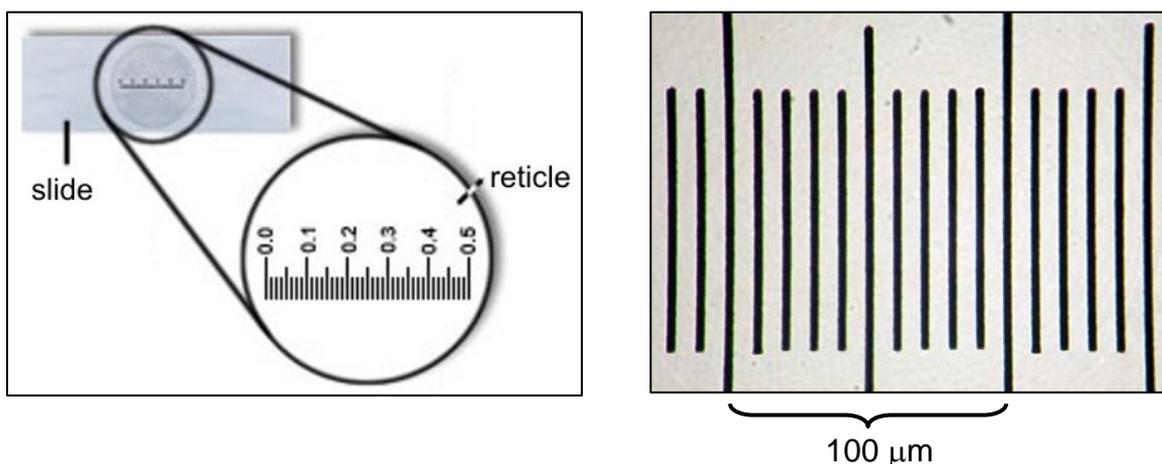


Figure 6 - Nikon microscope slide scale (left) and the corresponding microscopic digital image (right). The larger vertical bars define distance intervals of 100 μm .

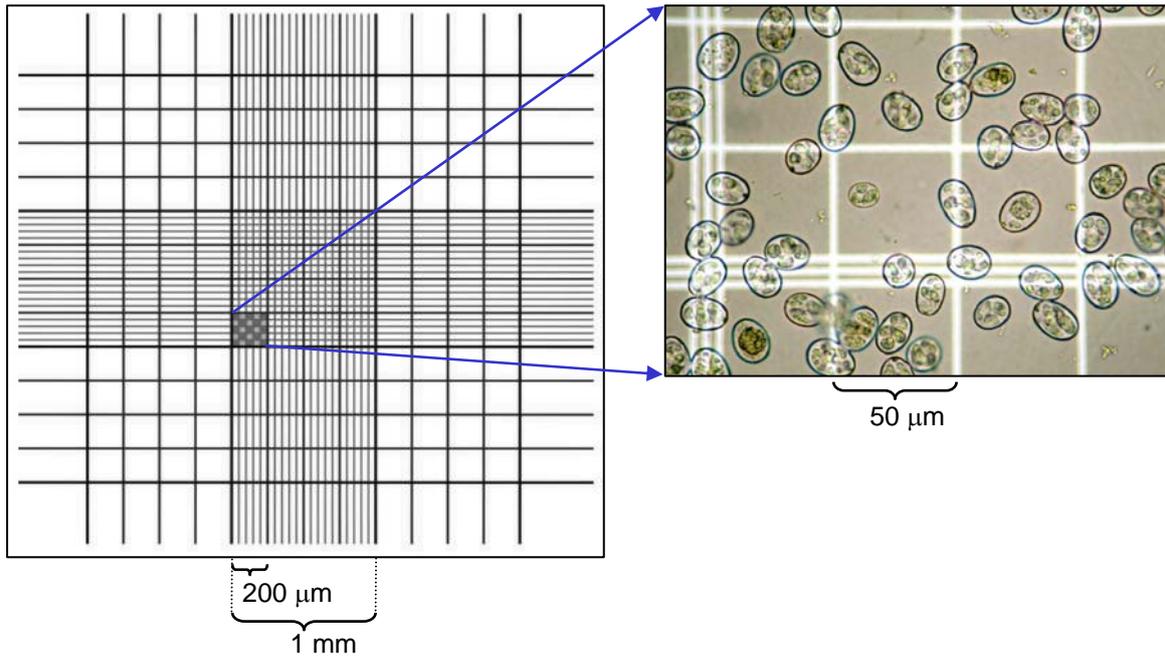


Figure 7 - The Neubauer counting chamber (hemocytometer) reticle (left) and the corresponding microscopic digital image (right). The small squares of the reticle present a side length of 50 μm . Oocysts of *E. acervulina* (larger diameter of $19 \pm 1.3 \mu\text{m}$) are displayed for size comparison purpose only.

Using the slide scale or a hemocytometer counting chamber, take a picture under the same conditions that you are going to use for capturing the oocyst images. Open the picture in any image editor (Gimp, Adobe Photoshop, Corel Photo-Paint, etc.) and determine the coordinates of two points (there are specific tools for this task) that define a given distance interval, that is, a known length in micrometers. For example, points representing the central part of two black vertical bars, in the scale of Figure 6, would correspond to a distance interval of 100 μm .

To calculate the distance (in pixels) between these points, we can use the equation below:

$$D = \sqrt{(x_2 - x_1)^2 + (y_2 - y_1)^2}$$

where:

D = distance

x_1, y_1 = coordinate of first point

x_2, y_2 = coordinate of second point

Due to image capture variations and, to improve accuracy, it is recommended to take different shots and calculate the distance between two reference points as the average of measures determined in different images. For example, we

computed the distances of a known length in a hemocytometer (see Figure 7), and listed the values in Table 1. The distance between the first pair of points, representing a length of 50 μm , is 560.47 pixels. Dividing the total number of pixels by 50, we determine the scale in pixels per micrometer (11.21). Computing the average from different images, we obtain a value of 11.20 pixels/ μm . Concluding, in this example, using a sensor of 3.9 MPixels, we should use 11.2 as the `Scale` in the COCCIMORPH system.

If the same equipment, magnification and resolution are used from this point, this step only needs to be done once. **However**, if you change the camera, microscope objective, capture resolution, or zoom position, you **must** necessarily calibrate your system once again.

Table 1 – Calculation of the scale. Coordinates of two points (x_1y_1 and x_2y_2) covering a length of 50 μm in a hemocytometer, and the corresponding distances, are listed. The scale is calculated by dividing the distance (in pixels) by the length (50 μm). The final scale value is obtained from an average of ten independent measures.

x_1	Coordinates		y_2	Distance (pixels)	Scale (pixels/ μm)
	y_1	x_2			
605	1541	1165	1518	560.47	11.21
22	1453	604	1424	582.72	11.65
225	632	792	617	567.20	11.34
242	1193	808	1172	566.39	11.33
1949	1656	1925	1143	513.56	10.27
1925	1143	1361	1157	564.17	11.28
1361	1157	1346	602	555.21	11.11
1377	1666	1949	1656	572.09	11.44
1346	602	1907	583	561.32	11.23
1907	583	1898	11	572.07	11.44
Average					11.20