An Algorithm to Extract Physical Characteristics of Nematodes from Microscopic Images of Plant Roots

Alexis Toribio, Luis Vargas, Guillermo Kemper and Alfonso Palomo

Abstract — **In Peru, phytoparasite nematodes are a type of specimen that adheres to vegetable roots, preventing plants from absorbing the necessary nutrients to grow. In order to apply contingency and prevention plans, it is necessary to study the families to which the nematode pests belong by analyzing their physical characteristics with a microscope. The proposed system represents an alternative to the current methods for the classification of phytoparasite nematodes through image processing. This system is an algorithm oriented to detect the physical characteristics of nematodes in tropical fruit crops (width and length). The algorithm involves image acquisition of nematodes through a microscope, obtain the luminance component of the image, illumination correction, binarization by histogram, objects segmentation, discrimination by area, surface detection and calculation of the Euclidean distance to approximate the physical characteristics of specimens in a sample of nematodes. Favorable results were obtained in the detection of the characteristics of the Meloidogyne type II species. The results were validated from those obtained by clinical analysis of the specialists, achieving up to 85% of success.**

Keywords—nematodes, plant roots, microscopic images, illumination correction, image processing.

I. Introduction

In the development of agricultural activities, People committed to agricultural activities must face a large number of challenges in order to obtain high quality and profitable products. One of the major problems faced is the overpopulation of phytoparasite nematodes in vegetable crops. This overpopulation takes place, mainly, because these creatures adhere to the roots of plants, preventing their absorption of nutrients, and thus affecting its growth. In Peru, several nematodes harmful families are known in different types of cultivation, being one of the most damaging and common the Meloidogyne type II nematode that concentrates on the roots of tropical crops, mainly fruits.

Several methods of analysis to detect the family of nematodes are currently used, being the most accurate one the real-time PCR, where the parasites are grouped by families analyzing their DNA. In Peru, due to the high cost of this equipment, we choose to study them by analyzing images obtained from a microscope. When an expert determines the physical characteristics, not only the family can be defined, but also the age and a better diagnosis of the state, in which the infested crop is found, is given. Because of this, is very important to determine the width and length of a specimen in order to provide important details to the possible solutions against an infestation of these microorganisms.

The proposed system seeks to provide a useful tool for laboratory use that delivers the physical characteristics of the microorganism (width and length), and with it, specialists are able to find more information for the identification of specimens. Furthermore, to train new professionals in plant pathology, this resource is proposed to deliver concrete measures that are difficult to approximate. In this sense, the software requires digital images obtained from the microscope, for which a specialized camera was used, Omax brand model a3530u with 3.2 megapixels in the default configuration.

Some works in this field have been developed, being of importance those mentioned below:

Carlos A. Silva in [1] explains an algorithm capable of detecting the characteristics of nematodes using neural networks. However, he does not explain a method to correct the noise of the illumination present in old microscopes. Moreover, this method fails to demonstrate when nematodes are superimposed on themselves.

On the other hand, Nikzad B. Rizyvandi in [2] explains an algorithm capable of detecting the length of nematodes by separating them into small blocks and calculating the angle with the direction of each block. This solves the problem of nematodes superimposed with other nematodes, however, those that are superimposed on each other are not well detected.

Brad T. Moore in [3] explains an application called "WormSizer" that calculates the dimensional characteristics of nematodes, such as size, trajectory, etc. Nevertheless, authors do not detail, in the results, the different situations in which nematodes can be found, such as the example of nematodes superimposed on each other.

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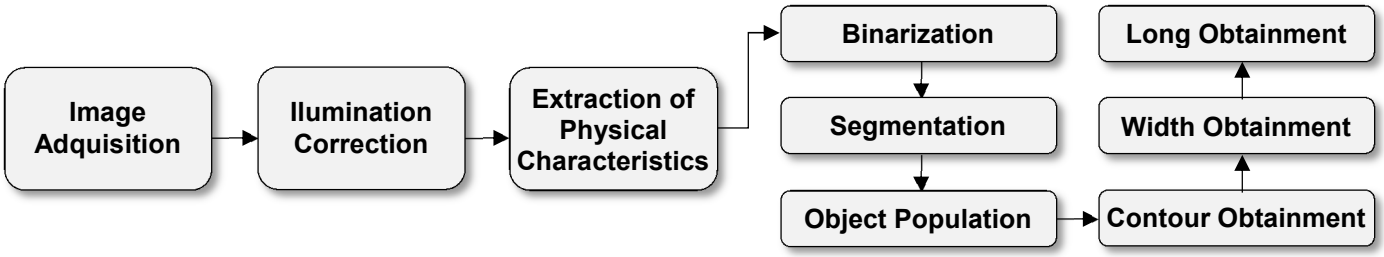

Fig. 1. Block diagram of the proposed method.

II. Proposed System

Fig. 1 shows the block diagram of the proposed algorithm in order to obtain the length and width of the nematodes. This process is described below.

A. Image acquisition

To acquire the image, the Omax camera model A3530U was used, which has a response speed of 8fps at 2048x1536 pixels, SNR of 43db, resolution of 2048x1536 pixels, pixel size of 3.2 µm x 3.2 µm and uses a model of RGB capture color.

B. Illumination correction

The objective of this stage of the algorithm is to eliminate the radial noise caused by the light source of the microscope. To achieve this, the luminance component $I_Y(x,y)$[1] must first be obtained.

Then, it's required to attenuate the high-frequency components $I_Y(x,y)$, that is, the pixels that make up the nematodes, and thus, extract the background image. To do this, a Gaussian filter is used, expressed as:

$$G(x,y) = \frac{1}{2\pi\sigma^2} e^{-\frac{x^2+y^2}{2\sigma^2}} \quad (1)$$

Where σ is the standard deviation and x and y are spatial coordinates. For this case, the value of σ = 160 was used and the square matrix is 641x641.

We define $I'_Y(x,y)$ as the resulting image of the Gaussian filtering. Then we get the negative of $I'_Y(x,y)$ expressed as:

$$\hat{I}_Y(x,y) = 255 - I'_Y(x,y) \quad (2)$$

Finally, the corrected image expressed as:

$$I_P(x,y) = \frac{I_Y(x,y) + \hat{I}_Y(x,y)}{2} \quad (3)$$

Fig. 2a and 2b show the original luminance picture $I_Y(x,y)$ and the corrected image $I_P(x,y)$ respectively.

C. Extraction of physical characteristics

This stage of the algorithm is made up of several sub-stages that explain the method of binarization, segmentation, filling the structure of the subject of interest, and determining the width and length of the nematodes.

1) Binarization

The binarization is applied to segment the objects of interest. For the selection of the corresponding threshold, the histogram of the image $I_P(x,y)$ is used.

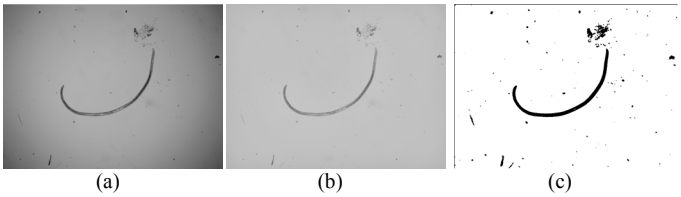

Fig. 2. Images a) $I_Y(x,y)$, (b) $I_P(x,y)$ y (c) $I_b(x,y)$.

Fig. 3 shows the histogram of the image $I_P(x,y)$. We define the thresholding value reference where the histogram mode is located. This value is defined as r_0.

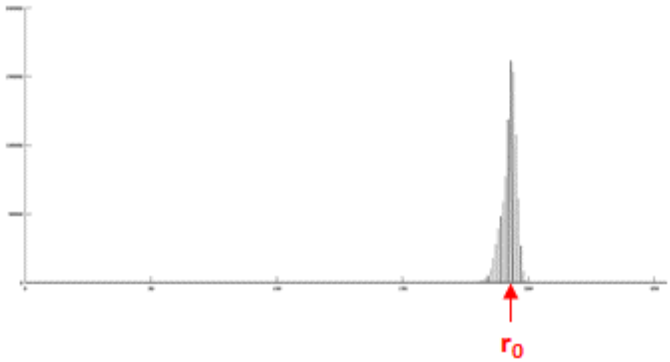

Fig. 3. Histogram of the image $I_P(x,y)$

Then, the binarized image $I_b(x,y)$ is obtained from the following thresholding expression:

$$I_b(x,y) = \begin{cases} 0 & , \quad r_0 - 15 \leq I_P(x,y) \leq r_0 + 15 \\ 255 & , \quad other\ case \end{cases} \qquad (4)$$

2) Segmentation

To segment the objects that identify the nematodes, connectivity 8 [4] labeling was used. This process allows to segment each labeled object and evaluate its size in number of pixels. In this case, an object is eliminated from the image if its size was smaller than 3500 pixels. This data was obtained by tests in the laboratory.

3) Object Filling

Each labeled object is segmented into a binary sub-image to be evaluated. However, to avoid errors in the geometric calculations required, it is necessary to fill some objects when there is a case of nematodes twisted or superimposed on themselves. Observe in Fig. 4, where the internal voids of the binarized and segmented object are notorious.

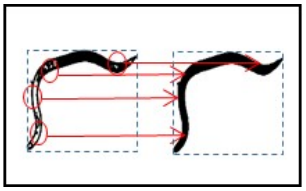

Fig. 4. Filling the empty spaces inside the nematode contour.

The procedure used for the filling process is described below:

Step 1: Each object is segmented into an image space (box). From the center point of the upper edge of the image, travel down one column until you find a nematode pixel $p(x_1, y_1)$. The procedure is repeated from the center point of the left edge of the image, running through the corresponding row until finding the first nematode pixel $p(x_2, y_2)$. From $p(x_1, y_1)$, lines are drawn at different angles looking for the smallest one that traverses the body of a nematode (if in the path there is a sequence of white pixels, the path is followed while the number of these pixels does not exceed 1.3 times the average width of a nematode, which for this case was established in 33 pixels from the tests performed), then this value is stored. The procedure is repeated with the pixel $p(x_2, y_2)$ and the obtained value is averaged with that obtained from $p(x_1, y_1)$. The result of the average is an estimate of the apparent width A_p of the nematode.

Step 2: Horizontal and vertical sweeps are made to the image containing the nematode in order to find rows and columns of internal white pixels of nematodes whose size is smaller than A_p . In this case, all the coordinates of the internal white pixels found are stored.

Step 3: Finally, value 0 is assigned to all the pixels whose coordinates were stored in the previous step. The resulting image is shown in Fig. 4.

4) Obtaining the contour

Obtaining the contour of the objects of study is crucial to extract the physical characteristics in the most complicated cases. Initially, a random pixel $p(x_0, y_0)$ is taken from its edge, its coordinates are stored in a matrix **V1** and the procedure below is followed:

Step 1: Starting from $p(x_0, y_0)$ the closest black edged pixel of its 8 neighborhood is searched in a clockwise direction. The resulting pixel of the search is defined as $p(\dot{x}_0, \dot{y}_0)$, which is stored in **V1**.

Step 2: Take $p(\dot{x}_0, \dot{y}_0)$ as the starting point and repeat the whole procedure from step 1.

Step 3: At the end of the path of the matrix **V1**, it should contain all the coordinates of the edge pixels, which are signaled in Fig. 5.

5) Obtaining the width

With the filled nematodes and matrix **V1**, the following procedure is performed to obtain the width of the nematode:

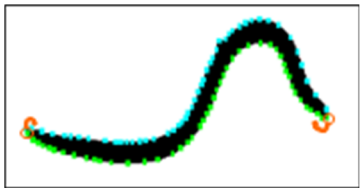

Fig. 5. Obtaining all the points of the external border

Step 1: A vertical sweep (from the top edge) of the first column of the image containing a nematode is performed. Sweep stops when the first black pixel is found. Then from that pixel, the sweep of the column is continued until finding a white pixel. The number of black pixels found is stored in a vector n1. The procedure is repeated for all columns by storing the values obtained in **n1** (see Fig. 6).

Step 2: Repeat the procedure in step 1 for a horizontal sweep. In this case, the vector obtained is **n2** (see Fig. 6).

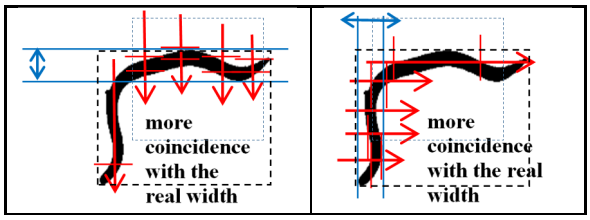

Fig. 6. Vertical and horizontal sweeping of the nematode.

Step 3: Determine the highest occurrence value in **n1**. This value is defined as n_0 and is stored as the first value of a vector **n11**. Then the values of **n1** that are in the interval $[n_0 - 8, n_0 + 8]$ are taken and also stored in **n11.**

Step 4: The procedure of step 3 is applied to vector n2, resulting in vector **n22.**

Step 5: The arithmetic mean of the values stored in **n11** and **n22**2 The obtained values are defined as $m1$ and $m2$ respectively. These values constitute the estimates of the nematode width of vertical and horizontal perception.

Step 6: From the arithmetic means obtained, the final value of the width of the nematode is calculated, which is defined as w_p. To obtain this value, the following conditions were applied:

- If $m1 < m2/4$ then $w_p = m1$
- If $m2 < m1/4$ then $w_p = m2$
- If none of the above conditions were met, $w_p = \frac{m1+m2}{2}$

These conditions filter and avoid possible errors in the estimation, which can occur for cases such as those shown in Fig. 7.

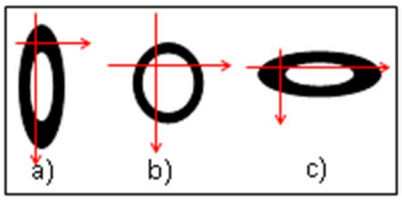

Fig. 7. Special cases, in a) elongated closed nematode, b) symmetric closed nematodes, c) widened closed nematode.

6) Obtaining the length

In order to obtain the length of a nematode, it must first be classified if it is an open or a closed nematode. The procedure for this is detailed below:

Step 1: A vertical sweep (from the upper edge) of the first column of the image containing the nematode is performed. We call the first black pixel found as an input pixel. Continue with the nematode body sweep (black pixels) until the last black pixel is found (output pixel). If the nematode is closed (see Fig. 7), a second input pixel and a second output pixel will be found when sweeping continues. In this case, the coordinate of the first output pixel is stored in a matrix **V2.**

Step 2: The procedure is repeated for all columns of the image that contains the nematode**.**

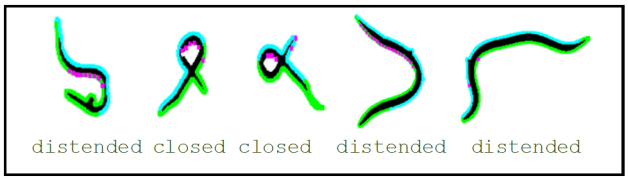

Fig. 8. Detection of superimposed pixels.

Step 3: All identical coordinates, which were also stored in **V1**, are removed from **V2**.

Step 4: If **V2**, finally, doesn't retain any value, the nematode is considered open and only steps 5 and 6 are executed. Otherwise, it is considered closed and steps 5, 7 and 8 are executed.

Step 5: With the coordinates stored in **V1** the Euclidean distances between consecutive neighboring pixels are determined. Then these distance values are added obtaining the value $ds1$.

Step 6: From $ds1$ the final length l_p of the open nematode is obtained (in number of pixels):

$$l_p = (ds1 - w_f)/2 \tag{5}$$

In this way, the contribution of the width of the specimen in one of the ends, which corresponds to its head, is eliminated.

Step 7: With the coordinates stored in **V2** the Euclidean distances between consecutive neighboring pixels are determined. Then these distance values are added obtaining the value $ds2$ (length of the internal contour).

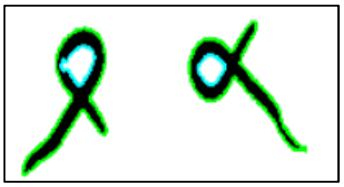

Fig. 9. Detection of two sections, outside and inside, in the closed nematode.

Step 8: Two sections of the nematode are recognized, one outside, whose value is ds1, and another inside, whose value is ds2 as shown in the Fig. 9. These values, represented in light blue (inside, as ds2) and green (outside, as ds1), will be added together and will result in the final length l_p (in pixels) of the closed nematode, according to:

$$l_p = (ds1 + ds2 - w_f)/2 \tag{6}$$

III. RESULTS

To evaluate the performance of the proposed algorithm, the results of the algorithm were compared with those obtained by the specialized ToupCam software. In this case, the measurements were obtained manually. This process was done with a software tool, put at disposition after taking a microscopic photo on which the user can input the optical zoom data used. With this information, small interconnected lines along width and length directions can be drawn over the picture of the specimen. These lines are added up in the software, giving two values that multiply internally with a constant value, providing, finally, two measurements in micrometers (l_r and w_r) that correspond to the width and length respectively. For the evaluation, 120 nematodes were considered properly segmented from the analyzed images. Then, for each nematode, the number of pixels that make up the length and width was measured. These measurements were obtained using software ToupCam (l_r and w_r) and the proposed algorithm (l_p y w_p).

All the values obtained in number of pixels were converted to micrometers using a Neubauer camera with an aspect ratio of 649 pixel/millimeters. With this conversion, the values l_r', w_r', l_p' y w_p' were obtained.

Finally, the error percentage for the length ($\%E_l$) and the width ($\%E_w$) was obtained from the following expressions:

$$\%E_l = \left(\left|l'_r - l'_p\right| \times 100\right)/l'_r \quad (7)$$

$$\%E_w = \left(\left|w'_r - w'_p\right| \times 100\right)/w'_r \quad (8)$$

Fig. 10 presents the curves of the length and width errors for 120 nematodes evaluated. The average error rate for the length was 15% while for the width was 11%.

These values were accepted by the specialists of the nematology laboratory of the UNALM since the error did not prevent the correct typing of the specimens.

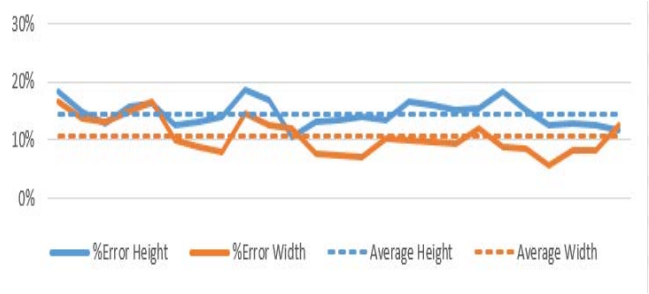

Fig. 10. %Error Height(blue) %Error Width(orange).

IV. Conclusions

The algorithm showed to be useful in the nematology laboratory since the error percentage obtained from the tests was accepted by the experts in this field. Moreover, the length and width components are of vital importance for the detection of Meloidogyne type II nematodes. Finally, the algorithm proved to be robust since the noise in the illumination did not affect the physical characteristics obtained due to the pre-processing algorithm.

The width and length of nematodes are two parameters used by specialists in order to classify this specimen into its family. The objective of the proposed algorithm is to obtain these two parameters, to be used as the input data in AI algorithms (neural networks - deep learning) in order to sort out Meloidogyne nematodes in the next step of the process to develop.

The whole algorithm was designed and tested using MATLAB because of its simplicity, and built with an AI function it has. The final product was developed using c# and phyton. This was done because c# contains strong built-in libraries to make the UI friendlier, and python has a strong processing power, as well as a large number of libraries, to run AI algorithms.

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