TECHNOLOGICAL INNOVATION FOR AUTOMATIC IMAGE ACQUISITION USING A MICROSCOPE

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KEYWORDS	ABSTRACT
Laboratory technician *MATLAB* *Digital images* *Algorithm* *Microscope* *Autofocus* *Image Processing*	*This project was developed because in most laboratories, the main problem is the time a laboratory technician spends finding the best focused image in the microscope. Its objective was to design a program that allows to find the best focused image of a set of images taken from a field of a biological sample using the microscope. To carry out this procedure, the MATLAB software was used, which allows the analysis and processing of digital images. The present work was developed in three stages: Research, development, and simulation, for which the value of the degree of focus and the execution time were considered. In the end, the algorithm that managed to find the focused image and with the fastest execution time was selected.*

1. Introduction

The reading of biological samples in the laboratories of hospitals, clinics or other medical centers using the microscope is performed through the manual technique, which in technological terms refers to a simple, inaccurate and unreliable system. This is because the reading of laboratory samples under the microscope depends on their preparation and analysis by experienced personnel, who may take erroneous notes such as excess or lack of bacteria observed due to eyestrain because they are in front of the microscope for a long time and repeat the same procedure several times. The manual method, which is highly variable, comprises in addition to misdiagnosis to patients, which is inefficient and slow taking into account the hundreds of daily samples left in the laboratories and not all samples are delivered the same day they are dropped off (Asmar & Drancourt, 2015).

Sample analysis in laboratories is a daily task. The microscope is used as routine field work in different areas such as uroanalysis, microbiology and hematology in medical laboratories, jet room and spinning laboratories in industries, among others. Large laboratories dedicated to research perform daily analysis of different samples and increasingly need to obtain more accurate and faster results. The Uroanalysis area performs more than 3500 tests per month; the Microbiology area, more than 7200 and the Hematology area, more than 3300 tests per month. In this context, engineering is very involved since they have developed efficient methods for reading a laboratory sample automatically using different algorithms (Alanya-Beltran et al., 2023; Ahmed et al., 2023). These algorithms are based on digital image processing. The automation of microscopes is performed in hardware, which allows the displacement of the microscope axes to perform the total scanning of the sample, and the capture of images through photographic or CCD cameras (Tozzoli et al., 2012). Software automation allows the analysis of the images taken by the camera and autofocus algorithms are used for this purpose. Its use in microscopes is very important because it improves the analysis of a sample and benefits laboratories by contributing to the development and improvement of results in favor of health (Pimentel-García et al., 2023).

Digital processing applied to images aims to improve image quality and as a consequence the possibility of visualizing or analyzing image details that cannot be perceived in their original form. It is based on the development of algorithms that are currently very complex and elaborate, and are used for digital image processing (Alanya-Beltran et al., 2022).

First, it starts with the acquisition of an image, i.e., it is a conversion of the real image to a digital image and after that sending it to the computer; thus, this step is very important, the acquired images must have very good resolution to be able to evaluate them correctly (Bacca-Quintero et al., 2018). Then, the digital image is represented by a matrix that can be two-dimensional or three-dimensional. A digital image is composed of a finite number of elements called pixels and are represented, in this two-dimensional case as a function f(x, y); each value of this function is equivalent to the gray level of the image at that point (Gómez and Rubio, 2023). Finally, grayscale images are analyzed by using autofocus algorithms and different techniques such as fusion and segmentation and the application of filters (Fu et al., 2017). This image processing technique obtains and enhances images taken from the microscope and is applied in studies in medicine, such as radiographs, images of microbacteria and also in the analysis of images sent from a satellite or satellite images (Graterol-Ruiz, 2020; Scardina et al., 2020).

In Peru, few laboratories have automated microscopes that can adjust the focus of a sample. However, in other countries, such as in the United States, there are very advanced microscopes that, in addition to analyzing a blood sample, for example, proceed to the study of the sample and issue their results depending on what they are being programmed for. It is also used for DNA tests, to rule out different diseases such as AIDS, cancer or others and also for pregnancy diagnosis.

Practically the human intervention is in less percentage to obtain the result, that is, it is a completely automated process (Dhinakaran et al., 2022). The use of autofocus and automation in microscopes has many

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advantages among which are presented, 1) Fast results: The results of the analysis will be performed faster; 2) Efficiency: The final image will be the one that can be best analyzed because they are the ones that are better focused; 3) Increased sample analysis: much more analysis can be performed per day. Automation and application of an autofocus system allow the laboratory to handle a high number of requests in a short time and achieve high accuracy (Bulacio et al., 2015). Automation combines productivity and quality as indispensable tools; for example, a laboratory in the late 1970s obtained approximately 10,000 results per year and technician; then at the beginning of the 21st century, the same laboratory generated 100,000 results per year and technician, with automated and non-automated technological level.

Currently there are several companies that have already entered the business of microscopes with automatic sample analysis. These include SONY, WERTH, FEI, JEOL, NIKON, HITACHI, AXIO and others. All these companies have microscopes that work automatically and also, they have used both different software and hardware, each one more innovative than the other or that present greater advantages in some aspect such as resolution and magnification or zoom. Therefore, the research aims to design a program that allows to select the best focused image of a sample in order to obtain results faster and in an efficient way, which is beneficial for patients with serious diseases that require results quickly and for those laboratories with low resources.

2. Development of the research

For the development of the research, first it is required to perform research on the autofocus algorithms, which are used to perform the focusing process automatically, likewise, multiple autofocus algorithms have been proposed that present some methodologies such as the use of operators, which are based on the calculation of the first and second derivative, called gradients; the calculation of variances to estimate the degree of defocus in the images and the differentiation of images (Liang et al., 2022). The table shows the algorithms to be used and the methodology on which it is based.

Table 1. Comparative table of the algorithms to be used

	Algorithm to be used	Methodology	Based on
1	Absolute gradient count (AGC)	Differentiation between pixels	Image differentiation
2	Tenengrad function	Edge detection	First derivative and convolution masks
3	Variance of the gradient magnitude of the Sobel operator	Variance	Image contrast
4	Laplacian function	Edge detection	Second derivative and convolution mask
5	Variance of the gradient magnitude of the Laplacian operator	Variance	Image contrast
6	Image entropy function	Entropy	Histograms

Source: Own elaboration

To be able to develop all the algorithms mentioned above, it is necessary that the image is in two dimensions; with this the value of each element of the matrix (pixel) can be represented as $f(x, y)$. This means that the value of each pixel is at position (x, y) of the matrix. The size of the matrix is determined by M x N, where the value of M indicates the number of rows and N indicates the number of columns. In addition, some of the mentioned algorithms work with the Otsu Threshold Value (U). Each algorithm considers a value of degree of focus (VGE) or also called focal value of an image.

In order to select the algorithm to be used, first the images were acquired, then the algorithms were developed and finally tests and comparisons were made. Following these steps will allow to find the best algorithm that is able to find the best focused image.

The first step is the transmission of the microscope image to the computer, for which a Halion M304 webcam and an Olympus CX31 binocular optical microscope were used. The webcam has been coupled to a lens of the microscope, in this way, it is possible to observe and capture the images of the sample in the computer, to be used later in the proposed algorithms. In order to capture the image, there are two ways to do it: The first is to do it manually, i.e., locate the sample and capture the image in the field you want to analyze, then a name is given to be able to identify it. This procedure is the one that will be used to perform the tests. The second is the automatic mode, in this way it is only necessary to place the number of fields of the sample to be analyzed. For the first image capture, the sample must be manually focused. The microscope has a hardware that allows the displacement of the microscope axes and a programming that allows the capture of images in each field in an automated way. In addition, for each field, pictures are taken at different heights and stored.

In the present work it is required to use software that allows to perform digital image processing. Among some of the softwares mentioned are Artensoft Photo Editor, Ultra and Matlab. The latter was chosen since I know that I had a previous knowledge of its use and because of its relative ease in programming. When developing the algorithms in this software, the program should not be too long but as short as possible, so that the time it will take to deliver the result does not influence too much. Also, it is necessary to add filters that allow to eliminate the noises present in the images. The six algorithms shown in Table 1 were used.

In order to determine the best autofocus algorithm, a comparison between all the proposed algorithms is needed. The parameters necessary to perform the comparison are: degree of focus presented by the image, and algorithm execution time. For the choice of the autofocus algorithm, three tests were performed. First, an image was created and three distorted images were generated from it. The four images were read and analyzed for each program. The programs developed deliver as a response the VGE of each of the images. In each of the programs these values were compared; verifying that the first image (the created image) presents the highest VGE. Secondly, an image of a photograph was taken and five different types of noises were applied: Gaussian blur, lighting effects, melting, HSV and RGB. These noises were applied because they are the most similar to the noises that occur when reading through the digital camera. The GIMP program was used to perform this procedure, which allows applying these types of noise at different levels to the images.

In each of the tests, the VGE of the images were compared, verifying that the original photograph presents the highest degree of focus. Finally, eight images taken from the microscope of the Microbiology laboratory of the Hospital Dos de Mayo were analyzed, one of which is in focus and the rest out of focus. As more than one algorithm was found to be able to capture the focused image, then the execution time was taken into account. The program that obtained the result in the shortest time was considered; this is also an important factor because it is applied to reality due to the number of samples to be analyzed.

3. Results

Four tests were performed to find the best autofocus algorithm. In the first test, comparisons of the values of degree of focus (VGE) of the original image and its modified images are performed. In the second one, the images that have been added with different types of noise are evaluated, and the comparison of their VGE with their original image is performed. In the third, a test was performed with sample images taken from the microscope comparing the VGE of the focused image with the VGE of defocused images. Finally, a test was performed in which the algorithm was selected.

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3.1. Test 1 of simulated image analysis

An image of size 400 x 400 pixels was created (Figure 1a), which is taken as a standard image, and distorted using the GIMP-2 program by applying Gaussian noise of blur radius (in pixels) 5(Figure 1b), 20(Figure 1b) and 80(Figure 1d).

Figure 1. Images of the first test

a

b

c

d
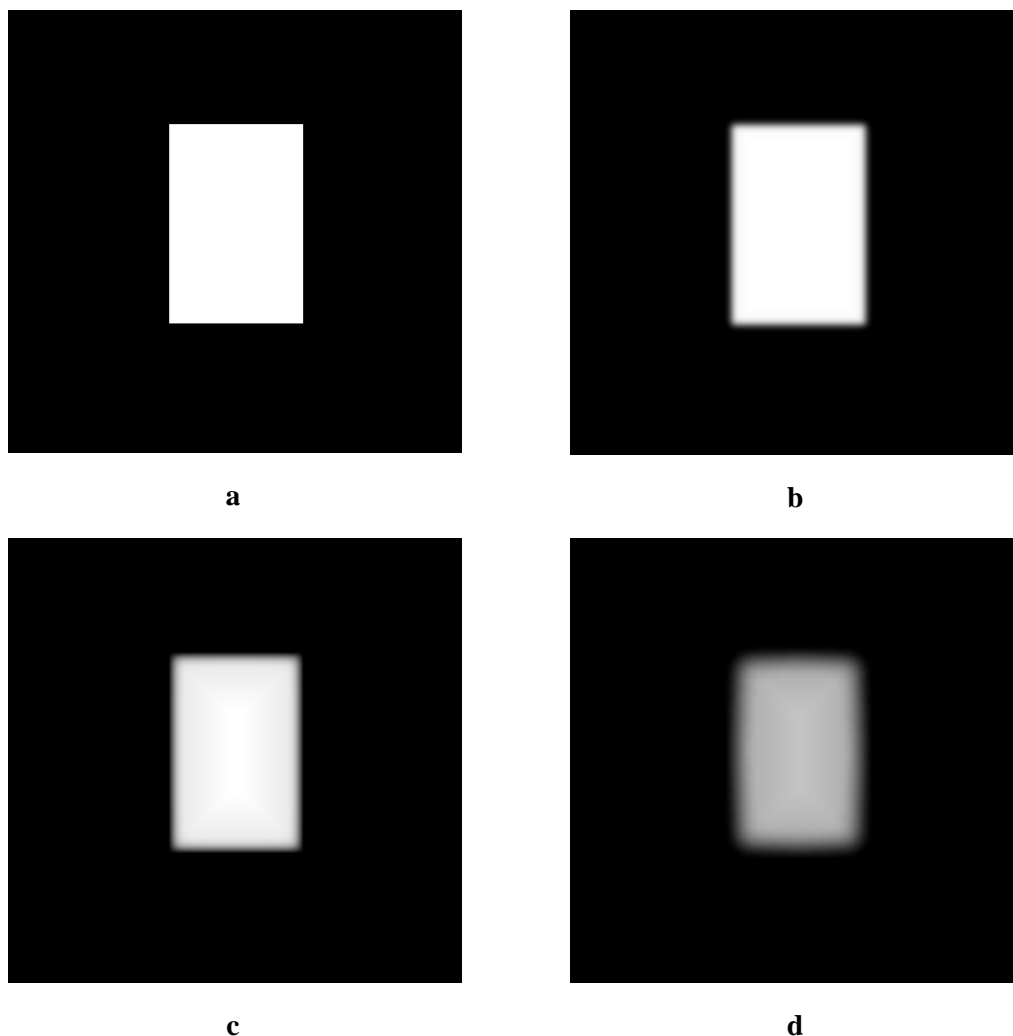
Source: Own elaboration

These images were analyzed using the developed algorithms to determine the VGE of each image. The Otsu threshold value is a comparison parameter for the first three algorithms presented in Table 2, the remaining algorithms do not require the aforementioned threshold value. Figure 1a has an Otsu threshold value of 0, Figure 1b of 121; Figure 1c of 112 and Figure 1d of 79. For this case the following VGE results were obtained for the four images in Figure 1 as shown in Table 2.

Table 2. VGE of the images of the first test after applying the developed algorithms.

	Algorithm to be used	1.a	1.b	1.c	1.d
1	Absolute Gradient Count (AGC)	1000	0	0	0

2	Tenengrad function	$8{,}43 \times 10^8$	$1{,}32 \times 10^8$	$1{,}11 \times 10^8$	0
3	Variance of the gradient magnitude of the Sobel operator	$8{,}70 \times 10^8$	$1{,}46 \times 10^8$	1.25×10^8	0
4	Laplacian function	33728	15515	13869	4360,7
5	Variance of the gradient magnitude of the Laplacian operator	1294150	57628	39231	2204.4
6	Image entropy function	0.52383	1.1429	1.1715	1.4836

Source: Own elaboration

It is observed that both the entropy function fails to find Figure 1a as the best focused image, this occurs because the images shown have more black level (gray value 0) than white (gray value 255). And the entropy function depends on this gray value. However, this algorithm will be used in the following test to observe its behavior. The rest of the algorithms did manage to obtain higher VGE value in Figure 1a.

### 3.2. Test 2: Noisy photo analysis

In order to perform this test, a photograph, called a master image, of size 768 x 1024, was taken as a reference, and then noise was added to it. In order to perform the previous step, it was necessary to use the GIMP-2 software, which allows to modify the image by adding different types of noise, blurs, etc. The distortions that were added to the image are Gaussian blur, lighting effects, HSV noise, RGB noise and melting effect.

Subsequently, each of the algorithms indicated in Table 1 was applied to each of the images to which the different types of noise were added. Finally, the VGE were obtained and the results obtained were analyzed, resulting that the Tenengrad function and the Sobel Operator algorithms

3.3. Test 3: Analysis of images taken from the microscope

This test was performed using a computer, a webcam and an Olympus CX31 binocular microscope. The webcam has been attached to a lens of the microscope. First, a sample, which is used for bacillus detection, is placed on the microscope stage and then the image is adjusted until an image is found to be in focus. The image is then captured and stored in the computer. Then, the stage height is varied and the observed images are captured and stored in the computer. The images taken are shown in Figure 2.

Figure 2. Images taken from the microscope

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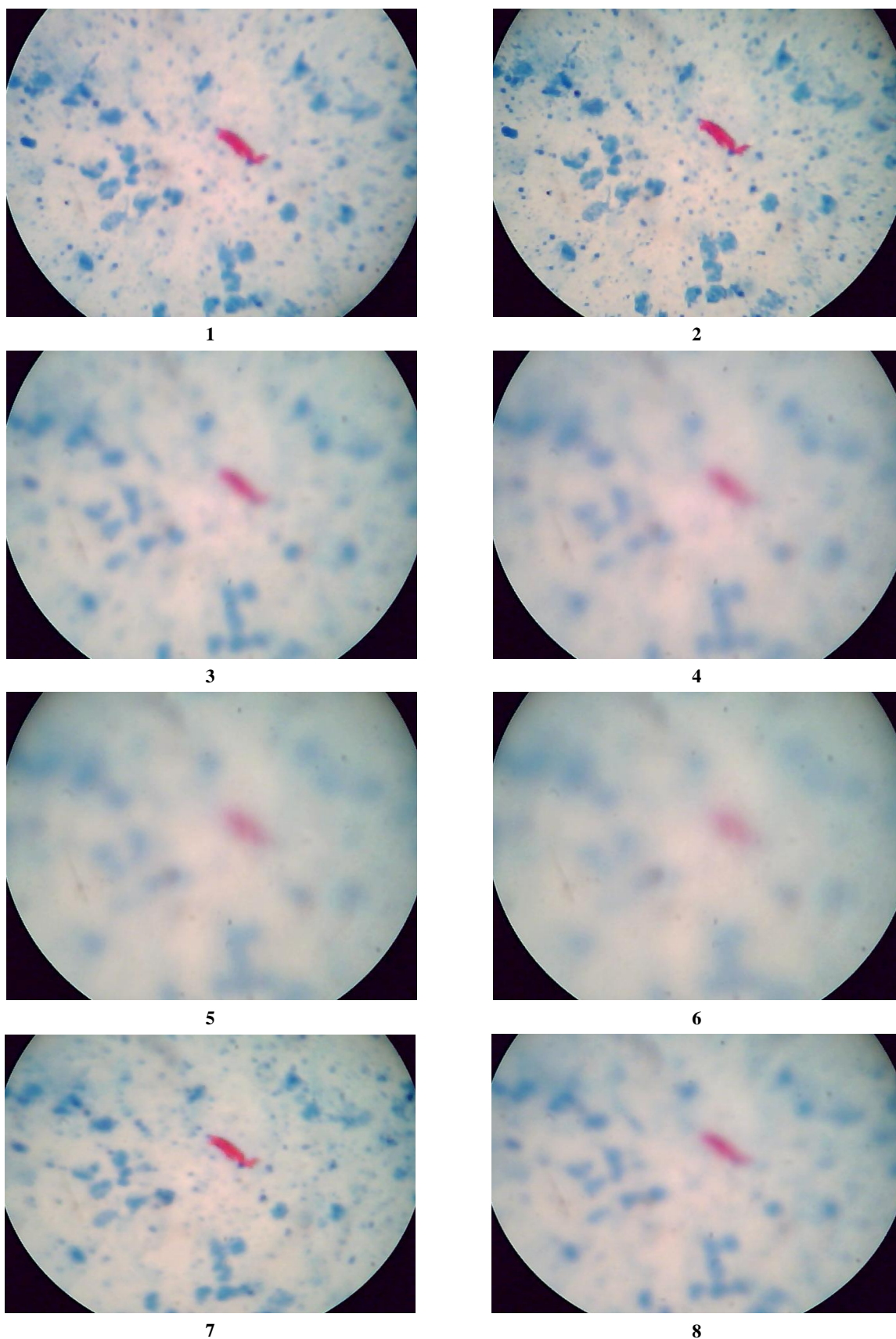
Source: Own elaboration

The images shown above were analyzed with all the algorithms proposed. The results obtained are shown in Table 3.

Table 3. VGE of images taken from the microscope

	Algorithm to be used	2.1	2.2	2.3	2.4	2.5	2.6	2.7	2.8
1	Absolute gradient count (AGC)	86	83	131	142	152	142	110	134
2	Tenengrad function	162.9 x107	155.5 x107	154.3 x107	153.4 x107	135.4 x107	138.7 x107	142.9 x107	142.6 x107
3	Variance of the gradient magnitude of the Sobel operator	145.2 x107	131.1 x107	144.1 x107	144.6 x107	128.1 x107	131.4 x107	130.4 x107	133.2 x107
4	Laplacian function	103010.67	110629.67	114702.67	116529.67	110878.67	111983.33	109436.67	112739.33
5	Variance of the gradient magnitude of the Laplacian operator	867275.28	1170361.39	1419653.39	1502952.33	1276885.94	1328924.33	1162089.00	1354007.28
6	Image entropy function	6.83	6.73	6.67	6.64	6.70	6.64	6.76	6.70

Source: Own elaboration

After observing the results, it can be seen that only the Tenengrad, Sobel operator gradient magnitude variance and image Entropy algorithms have the highest VGE value in figure 1, which was the focused image. At the end of the first three tests, the Tenengrad, Sobel operator gradient magnitude variance algorithms were able to determine the focused image in all the situations in which it was presented. Thus, these two algorithms were selected for being the most reliable when trying to find the best focused image in the presence of noise and in real laboratory samples. As in both cases the focused image was found to be the one with the highest VGE, then one more test had to be performed in which the point to be compared was the execution time of the program; that is, the time it took to find the values of the degree of focus.

3.4. Execution time of the selected algorithms

To find the execution times whose result is in seconds, only a few commands (tic/toc) were added to the original program of each algorithm developed in the Matlab software. When performing this procedure, a single value was not always obtained, but varied from 0.01 to 0.8 seconds. Therefore, a repetition of 50 times was performed for each image in each test and an average value of the execution time was found for each of them. The results obtained are shown below.

Table 4. Results of the time test

	Algorithm to be used	2.1	2.2	2.3	2.4	2.5	2.6	2.7	2.8
1	Tenengrad function	0.139	0.1378	0.1348	0.138	0.1345	0.1331	0.1366	0.1352
2	Variance of the gradient magnitude of the Sobel operator	1.2482	1.2335	1.2212	1.2299	1.2214	1.2294	1.2341	1.2288

Source: Own elaboration

The results show that the Tenengrad algorithm performs the test in a total time of 1.0890 seconds and the Gradient Magnitude Variance Sobel (VMS) algorithm performs it in 9.8465 seconds. This equates on average per image to 0.1361 seconds for the first case and 1.2308 seconds for the second case. Therefore, the Tenengrad algorithm was selected because it takes 1.0947 seconds less than the other algorithm. This is 9.04 times faster than the VMS algorithm.

The Tenengrad algorithm is the most suitable algorithm to implement in the autofocus software. This test has been relevant because in reality this procedure has to be performed many times. For example, by analyzing a sample in 100 fields and in 20 different height positions for each one; then, the value of degree of focus will be found in 2000 images. This means that the time taken by the first algorithm would be 2000x 0.1361 seconds equivalent to 272.2 seconds (4.53 minutes) for each sample. Compared to the second algorithm, the second algorithm takes 2461.6 seconds (41.02 minutes).

In conclusion, after performing the tests to find the best autofocus algorithm, the Tenengrad algorithm was chosen because it was able to identify the focused image in each of the first three tests and also because its execution time is faster than the other selected algorithm.

4. Conclusions

After a comparison of 6 algorithms in which different tests were performed, the Tenengrad algorithm was selected because it has a higher efficiency in the detection of the best focused image and also because it is the fastest algorithm compared to the Gradient Magnitude Variance algorithm of the Sobel operator.

The Tenengrad algorithm was applied to a sample of laboratory bacilli and its correct operation was verified. The applied program allows an easy adaptation. Therefore, a base program has been obtained with which it is possible to carry out future research work requiring the use of this program. The processing time required to recognize the best focused image is 9 minutes compared to that required for a manual process which varies between 8 and 15 minutes. The Entropy algorithm of an image had been proposed as it presented great expectations for finding the focused image, however, it did not pass the first test and had to be discarded.

It is recommended to use the software installed on the microscope to compare the variation of the focus degree value of a focused image with respect to the gradual movement of the microscope knobs. In addition, to develop the selected algorithm using a different program that allows to analyze images in real time, such as 'Mathematica', which is used by international companies and has been used in cancer diagnosis. Also, to use a new algorithm which works with frequencies and uses the Fourier Transform for image analysis. This would allow a faster response. Finally, to perform the same procedure for other types of tests such as blood tests, since in the present work only samples intended for tuberculosis analysis were used.

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