Acquisition of digital images and identification of *Aedes aegypti* mosquito eggs using classification and deep learning

Pedro Saint Clair Garcia*, Rafael Martins†, George Luiz Lins Machado Coelho†, Guillermo Cámara-Chávez*
*Computer Science Department
†Biology Department
Federal University of Ouro Preto
Campus Universitário Morro do Cruzeiro
Ouro Preto - MG, Brazil

***Abstract*—The mosquito *Aedes aegypti* can transmit some diseases, which makes the study of the proliferation of this vector a necessary task. With the use of traps made in the laboratory, called ovitraps, it is possible to map egg deposition in a community. Through a camera, coupled with a magnifying glass, are acquired images containing the elements (eggs) to be counted. First, the goal is to find pixels with a similar color to mosquito eggs; for that, we take advantage of the slice color method. From these already worked images, a process of transfer learning with a convolutional neural network (CNN) is carried out. The intention is to separate which elements are eggs from the others. In 10% of the test images, the count performed by the model, and the ground truth of the number of eggs was considered weakly correlated. This problem occurs in images that have a high density of eggs or appear black elements that resemble mosquito eggs, but they are not. For the remaining 90% of the test images, the counting was considered to be perfectly correlated.**

I. Introduction

One way to transmit diseases on the planet is through vectors. Each year there are more than one million vector-borne pathologies, such as malaria, dengue or yellow fever. The dengue virus [1] was first isolated in 1943 by Ren Kimura and Susumu Hotta and later in 1944 by Albert B. Sabin and Walter Schlesinger [2]. The scientific community assumes that dengue viruses have evolved into non-human primates and then jumped from those primates to humans in Africa or Southeast Asia between 500 and 1,000 years ago [2]. According to the World Health Organization [3], insect-borne diseases account for 17% of all infectious diseases, Aedes Aegypti is one of the most distributed mosquitoes in the world and has considerable medical importance as a vector of dengue fever and yellow fever.

The first records of the disease in Brazil were in 1916 [4], in the city of São Paulo, SP, And 1923 in Niterói, RJ, [5]. The first evidence of a big epidemic in Brazil, with 11 thousand infected, was in 1981-1982, in Boa Vista, at the time Federal Territory of Roraima [6].

In Brazil, the main diseases that can be transmitted by this vector are Dengue [7]–[10], Zica [11], and Chikungunya [12]. These diseases are transmitted during the mosquito cycle. After egg deposition, they hatch, turn into larvae, and reach adulthood. During this process, the eggs are placed in places containing standing water on millimetric surfaces in vessels, tires, bottles, etc [7], [8]. One of the most efficient ways of evaluating, detecting and monitoring the presence of this vector is through the counting of eggs present in a given location. In regions with medium proliferation density, the most efficient alternative is the use of ovitraps [13], which are traps for the mosquito to deposit its eggs, facilitating the collection of information on the proliferation of the transmitter [14]. After this egg deposition, images can be generated and analyzed. In most cases, it is done by a manual process, where time is demanded and assertiveness is inefficient, another possible way would be to treat the image automatically.

In this context, there are ways to count the number of eggs from an image. A classic method is through segmentation [15]–[17]. Usually, the images are acquired in RGB format. In most cases, they are used in this form, but in some research works, they are transformed into another representation before segmenting. With the image in a suitable color system, the segmentation process is carried out, where it is possible to use algorithms based on thresholding [15], [16], a connection between neighbors [16], evolutionary programming [16], entropy [17], among others. The purpose of these algorithms is to separate the pixels that belong to the eggs from others. In some cases, a filter is run to eliminate some pixels belonging to objects smaller than a predetermined minimum size. This is necessary to eliminate some noise and impurities in the image. Finally, a relation between the total number of pixels belonging to the eggs and the average size of a unit results in the number of eggs present in the image.

Another way to facilitate this counting process is through applications that can interact with the user through graphical interfaces [18], [19]. In this software, some operations are made automatic, but the user can modify some settings such as the threshold value and the minimum size of a unit egg area. In these environments, it is also possible to visualize the counting process, which allows the operator to analyze whether all objects have been found, whether to remove some element from the count or to add.

In the year 2012, Alex Krizhevsky, Geoff Hinton, and Ilya Sutskever conquered ImageNet contest. Since then, CNN's have become standard in object detection and classification in images [20]. In these days, in some cases, CNN's are better than humans in some categorization processes. In this same line, some papers describe a way of detecting objects, for example, the presence of malaria in images [21] or biological parasites in feces, bloodstream or human saliva [22]. The authors analyze the efficacy of detecting the malignant parasite, raising the numbers of parasites in the images as a whole, this is enough to tell if the individual has the disease. In [23], the authors used an image representation technique, a segmentation and filtration process, and the proportion in pixels of the egg unit size. In sequence, feature extractors are applied along with learning techniques to identify overlapping eggs, information used in the counting process that significantly increases the accuracy of the results. Another way to obtain a result is by making a protocol for a rapid estimate of the number of aedine mosquito eggs [24]. Thus, there is an important technology to be studied, where the objective is to develop an automatic method to identify the eggs present in the image using a process based on color system and convolutional neural networks (CNN).

To facilitate the control and proliferation of the Aedes Aegypti mosquito, this research work proposes a simple image acquisition process that can be replicated in any laboratory, with minimal infrastructure, the construction of a base of images to be studied and amplified with the time, besides proposing a tool based on deep learning approach to aid ecological studies and population modeling, resulting in a better understanding of the epidemiology caused by Aedes Aegypti or to help formulate better control strategies. In addition, an automatic method will generate greater reliability and better accuracy in statistical analyses.

II. Materials and Methods

The methodology is based on egg collection through ovitraps, in a process of aquisition image and in the model denominated Decision Tree Classifier with CNN. All of these processes will be described here.

A. Ovitraps

Egg deposition was performed through traps called ovitraps. This method for egg trapping is listed among the dengue vector monitoring methodologies advocated by the World Health Organization. It is currently considered to be the most efficient technique, especially when egg density is not high and has a low operating cost [8].

The traps were made using plastic containers of black-frosted color with a capacity of 500 mL, an amount of water is poured into the vessel and, a wood fiber is attached to the vessel. Figure 1 shows the equipment. 1

The ovitraps are scattered a community. After a certain period of time, they are collected and taken to the laboratory, undergoing a drying step and are already suitable for the counting process.

Fig. 1. Assembly of Ovitraps used to capture mosquitoes' ovideposition.

B. Image Acqquisition

The acquisition of images is done through laboratory assembly. A *"Canon PowerShot A650 IS, Image Stabilizer AiAf"* camera is used, coupled to a microscope magnifier with a 40-fold magnification lens, supported by a tripod as shown in Figure 2.

Fig. 2. Image acquisition system

The camera has been configured and the parameters must be protected against unwanted modifications. The magnifying glass has four lighting settings, only the upper light was used with minimal intensity to reduce the number of shadows. The main lens configuration was defined by varying the coupling height and focal length so that the image was as sharp as possible. The images are taken with a time interval of three seconds, this prevents blurring of the image because the image will be captured automatically.

C. Decision Tree Classifier with Convolutional Neural Network

A database was created with the objective of adapting to any laboratory, requiring a camera, a magnifying glass and a tripod. Once the ovitraps were scattered and collected to the laboratory, the image acquisition where low, medium and high-density egg samples were obtained, see Figure 3.

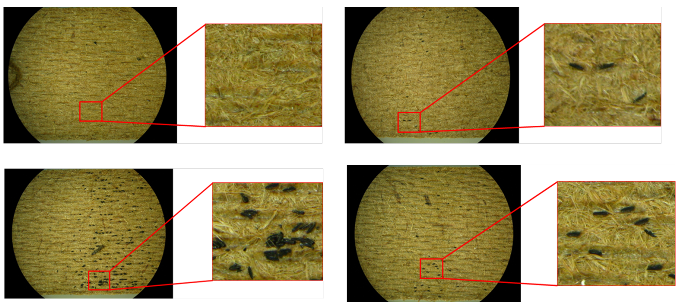

Fig. 3. Figure showing the various forms of egg deposition in ovitraps. In the upper left corner, it does not contain eggs. In the upper right, low density of eggs. In the lower rigth, medium density. In the lower left, high density.

Some of them had imperfections, that is, adverse elements that are not eggs, pallets or field delimiting the lens, according to Figure 4.

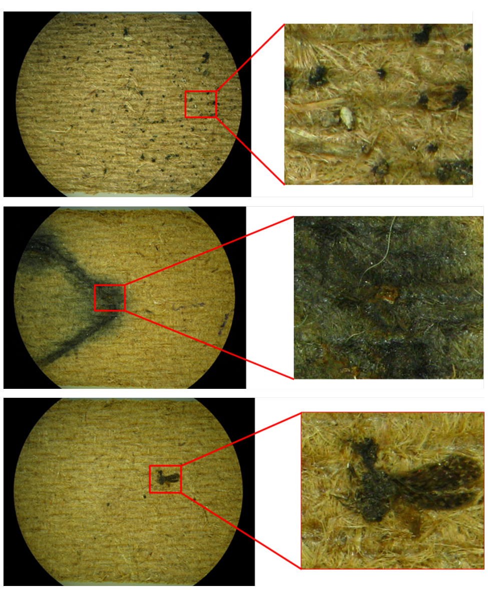

Fig. 4. Figure showing the various forms of ovitraps imperfections. In the upper, imperfections caused by sand grains sedimented on the palette, probably by influence of the wind. In the middle, imperfection caused by the pallet attachment clamp on the edge of the pot. In the lower, imperfection caused by some insect that got stuck in the palette.

Images are extracted in RGB format. First, we execute a palette segmentation algorithm to remove black areas around the interest region, these black areas were originated during the image acquisition with the magnification lens. Figure 5 shows the result of the extraction of the area of interest.

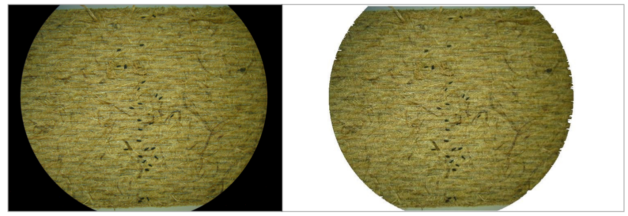

Fig. 5. Extraction of the area of interest. On the left, the image shortly after the acquisition process, a black part can be observed which was caused by the adaptation of the camera on the magnifying glass. On the right, the black part is transformed in white to avoid a color that resembles the egg.

The collected database was generated from the process of ovitraps and acquisition of images described in Section 2. The images of the database were collected during the year 2017. Every two months, a number of samples were taken in our laboratory, so six samples were obtained, which generated 425 images, containing approximately 14,500 eggs.

The database contains all types of egg densities combined with some forms of imperfections. The images have a 3000×4000 pixel resolution, and a macro setting to improve sharpness. The database was divided into 300 images to train, 30 images to validate and 30 images to test. Only images containing eggs were considered.

After extract the interest area, we collected examples of pixels belonging to the eggs and example of pixels that do not belong to the eggs, the Figure 6 (synthetic image) shows examples of eggs for extraction of positive pixels. From each pixel the components of the normalized RGB color system and CIELAB (L* a* b*) were extracted by creating the following information tuple: $(R, G, B, L, a, b, class)$, where:

- L, a, b belong to the CIELAB color system.
- R, G, B belong to the normalized RGB color system;
- $Class$ defines whether the pixel belongs to the egg (0 or 1).

The standard RGB and L * a * b * color spaces have been chosen because they represent the color characteristics and brightness of the image.

Fig. 6. Synthetic image used as examples of eggs for extraction of positive pixels.

With this information, a decision tree classifier was used to classify each pixel as belonging or not to the egg. After the built classifier, it is possible to generate a mask of the images by separating the pixels that supposedly belong to the egg. From these masks and human analysis new positive and negative examples are generated to feed the tree. After building the tree a refinement is performed to eliminate objects that are less than a minimum size. The generated result, together with the ground truth already built, is fed into a Convolutional Neural Network. With this formatted input, the weights of a 10-class trained network [25] were used as the initial weights in our proposed CNN. The network architecture contains the following sequence of layers.

1) Data input initial layer with zero center normalization
2) Convolutional layer with 32 filters of size $5 \times 5 \times 3$
3) A RELU layer
4) 3×3 max pooling layer
5) Convolutional layer with 32 filters of size $5 \times 5 \times 3$
6) A RELU layer
7) 3×3 max pooling layer
8) Convolutional layer with 64 filters of size $5 \times 5 \times 3$
9) A RELU Layer
10) 3×3 max pooling layer
11) 64 Fully connected layer
12) A RELU layer
13) Fully connected layer with 2 neurons
14) A softmax layer
15) Classification layer with 2 classes

The training was based on stochastic gradient descent with momentum, and the input data is the result of slice color process with a list of ground truth to each image. The learning rate has been reduced by a factor of 0.1 every epochs. The training has last for 100 epochs, and each iteration has used a mini-batch with 128 observations. The momentum was set to 0.9 and initial learning rate of 0.001.

The CNN classifies the data in two classes: eggs and background, trains an R-CNN (regions with convolutional neural networks) based object detector. In addition it generates to the bounding box of the detected egg.

Finally, with the trained net, the eggs are identified, as shown in the Figure 8.

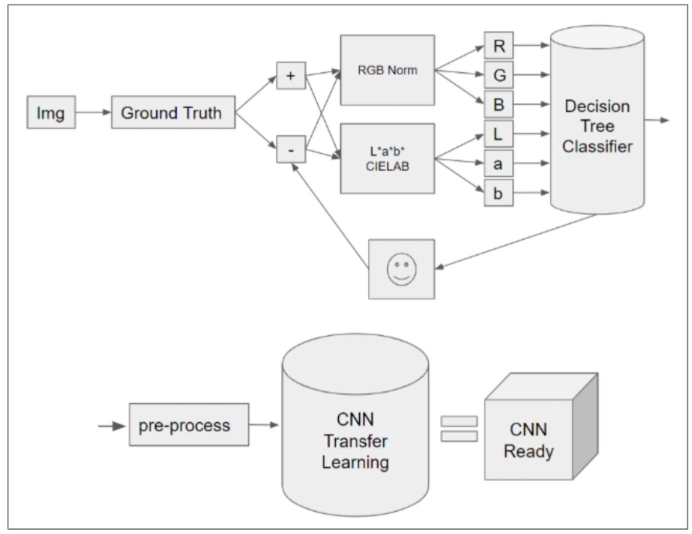

Fig. 7. Network acquisition process to identify dengue mosquito eggs in digital images. The process goes through 3 main stages; a) extracting information for negative and positive examples to the tree (classifier entry); b) classification and generation of the mask (input to the network) c) Detection of the egg from the transfer of learning in a convolutional neural network.

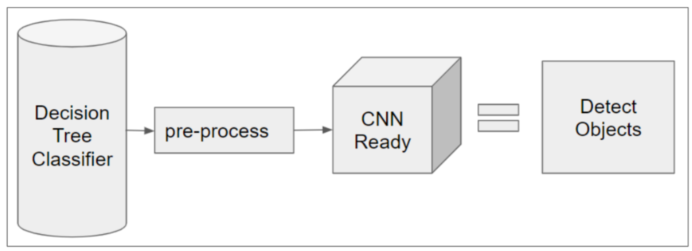

Fig. 8. Egg identification process. The image passes through the classifier filtering is used to eliminate small objects. Finally, the eggs are identified through the already trained network.

III. Results

In this section, we are going to compare the performance of our CNN approach with other methods in the literature used for counting mosquito eggs. Some of these methods transform the images from RGB color space to HSV, HIS, CMY [26]. First, we are going to present our dataset, then the literature methods used for comparison and finally, we present the result performance of our approach.

The methods used for comparison are based on threshold methods. We evaluate different thresholding approaches to find the best one. For example, we test the Otsu method [27], maximum entropy [28] and adaptive threshold [26]. Unfortunately, none of them adapt well to conditions were the images were captured, confusing penumbra pixels with eggs.

The model of the literature, that best adapts to the conditions of the laboratory was through the Red channel, of the RGB color space, with a manually configured threshold [16]. In parallel, an artificial image was generated, see Figure 6, Through this figure is possible to identify the average number of pixels in an egg. After segmentation, objects that are one-third smaller than the average size of an egg are eliminated. Thus, having the total number of pixels belonging to the eggs and the mean area of the eggs, the number of eggs in the image is calculated by dividing the total number of pixels by the mean average size. The criteria adopted for classic methods just count the number of pixels with a color similar to egg color, they do not take into account if these pixels are together or spread around the image, occupying regions, even much smaller than eggs.

As a condition demanded by the biologist, the entire palette must appear in the image because sometimes mosquitoes lay eggs close to the borders of the palette. As a consequence, in all images, an extra non-palette region is included in the image, as highlighted in Figure 9. For the classical model this becomes a problem because, due to light conditions, this part of the image gets darker, confusing these dark pixels with egg pixels.

Classical methods just count the number of pixels with

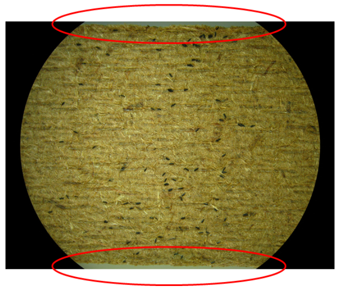

Fig. 9. Edge of the palette that should be consider in of the counting process.

a similar color to mosquito egg, they do not detect them. Consequently, we do not have any information about the number of false positives, false negatives, and true positives.

On the other hand, analyzing the count of the number of eggs based on the number of pixels, it is possible to correlate the number of real eggs in the image with the number extracted by the model, through a linear regression [29]. To trace the trend line, we set $b = 0$ (linear coefficient equal to zero) to identify the similarity of one count with another. $(Y = C * X)$. R is a linear correlation factor between the two counts. The more the value of C and R is closer to 1 the more assertive will be the count made by the model, the X and Y axes are the counts performed by the ground truth and the model respectively. R for this analysis was 0.2087, which gives a R^2 equal 0.45 and a C of 0.5177 which results in a moderate correlation.

In Figure 10, we show the relation between the number of eggs detected by the classic model and the true count.

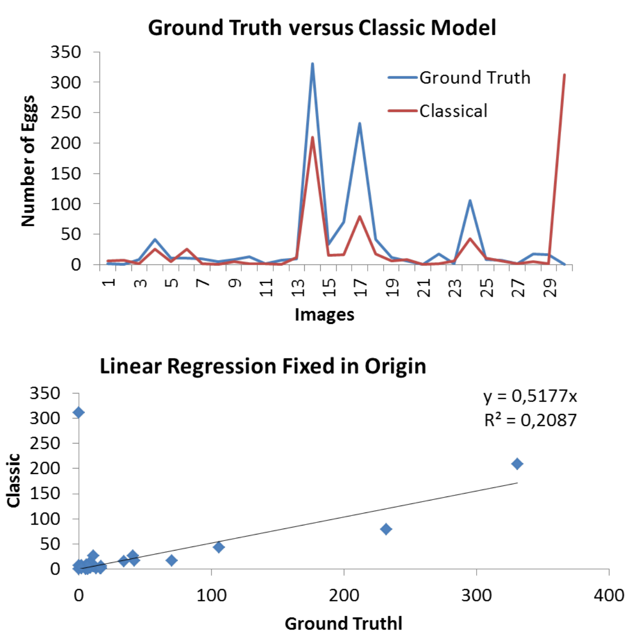

Fig. 10. Comparison between classical model and manual counting

Finally, we present the results of the proposed model. First, images are processed using the decision tree classifier method. After the image pre-processing, we train a CNN. Then, we evaluate the performance of our approach, not only by counting the number of eggs, but also by detecting them in the image. We use the Jaccard coefficient [30], also known as intersection over union, to measure how much the location of the model bounding box differs from its real location. This index shows that 91 % of the eggs are found, considering an intersection rate on the union of 0.3, in validation images. Therefore, the proposed network can identify most of the eggs.

Figure 11 shows precision and recall for each test image. It was considered as detected egg (true positive), if it has a Jaccard coefficient over 30% , otherwise, it is considered as a false positive. We can see that most of the images have a recall and precision over 0.7. Our proposed approach is more appropriate than classical models because besides evaluating the color, it also perceives characteristics of the eggs.

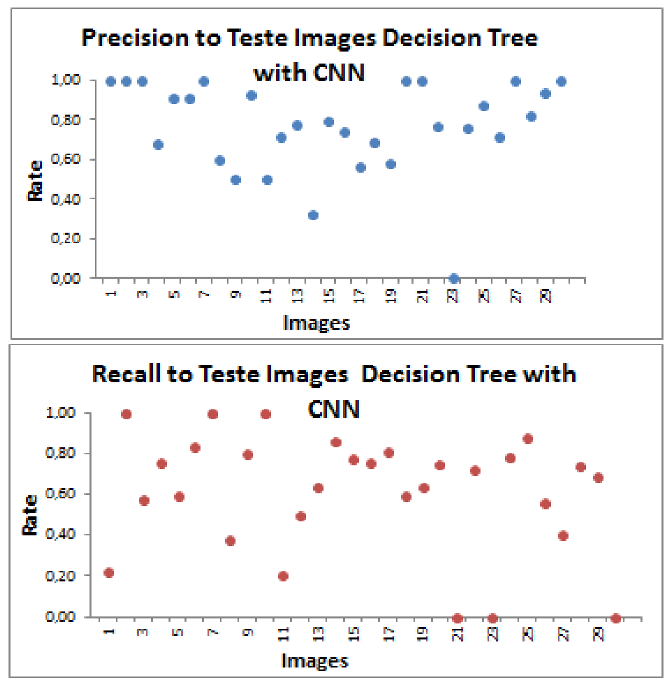

Fig. 11. Precision and Recall for the decision tree classifier mask generated with CNN

Figure 12 shows the correlation between the counting performed by our model with CNN and the real count, where C is 0.5468, R^2 has the value of 0.65 and R is close to 0.86 which can be considered as a strong correlation.

Another problem that makes this task a challenging one, is the similarity between eggs and other types of objects like other insect eggs, boulders of black color, the pattern of the palette, etc.

To analyze the method using CNN, the precision versus recall curve and the ROC curve [31] were used. These curves are generated using false positive, false negative and true positive metrics. These parameters allow calculating the precision, recall, rate of true positives and true negatives, which are the

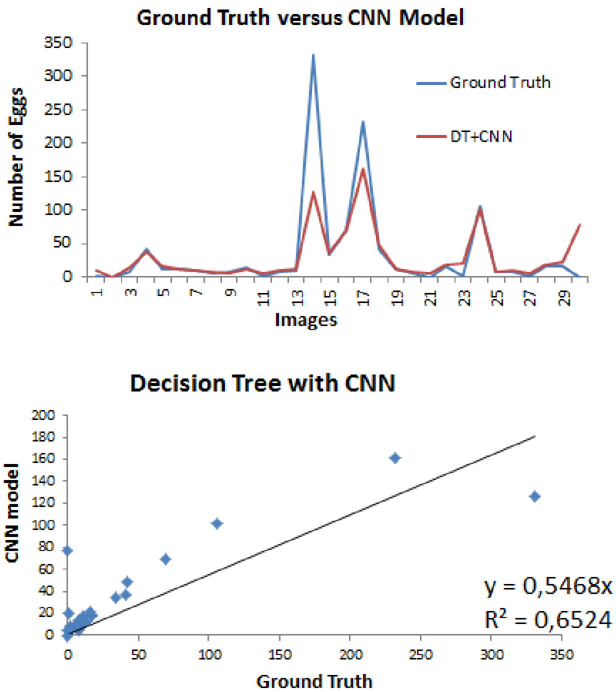

Fig. 12. Comparison between Decision Tree Classifier with CNN and manual counting

axes of the graphs, see Figures 13 and 15. Figure 11 shows that in both curves there is a drop in accuracy or true positive rate from a given point. This is due to the influence of 3 images on a total of 30 test images, see Figure 14. An image that contains 331 eggs only 126 were found, another with 232 was found 161 and finally, an image that does not have eggs was found 77.

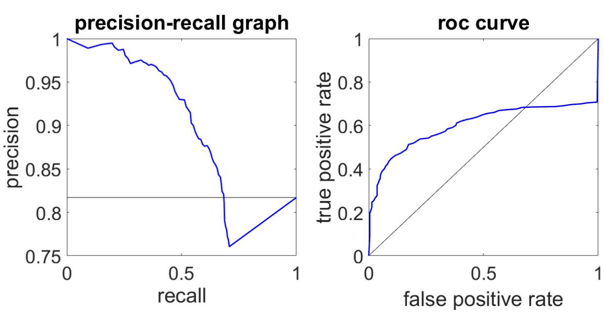

Fig. 13. ROC and Precision-Recall curve considering the entire test base.

By removing these three images from the set test there is a significant improvement in the curves analyzed, as can be seen in Figure 15. For this sample set, the objects found are actually the eggs to be counted, since both precision and recall have high rates.

For the correlation analysis, C was equal to 1.00008 R^2 of value 0.9469 and R near 0.9703 which can be considered as almost a perfect and equal correlation. See Figure 16.

With this analysis, it is possible to identify the images that

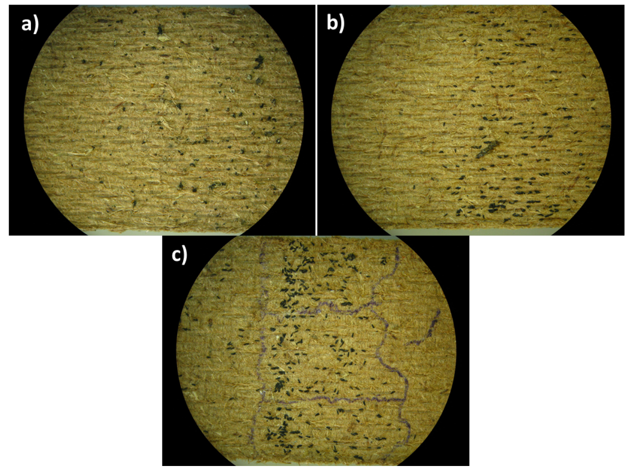

Fig. 14. Images with a high imprecision in count. In a) small stones of black color, in b) the has 232 and in c) has 331 eggs.

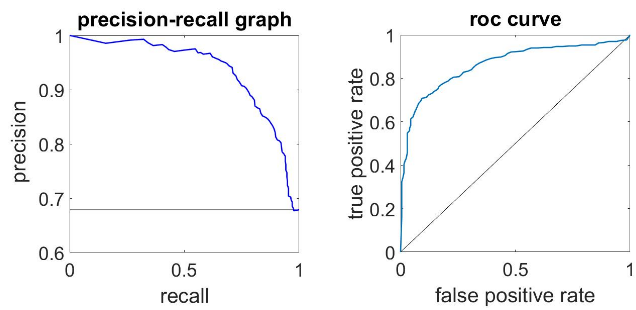

Fig. 15. ROC and Precision-Recall curve without the three images with a high difficulty in hitting the number of eggs.

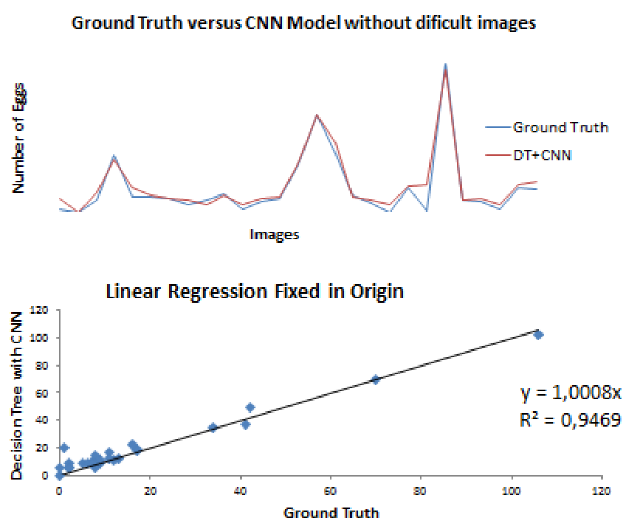

Fig. 16. Comparison between decision tree classifier color with CNN without the three images with a high difficulty in hitting the number of eggs and manual counting

have high density of eggs, and with this, high number of eggs superimposed one another.

IV. Conclusions

Research is currently underway to identify the main sources of disease spread, in some cases, it is necessary to have agility in the process. Therefore, the present work is a technical contribution to the epidemiology laboratory.

In the literature, there are some ways to count dengue mosquito eggs, but the basic processes did not adapt to the conditions found in the university laboratory. The process of acquiring the images is done in a specific way. The mask gerated by decision tree classifier is actually an adaptation of the existing models to the conditions found in the laboratory. Deep machine learning enhances these models, giving greater credibility to the work done automatically.

In this work, it is noted the great importance of knowing the deep machine learning, since the identification of objects in an image is something accomplished with some ease by this procedure. What makes this technique a highly effective tool and adaptable to the conditions encountered in a given problem. An important fact is that the more trained the machine is, the more it will increase its probability of success, it is enough that the data have standardization of the images, a high quantity, and variability.

Finally, dengue is a major Public Health problem that affects the whole world. In Brazil, climatic conditions have favored the proliferation of the mosquito, causing a significant increase in the number of cases in recent years. This new automatic way of counting brings more data reliability and agility in part of the dengue control process.

Acknowledgment

The authors would like to thank the Epidemiology Laboratory for sharing the work environment and providing a study object for the present study, CAPES and UFOP for their research support.

References

[1] CDC, "Centers for disease control and prevention. yellow book: 2018: Health information for international travel," *New York:Oxford University*, 2018.

[2] I. A. Lobo, "Dengue fever. cambridge, ma: Npg education. nature, scitable by nature education, a collaborative learning space for science." https://www.nature.com/scitable/ebooks/dengue-fever-22453392, June 2018. [Online]. Available: https://www.nature.com/scitable/ebooks/dengue-fever-22453392

[3] WHO, "Dengue control," http://www.who.int/denguecontrol/mosquito/en/, November 2017. [Online]. Available: http://www.who.int/denguecontrol/mosquito/en/

[4] M. Teixeira, M. Barreto, and Z. Guerra, "Epidemiologia e medidas de prevenção do dengue," *IESUS*, vol. 8, 12 1999.

[5] A. PEDRO, "O dengue em nictheroy." *Brazil-Médico*, vol. 1, 1923.

[6] C. H. Osanai, "A epidemia de dengue em boa vista, território federal de roraima, 1981-1982." Master's thesis, Escola Nacional de Saúde Pública, Rio de Janeiro, RJ., 1984.

[7] R. L. d. O. Rotraut A. G. B. Consoli, *Principais mosquitos de importância sanitária no Brasil*. FIOCRUZ, 1994.

[8] O. Forattini, "Ecologia, epidemiologia e sociedade," *Editora Artes Médicas, EDUSP - São Paulo*, 1992.

[9] K. M. Pepin, C. Marques-Toledo, L. Scherer, M. M. Morais, B. Ellis, and A. E., "Cost-effectiveness of novel system of mosquito surveillance and control, brazil," *Emerg Infect Dis*, 1914.

[10] K. B. F. Marzochi, "Dengue in brazil - situation, transmission and control: a proposal for ecological control," *SciELO Analytics*, 2013.

[11] K. G. Luz, G. I. V. dos Santos, and R. de Magalhães Vieira, "Febre pelo vírus zika," *Journal of General Virology,2*, 2015.

[12] M. R. Donalisio and A. R. R. Freitas, "Chikungunya no brasil: um desafio emergente," *Journal of General Virology*, 2014.

[13] R. W. Fay and A. S. Perry., "Laboratory studies of ovipositional preferences of aedes aegypti." *Mosquito News*, vol. 25, pp. 276–281, 1965. [Online]. Available: https://www.biodiversitylibrary.org/part/129217

[14] FIOCRUZ, "Avaliação de armadilhas para a vigilância entomológica de aedes aegypti com vistas á elaboração de novos índices de infestação." *NOTA TÉCNICA N.º 3/2014/IOC-FIOCRUZ/DIRETORIA*, 2014.

[15] C. A. B. Mello, "Automatic counting of aedes aegypti eggs in images of ovitraps," *Ganesh R Naik (Ed.)*, 2009.

[16] M. N. B. Portela, "Contagem automática de ovos de aedes aegypti em imagens de ovitrampas," *Escola Politécnica de Pernambuco*, 2009.

[17] G. G. ao, "A new algorithm for segmenting and counting aedes aegypti eggs in ovitraps," *31st Annual International Conference of the IEEE EMBS Minneapolis, Minnesota*, 2009.

[18] J. Gaburro, "Assessment of icount software, a precise and fast egg counting tool for the mosquito vector aedes aegypti," *Parasites e Vectors*, 2016.

[19] A. Mollahosseini, "Auser-friendlysoftwaretoeasilycount anophelesegg-batches," *BioMed Central*, 2012.

[20] A. Krizhevsky, I. Sutskever, and G. E. Hinton, "Imagenet classification with deep convolutional neural networks," in *Advances in Neural Information Processing Systems 25*, F. Pereira, C. J. C. Burges, L. Bottou, and K. Q. Weinberger, Eds. Curran Associates, Inc., 2012, pp. 1097–1105. [Online]. Available: http://papers.nips.cc/paper/4824-imagenet-classification-with-deep-convolutional-neural-networks.pdf

[21] J. Hung, A. Goodman, S. Lopes, G. Rangel, D. Ravel, F. Costa, M. Duraisingh, M. Marti, and A. Carpenter, "Applying faster r-cnn for object detection on malaria images," *Vis Pattern Recognit Workshops*, 2018.

[22] J. A. Quinn, R. Nakasi, P. K. B. Mugagga, P. Byanyima, W. Lubega, and A. Andama, "Deep convolutional neural networks for microscopy-based point of care diagnostics," *arXiv:1608.02989v1 [cs.CV]*, 2016.

[23] P. V. V. Paiva, "Contagem automática de ovos do carrapato rhipicephalus (boophilus) microplus em imagens microscópicas," *Universidade Gederal de Alagoas*, 2015.

[24] J. W. Mains, D. R. Mercer, and S. L. Dobson, "Digital image analysis to estimate numbers of aedes eggs oviposited in containers," *The American Mosquito Control Association*, 2008.

[25] mathworks. (2018, 01) Deep learning example: Traning from scratch using cifar-10 dataset. matlab mathworks. [Online]. Available: https://www.mathworks.com/examples/matlab/community/36291-deep-learning-example-traning-from-scratch-using-cifar-10-dataset

[26] R. C. Gonzalez, *Digital Image Processing*. Pearson Education, Inc, 2008.

[27] N. Otsu, "A threshold selection method from gray level histograms." *Automatica,11(285 296), pp. 23 27.*, 1975.

[28] C. Qi, "Maximum entropy for image segmentation based on an adaptive particle swarm optimization," *Applied Mathematics & Information Sciences*, 2014.

[29] S. M. B. B. Correa, *Probabilidade e Estatística*. Pontifícia Universidade Católica de Minas Gerais, 2006.

[30] R. Real and J. M. Vargas, "The probabilistic basis of jaccard's index of similarity," *DDepartment of Animal Biology, Faculty of Science, University of Malaga, Malaga 29071, Spain;*, 1996.

[31] J. Davis and M. Goadrich, "The relationship between precision-recall and roc curves," *Department of Computer Sciences and Department of Biostatistics and Medical Informatics, University of Wisconsin-Madison, 1210 West Dayton Street, Madison, WI, 53706 USA*, 2006.