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Chagas Parasites Detection through Gaussian Discriminant Analysis

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Abstract

Visual detection of Chagas parasites through microscopic inspection is a tedious and time-consuming task. In this paper we provide an algorithm based on Gaussian discriminant analysis for the task of Chagas parasites detection in blood sample images. We give details of the algorithm and our experimental results. With this method we obtained a true-positive rate of 0.9833 and a false-positive rate of 0.1563.

1 Introduction

According to the World Health Organization [18], Chagas disease, also known as American trypanosomiasis, is a potentially life-threatening illness caused by the protozoan parasite, Trypanosoma cruzi (T. cruzi). It is found mainly in Latin America, where it is mostly transmitted to humans by the faeces of triatomine bugs, known as 'kissing bugs' , among other names, depending on the geographical area. An estimated 10 million people are infected worldwide, mostly in Latin America where Chagas disease is endemic. More than 25 million people are at risk of the disease. It is estimated that in 2008 Chagas disease killed more than 10,000 people.

Some of the key factors of the Chagas disease are: 1) An estimated 10 million people are infected with Trypanosoma cruzi worldwide, mostly in Latin America, 2) Chagas disease is curable if treatment is initiated soon after infection, and 3) Blood screening is vital to prevent infection through transfusion and organ transplantation.

Chagas disease presents itself in two phases. The initial acute phase lasts for about two months after infection. During the acute phase, a high number of parasites circulate in the blood. In most cases, symptoms are absent or mild, but can include fever, headache, enlarged lymph glands, pallor, muscle pain, difficulty in breathing, swelling and abdominal or chest pain. In less than 50% of people bitten by a triatomine bug, characteristic first visible signs can be a skin lesion or a purplish swelling of the lids of one eye. During the chronic phase, the parasites are hidden mainly in the heart and digestive muscle. Up to 30% of patients suffer from cardiac disorders and up to 10% suffer from digestive (typically enlargement of the oesophagus or colon), neurological or mixed alterations. In later years the infection can lead to sudden death or heart failure caused by progressive destruction of the heart muscle.

1998 *ACM Computing Classication System*. I.5.1 [Pattern Recognition] Models.
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1.1 The Chagas parasite detection problem

Depending on the phase of the disease some tests can be useful for making a diagnosis. According to [10] the most typical tests used for the diagnosis of the Chagas disease are: blood culture, chest x-ray echocardiogram, electrocardiogram (ECG), enzyme-linked immunoassay (ELISA), and peripheral blood smears.

Among these methods, we are interested in the peripheral blood smears. A peripheral blood smear is basically a glass microscope slide coated on one side with a thin layer of venous blood. The slide is stained with a dye, usually Wrights stain, and examined under a microscope. Visual detection of the Chagas parasite through microscopic inspection of peripheral blood smears is a time-consuming and laborious process, however it is the most widely used technique for parasitemia determination.

1.2 Previous work

Up to date, one of the most effective ways of detecting the Chagas disease in its initial phase is through the ELISA test. However, some other methods have been shown to work with very high accuracy as well. In Ponce et al. [12] the Chagas Stat-Pak rapid immunochromatographic test is introduced. Such rapid method was tested on 5,998 serum samples taken from Central America. This method showed 0.996 and 0.999 sensitivity and specificity, respectively, which is a performance comparable to that obtained with ELISA.

On the other hand, Chagas detection using machine learning and automatic image analysis is, to the best of our knowledge, not yet formally studied as it can be deduced from the lack of publications on this topic. However, some studies relating machine learning methods and the T. Cruzi parasite have been done, as reported by Gonzalez et al. in [6]. In this work different machine learning methods were trained and used to automatically classify coding gene sequences based on their functional roles as adhesins, a type of protein. In such research work the feature vector employed for the machine learning algorithms consisted of a 47-dimensional vector of features extracted from nuclear protein sequences. Therefore, they never employed digital images or features extracted from digital images for their machine learning classification methodology. Alternatively Romero et al. [13] introduced an off-axis holographic microscope calibrated to automate the task of detecting the presence of Trypanosoma cruzi in blood through the measurement of the phase shift produced by such microorganisms on the transmitted wave front. The detection method is based on temporal differences between digital holograms and none machine learning method is employed. A similar work for the automated detection of Trypanosoma cruzi based also on temporal differences is given in [1].

Although none studies using machine learning and computer vision methods have been reported yet for the detection of the Chagas parasite, they have been extensively used for the detection of the Malaria parasite [9, 4, 16, 7, 3, 14, 15]. In [9] an image classification system is designed to identify malaria parasites present in thin blood smears and differentiate the species of malaria. Morphological and threshold selection techniques are used to identify erythrocytes and possible parasites present on microscopic slides. Image features based on color, texture and the geometry of the cells and parasites are generated, as well as features that mimic features used by human technicians. A two-stage tree classifier using back propogation feed forward neural networks distinguishes between true and false positives, and then diagnoses the species of the infection.

The study provided in [4] presents a method for quantification and classification of erythrocytes in stained thin blood films infected with Plasmodium falciparum (malaria parasite). The proposed approach is composed of three main phases: a preprocessing step, which corrects luminance differences. A segmentation step that uses the normalized RGB color space for classifying pixels either as erythrocyte or background followed by an Inclusion-Tree representation that structures the pixel information into objects, from which erythrocytes are found. Finally, a two step classification process identifies infected erythrocytes and differentiates the infection stage, using a trained bank of classifiers. Additionally, user intervention is allowed when the approach cannot make a proper decision. Four hundred fifty malaria images were used for training and evaluating the method. Automatic identification of infected erythrocytes showed a specificity of 0.997 and a sensitivity of 0.94. The infection stage was determined with an average sensitivity of 0.788 and average specificity of 0.912.

In [16] the images acquired from Giemsa-stained peripheral blood samples are transformed to match

a reference image color characteristics. Then, a parasite detector is built using a Bayesian pixel classifier that marks stained pixels. The class conditional probability density functions of the stained and the non-stained classes are estimated using the non-parametric histogram method. The stained pixels are further processed to extract features (histogram, Hu moments, relative shape measurements, color auto-correlogram) for a parasite or non-parasite classifier. A distance weighted K-nearest neighbour classifier is trained with the extracted features achieving 0.74 sensitivity, 0.98 specificity, 0.88 positive prediction, and 0.95 negative prediction values for the Malaria parasite detection.

Another technique is proposed in [7], based on pattern matching with parameter optimization and cross-validation against the expected biological characteristics of red blood cells. In a final stage, the parasitaemia measurement is carried out by partitioning the uninfected and infected cells using an unsupervised method. The obtained estimates were analyzed with respect to manually acquired results from professionals. Red blood cells detection resulted in precision and recall rates of 0.80 - 0.88 and 0.92 - 0.98, respectively. By using a training-based method, the precision and recall rates were improved to 0.92 and 0.95, respectively.

The first aim of the system presented in [3] is to detect malaria parasites by means of an automatic thresholding based on a morphological approach. A major requirement of the whole system is an efficient method to segment cell images. The methods employed make use of knowledge of the red blood cell structure, that is not used in other existing watershed-based algorithms. Two different classification methods are provided, one based on morphological operators and another one based on color histogram similarity.

In [15] a software called MalariaCount is presented. It automatically generates parasitemias from images of Giemsa-stained blood smears. The potential application and robustness of MalariaCount was tested in normal and drug-treated in vitro cultures of Plasmodium falciparum. The results showed a tight correlation between MalariaCount and manual count parasitemia values. These findings suggest that MalariaCount can potentially be used as a tool to provide rapid and accurate determination of parasitemia in research laboratories where frequent, large-scale, efficient determination of parasitemia is required.

In addition to detection algorithms, some authors have developed complete automated systems. In [8] an automated system to identify and analyze parasite species on thick blood films by image analysis techniques is presented. The system comprises two main components: (1) Image acquisition unit and (2) Image analysis module. The authors have developed an image acquisition system that can be easily mounted on most conventional light microscopes. It automatically controls the movement of the microscope in 3-directional planes. The vertical adjustment (focusing) can be made in a nanometer range (7-9 nm). Images are acquired with a digital camera that is installed at the top of microscope. The captured images are analyzed by an image analysis software which utilizes the state-of-the-art algorithms to detect and identify malaria parasites.

Other works related to the application of pattern recognition methods for images taken with microscopes are those used for the detection of special types of cells such as cancerous cells [17] and cervical cells [11]. In the first work the authors used the shape, size and texture of the cells to perform a classification, meanwhile in the second work a K-means clustering algorithm for designing binary tree classifiers is used, along with the Bhattacharyya distance metric.

2 Materials and methods

The results we provide in this paper were obtained using a typical pattern recognition methodology [2], using training and testing sets of images. We had available for our study a total of 120 color images of size 256×256. 60 of these images were specially selected to contain a Chagas parasite. Meanwhile the other 60 remaining images were selected to contain none parasites. Using these images we performed machine learning to get our basic classifier algoritm, which was then used for the more general Chagas parasite detection process. In the next section we provide the details of the Chagas detection process, leaving the details of the machine learning training and testing part for the next section.

2.1 Method overview

Our image analysis and detection process involves two main stages: (1) the image preprocessing and (2) a process for the detection of Chagas parasites.

1. Preprocessing the image consists in converting the original 256×256 RGB (Red-Green-Blue) images to 256×256 images formed exclusively with the information provided by the green component of the original image, resulting in what we call green-channel images.

2. The process of detecting Chagas parasites is the main contribution of this paper and it requires the specification of three procedures:

   (a) One procedure is needed for extracting features which are good at representing important characteristics of a set of pixels, relevant to the parasite detection problem.

   (b) A second procedure is required for classifying a set of pixels represented by a feature vector, as being a parasite or a non-parasite.

   (c) The third and last procedure is used to guide the search for parasites in the green-channel images.

2.2 Image acquisition

A group of mice were infected with an inoculation of 5 blood trypomastigotes of T. cruzi via intraperitoneal. Once the mice were infected, the parasitaemia detection started in average between 11 and 15 days afterwards. At this time the blood smears were prepared and stained using Wright stain, which allows the observation of the morphology of different blood cells, as well as parasites such as T. Cruzi, Leishmania sp., Plasmodium sp., etc. After the staining process the blood smears were placed vertically and were left to dry. Finally, an optical Nikon Eclipse E600 microscope was used to take images under typical laboratory illumination conditions. First at 10X and then at 100X, with a resulting size increase of 1000 times. Note that the images received no special treatment other than their conversion to RGB format.

2.3 Feature extraction

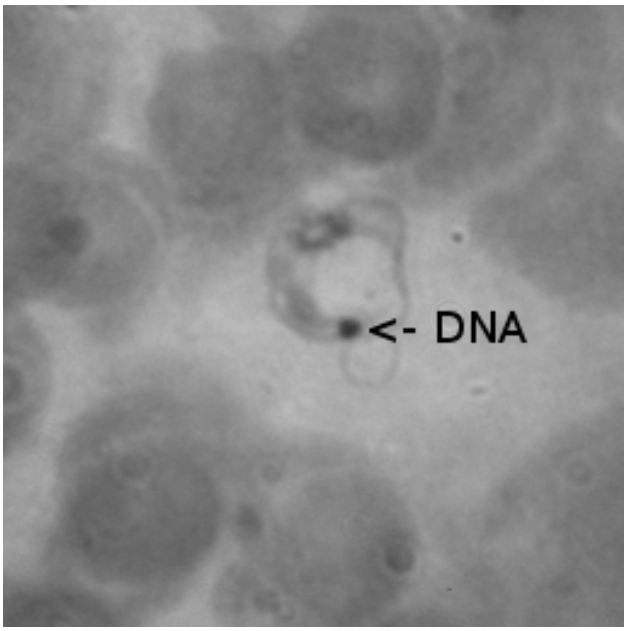
Feature extraction is an essential preprocessing step to pattern recognition and machine learning problems. It consists in transforming some input data into a reduced set of features, also called features vector. If the features extracted are carefully chosen it is expected that the features set will extract the relevant information from the input data in order to perform the pattern recogniton task using this reduced representation instead of the full size input.

The issue of choosing the features to be extracted was guided mainly by the following two concerns:

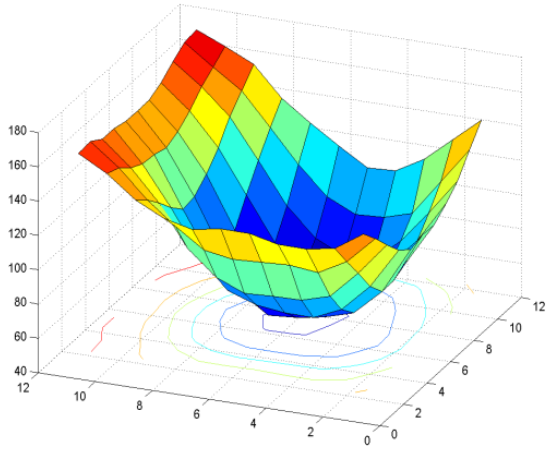
- the features should carry enough information about the image and should not require any domain-specific knowledge for their extraction.

- they must be easy to compute in order for the approach to be feasible for a large image collection and rapid computation.

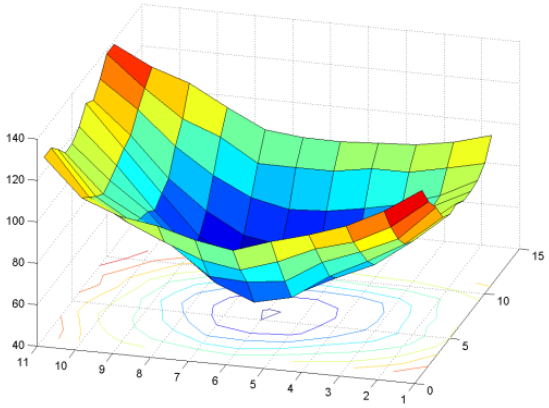
Based on these premises, we focused our attention on one dark spot of pixels appearing in all parasites' bodies. This spot which is shown in Fig. 1a, corresponds to an accumulation of DNA of the Chagas parasite. With a closely observation, we can see that it has one particular shape, shown as a surface in Fig. 1b. To capture this particular shape we decided to take a matrix of 11×11 pixels, centered around the pixel with the lowest value. Then this matrix is converted into a 121-dimensional vector by taking every row of it, transposing them and attaching one after the other. Such a vector of size 121 was used as our features vector. Figures 1c and 1d illustrate the DNA characteristical spot of two other parasites.



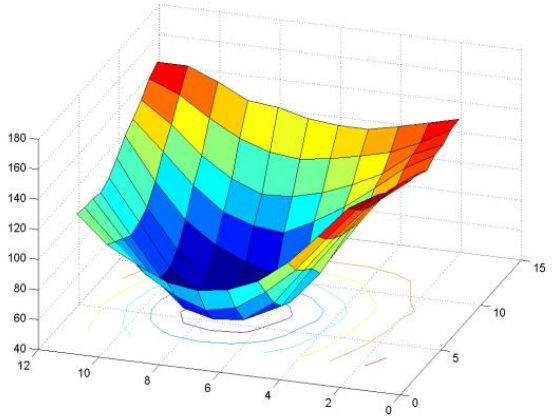
(a)



(b)



(c)



(d)

Figure 1: (a) Dark spot of pixels generated by an accumulation of DNA. (b, c, d) Three DNA spots corresponding to different parasites, seen as surfaces of 11×11 pixels.

2.4 Classification

Consider a classification problem in which we want to distinguish between a set of pixels corresponding to a Chagas parasite ($y = 1$) and a set of pixels that does not correspond to a Chagas parasite ($y = 0$), based on some features of such set of pixels. In Gaussian discriminant analysis first we need to build a model of what a Chagas parasite looks like and what something that is not a Chagas parasite looks like. Finally, to classify a new set of pixels in parasite or non-parasite we just need to match the new set of pixels with our two models, to see whether it looks more like a parasite or more like the non-parasites we have seen in the training set. Our algorithm try to model $p(x|y)$ and $p(y)$. For instance, if y indicates whether a example is a parasite ($y = 1$) or a non-parasite ($y = 0$), then $p(x|y = 1)$ models the distribution of parasites' features, and $p(x|y = 0)$ models the distribution of non-parasites' features. After modeling $p(y)$ and $p(x|y)$, which are called the class priors, our algorithm can then use the Bayes rule to derive the posterior distribution on y given x:

$$p(y|x) = \frac{p(x|y)p(y)}{p(x)}. \tag{2.1}$$

Here, the denominator is given by $p(x) = p(x|y = 1)p(y = 1) + p(x|y = 0)p(y = 0)$. However, if we are calculating $p(y|x)$ in order to make a prediction, then we do not actually need to calculate the denominator, since

$$\arg\max_y p(y|x) = \arg\max_y \frac{p(x|y)p(y)}{p(x)} \tag{2.2}$$

$$= \arg\max_y p(x|y)p(y). \tag{2.3}$$

In our model we assume that $p(x|y)$ is distributed according to a multivariate normal distribution. Such a strong assumption is later shown to provide quite good results for our data. It is well known in machine learning that choosing an optimal model for a given data set can become sometimes more an art than a science, depending on the chosen algorithm. However, it is widely accepted the use of cross-validation as a robust way to measure the performance of machine learning methods.

The multivariate normal distribution in n-dimensions is parameterized by a mean vector $\mu \in \mathbb{R}^n$ and a covariance matrix $\mathbf{\Sigma} \in \mathbb{R}^{n\times n}$ where its density is given by:

$$p(x; \mu, \mathbf{\Sigma}) = \frac{1}{(2\pi)^{n/2}|\mathbf{\Sigma}|^{1/2}} e^{-\frac{1}{2}(x-\mu)^T \mathbf{\Sigma}^{-1}(x-\mu)}. \tag{2.4}$$

Then, writing out the distributions for each class of target, parasite or non-parasite, we have:

$$p(x|y = 0) = \frac{1}{(2\pi)^{n/2}|\mathbf{\Sigma}|^{1/2}} e^{-\frac{1}{2}(x-\mu_0)^T \mathbf{\Sigma}^{-1}(x-\mu_0)}. \tag{2.5}$$

$$p(x|y = 1) = \frac{1}{(2\pi)^{n/2}|\mathbf{\Sigma}|^{1/2}} e^{-\frac{1}{2}(x-\mu_1)^T \mathbf{\Sigma}^{-1}(x-\mu_1)}. \tag{2.6}$$

To estimate the parameters μ_0, μ_1 and $\mathbf{\Sigma}$ we compute:

$$\mu_0 = \frac{\sum_{i=1}^m 1\{y^{(i)} = 0\}x^{(i)}}{\sum_{i=1}^m 1\{y^{(i)} = 0\}}, \tag{2.7}$$

$$\mu_1 = \frac{\sum_{i=1}^{m} 1\{y^{(i)} = 1\} x^{(i)}}{\sum_{i=1}^{m} 1\{y^{(i)} = 1\}}, \tag{2.8}$$

and

$$\mathbf{\Sigma} = \frac{1}{m} \sum_{i=1}^{m} (x^{(i)} - \mu_{y^{(i)}})(x^{(i)} - \mu_{y^{(i)}})^T, \tag{2.9}$$

where m is the number of training examples. At this point we know how to compute $p(x|y)$, now we just need to compute $p(y)$, which is done as follows. For the prior probability of finding a Chagas parasite we compute:

$$p(y = 1) = \frac{1}{m} \sum_{i=1}^{m} 1\{y^{(i)} = 1\}, \tag{2.10}$$

and for the prior probability of finding a non-parasite we do:

$$p(y = 0) = \frac{1}{m} \sum_{i=1}^{m} 1\{y^{(i)} = 0\} = 1 - p(y = 1). \tag{2.11}$$

Once we have computed the 4 probabilities: $p(x|y = 0), p(x|y = 1), p(y = 0)$ and $p(y = 1)$, we just need to evaluate the Bayesian models given by equation (2.3), in order to make a classification of a features vector as being a parasite or a non-parasite.

2.5 Search

After a visual examination of several images containing parasites, we realized that in many cases the DNA spot was the darkest point in the image, meaning that if we view each green-channel image as a surface created from its pixels' values, the DNA spots were very often local minima, sometimes they were actually the global minimum, as it is the case in the surface provided in Fig. 2. Therefore it is very convenient to start looking for parasites in places of the image where dark points exist, i.e. local minima. Our search algorithm is given next.

1. Find the global mimimum x.
2. Compute evaluation with the parasite model.
3. Compute evaluations with the non-parasite model.
4. Make classification.
5. If x is classified as a parasite
   print 'I have found a parasite' and stop.
   else
   eliminate current global minimum.
6. If the upper search limit has been reached
   print 'I have not found a parasite' and stop.
   else
   go to step 1.

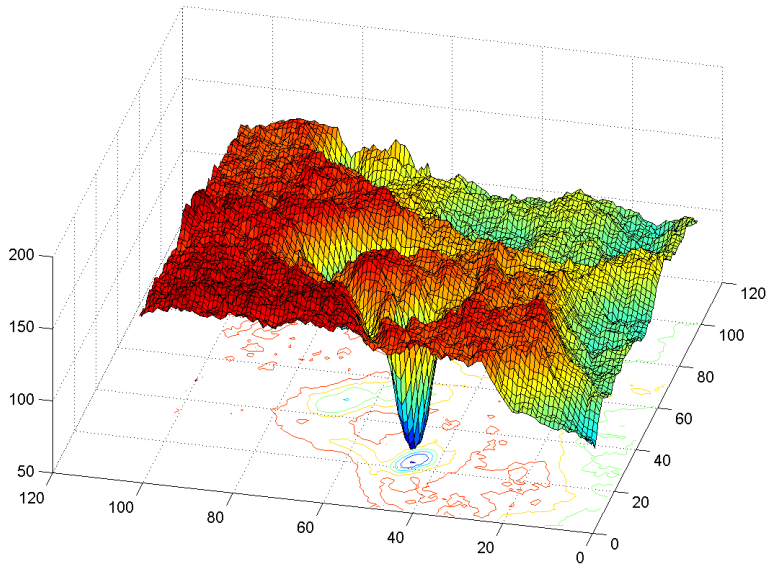


Figure 2: A green-channel image plotted as a surface. The global minimum of this image corresponds to the DNA spot of the Chagas parasite.

It is important to mention that the size of the feature vector x was originally 121, and then it was reduced to 10 through the use of Principal Component Analysis (PCA) [5]. Moreover, the upper search limit is a threshold value of pixel intensities which is computed experimentally from the training set. For our experimental work this threshold was defined as 100. The main bottleneck of the search algorithm, in terms of performance, is its sequential implementation. The time complexity is mainly a function of the number N of local minima found below the upper search limit, this is $\mathbb{O}(N)$.

3 Experimental results

Three different setups were used to experimentally validate our classification algorithm and in all three of them we applied the cross-validation approach. The experiments were run in a computer with an Intel Core i5 processor (3.20 GHz) and 4GB of RAM. The time to scan a whole image of 256×256 pixels ranged between 30 and 180 seconds, depending on the number of existing local minima. In other words, images with many dark regions, contain many low-frequency pixels and therefore the search algorithm requires more time to check each of them until a parasite is found or the upper search limit is reached. The search algorithm can be implemented to be run in parallel hardware, such as GPUs, in order to obtain a substantial improvement in the processing time, however the work reported in this article is focused on finding an optimal machine learning model and a functional feature vector.

Cross-validation is one of several approaches to estimating how well the model we have just learned from some training data is going to perform on future unseen data, in this work we used n-fold cross-validation. In n-fold cross-validation the examples in the input data are randomly divided into n equal partitions or sets; $n-1$ partitions are used for training, and the remaining 1 partition is used for testing. The process is repeated n times, with a different partition being used for testing in each iteration. Thus, each partition is used at least once in training and once in testing, and the average results are reported.

In the first setup each training example was a 121-dimensional vector, containing the features explained in the previous section. There were two Gaussian models, one model for what a parasite looks like and another for what something that is not a parasite looks like. Results are shown in Table 1.

	Test 1	Test 2	Test 3	Test 4	Mean
True positive	0.9333	1.0000	0.8666	0.8666	0.9166
False positive	0.4666	0.4000	0.0666	0.2666	0.2999
True negative	0.5333	0.6000	0.9333	0.7333	0.6999
False negative	0.0600	0.0000	0.1333	0.13330	0.0833

Table 1: Cross validation results using 4 partitions. Each partition with 15 positive and 15 negative examples. The input feature vector is in dimension 121.

In the second experimental setup we reduced the dimensions of the features vector from 121 to 10 by the application of PCA. We kept the use of two models, one for parasites and one for non-parasites. Results are shown in Table 2.

	Test 1	Test 2	Test 3	Test 4	Mean
True positive	1.0000	1.0000	0.9333	1.0000	0.9833
False positive	0.2000	0.0000	0.2000	0.1333	0.1333
True negative	0.8000	1.0000	0.8000	0.8666	0.8666
False negative	0.0000	0.0000	0.0666	0.0000	0.0166

Table 2: Cross validation results using 4 partitions. Each partition with 15 positive and 15 negative examples. The input feature vector was reduced to 10 dimensions through the use of PCA. Results in this table were obtained using two Gaussian models, one for parasites and one for non-parasites.

For the last series of experiments we kept the reduced size of the features vector, but instead of using two Gaussian models, we added one more. The third model introduced allowed us to make the strong assumption that those things appearing in the images and that are not parasites, could be clustered in two different groups, non-parasites type A and non-parasites type B. Results are shown in Table 3.

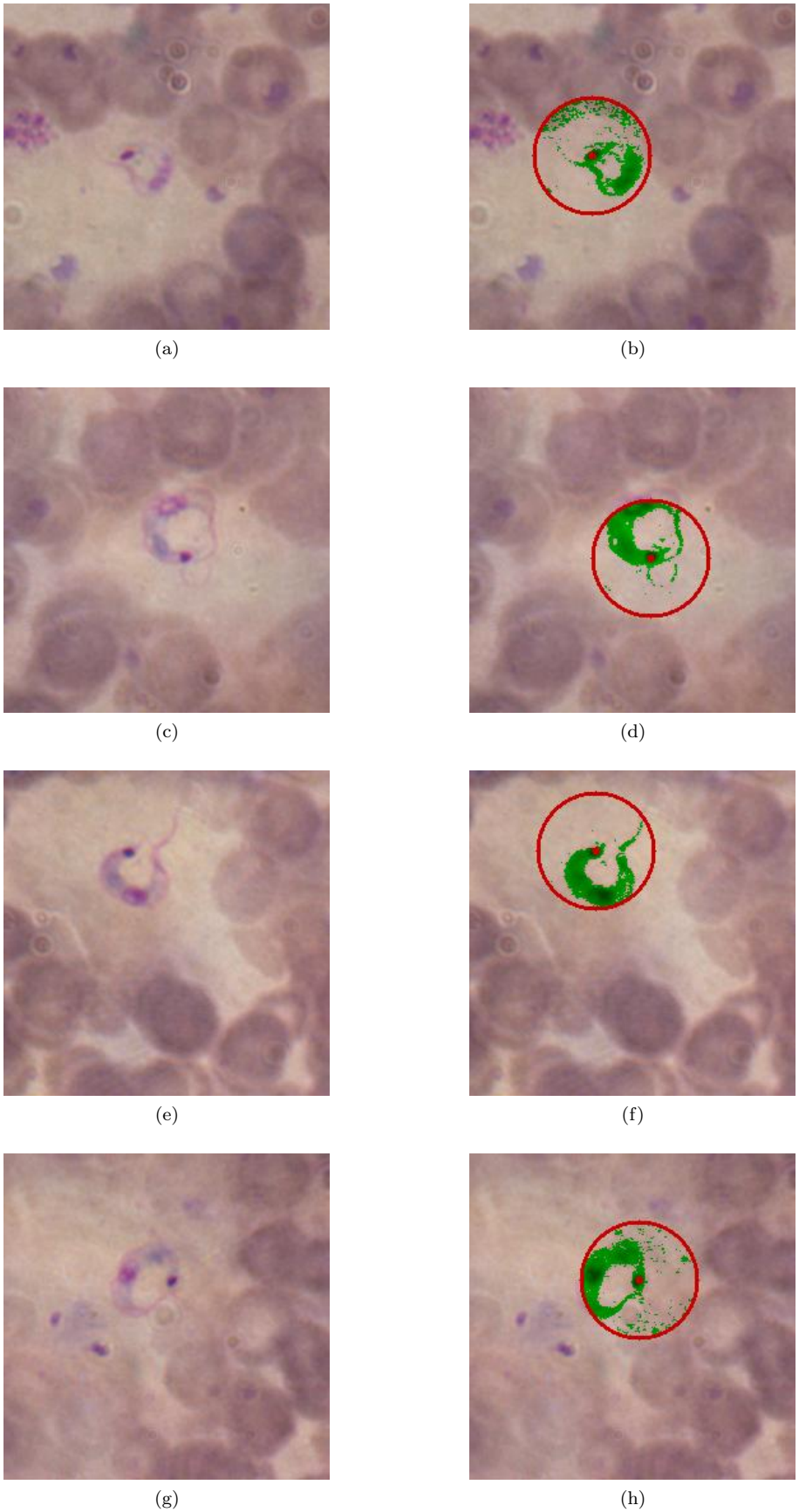
	Test 1	Test 2	Test 3	Test 4	Mean
True positive	1.0000	1.0000	1.000	1.000	1.000
False positive	0.2500	0.3055	0.5000	0.0833	0.2847
True negative	0.7500	0.6944	0.5000	0.9166	0.7152
False negative	0.0000	0.0000	0.0000	0.0000	0.0000

Table 3: Cross validation results using 4 partitions. Each partion with 15 positive and 15 negative examples. The input feature vector is in dimension 10. Three Gaussian models were used, one for parasites and two for non-parasites.

Among the three different ways of implementing our classifier model, the best one is given by the second experimental setup. This approach was tested with all 120 images available and the results are given in Table 4. Negatives are those situations when the algorithm finds none parasite in the image. Positive means that the algorithm found a parasite in the image. False means that the algorithm made a mistake in its detection and true means that the algorithm is right in its detection. The most important performance is given by the false-negatives, because is the percentage of images that the algorithm failed to find a parasite when it had one. In Fig. 3 we provide four sample images that illustrate the detection results of our algorithm.

	False	True
Negatives	0.0167	0.8437
Positives	0.1563	0.9833

Table 4: Final detection performance.



(a) (b) (c) (d) (e) (f) (g) (h)

Figure 3: Results of the Chagas parasite detection algorithm with a sample of images.

4 Conclusion

The best results were obtained using two Gaussian models, one for parasite images and one for non-parasites. This approach together with the search algorithm were used to test the detection performance with 120 images of 256×256 pixels and the resulting performance rates are 0.0167 false-negatives, 0.1563 false-positives, 0.8437 true-negatives and 0.9833 true-positives. Moreover, PCA was helpful to reduce the feature vector dimension from 121 to 10, keeping always a good performance. As we mentioned before, to the best of our knowledge, there are not published works reporting on the application of machine learning methods to the problem of automated detection of Trypanosoma cruzi parasites from blood images, therefore a comparison with other methods is not possible at this moment. Despite the promising results, one of the problems we are currently facing is the reduced number of positive examples available. Obtaining more training examples is not easy but it would allow us to improve our experimental work. Improving the feature vector by adding more characteristics such as the shape of the parasite or statistics related to histograms of the image seems also to be a promising path to follow.

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