EVALUATION OF SEGMENTATION METHODS APPLIED TO INTACT AND DAMAGED BOAR SPERMATOZOON HEADS

Eduardo Fidalgo, Javier de Pedro, Laura Fernández-Robles, Mª Teresa García-Ordás, Enrique Alegre
Industrial and Computer Science School. University of León.

Abstract

By using Haussdorf distance, this paper proposes the evaluation of four different segmentation methods. Despite the resulting images from these methods are not uniform, several pre-processing tasks have been carried out in order to make them suitable as inputs of the proposed evaluation method (Haussdorf distance). All methods have been applied over the same image dataset (intact/damaged spermatozoa). The evaluation has been made by comparing the results of the different methods with an expert segmentation of the dataset.

Keywords: Watershed transformation; Prior shape knowledge; Segmentation; k-Means clustering, Expectation Maximization, Normalized cuts, Normalized cuts UCM.

1 INTRODUCTION

The evaluation of semen quality is a major problem in the field of assisted reproduction. The pork sector has evolved in the last decades and it has become a major source of animal protein in the developed countries and with great future in emerging countries [8].

Due to the previous statement, all the research to ease or improve production in this species will have great economic and social impact. In the last years, there has been a major development in the field of pork reproduction in the management and control reproductive techniques, all related to the artificial insemination [5]. Artificial insemination has many advantages [3]:

- It allows working with a small number of animals on farms, saving time and money.
- It is possible to obtain improvements in every new generation.

There is a market where the farms responsibly buy semen samples to companies with production centers. These companies have to secure the maximum semen quality, performing reviews to avoid fertility problems and identify who the best donors are. Therefore, the evaluation of semen quality is a critical task in veterinarian.

Concentration, motion, morphology and acrosome integrity [6] are some of the parameters that are directly related with the semen quality. Some of them can be measured by the CASA systems (Computer Assisted Sperm Analysis) by using digital image processing [11].

In this work, the evaluation of 4 different segmentation methods have been applied into the same set of intact / damaged spermatozoon images. All the methods will be carried out over the same sets of images in order to compare results, identify problems and propose improvements when the evaluation shows unexpected or not valid results.

A description of the methods is explained in Section 2. Section 3 comments the Haussdorf distance as the way to evaluate the analyzed methods. Since the outputs of each method are not equal (in terms of size, presentation of the spermatozoon, etcetera), section 4 details the operations to be performed on each output so they can be properly used by Haussdorf distance. Section 5 shows and discusses the obtained results after applying the evaluation method and analysing them. A summary of the work performed and possible future directions are stated in section 6.

2 SEGMENTATION METHODS

Four different methods were used to segment the acrosome of the spermatozoa heads.

2.1 K-MEANS

The K-means algorithm is the main reference over different methods and permits to classify data into representative groups. There are four matrices that support the performance of this algorithm:

- Data matrix
- Distance matrix
- Centroid matrix
- Belonging matrix

All variants of this algorithm are fundamentally based on the way the distance between groups of data is measured, the criteria to select the group of some data and the manner we update all the information.

The pseudo-code that explains each step of the algorithm is:
1. Assignment: assigns each point of data to the cluster which has minor average distance.
2. Update: recalculate the centroid matrix using new data.
3. Ending: when no centroids produce any movement.

The first part shows this is a heuristic algorithm, which means the result depends largely on initial values, with no guarantees of optimality. For this reason, it is advisable to repeat this method several times to avoid the problem mentioned. The assignment or initialization step has two methods, Forgy and random partitions. The Forgy method consists of selecting K out of the total random observations selecting those data as initial centroid values. The random partitions method assigns to each observation one cluster randomly, which also assigns obviously all centroids randomly, jumping into the second step [10].

The second part of this algorithm uses one of the multiple formulae available to calculate one centroid for each cluster, taking into account new data and the structure of this data. It has been proposed using a matrix because computational calculus could be efficient, but the reader may use any other.

$$m_i^{(t+1)} = \frac{1}{|S_i^{(t)}|} \sum_{x_j \in S_i^{(t)}} x_j \qquad (1)$$

The distance matrix between data and centroids has to be obtained in every iteration in order to select the cluster which has its centroid with the smallest distance. In this research we have used Euclidean distance, but Hamming or Levenshtein are also valid [6].

The value selected is stored into the belonging matrix, so if from one iteration to another there is no change in the centroid matrix we can assume they are stabilized and the algorithm can finalize [3].

One of the most interesting results obtained is based on the use of L1 distance for the segmentation of black and white images because this provided important similitude between human and machine segmentation with no high ratio error [1].

2.2 NORMALIZED CUTS

Originally, normalized cuts method (NC) was used in data clustering, artificial intelligence and pattern recognition tasks. However, in this article, this method has been used for segmentation purposes as explained in [8].

This method proposes a segmentation criteria based on theorems from Graph Theory, modelling image data as a graph where every vertex is a set of points, which have some similarity properties.

The success or fail of this method depends on the criteria used for classifying those vertexes and the statistical method used for assigning labels that create the partitions of this segmentation [12].

The criterion for classifying graph vertex is called similarity function. In this case, the minimal cut has been used (exponential problem solved with Wu and Leavy [15] algorithms) because the optimal partition in the graph is the one that minimizes the cut value. Following this cut formulae:

$$cut(A,B) = \sum_{w \in A, v \in B} w(u,v) \qquad (2)$$

We can understand how to make a bipartition of the graph, the main object of the scene on the one hand and the background on the other hand.

Although it could be interesting dividing the graph using only a sum, this method is too much dependent on how the image is taken and how its objects are located, so those parameters have been normalised by computing the cut cost as a fraction of the total edge connections to all nodes in the graph [11].

$$Ncut(A,B) = \frac{cut(A,B)}{assoc(A,V)} + \frac{cut(A,B)}{assoc(B,V)} \qquad (3)$$

Where assoc is defined as the total connections sum from nodes in subset X to all nodes (that means the subset V). This measure can show the disassociation between nodes of the graph. Moreover, with the same spirit as last formulae we can define:

$$Nassoc(A,B) = \frac{assoc(A,A)}{assoc(A,V)} + \frac{assoc(B,B)}{assoc(B,V)} \qquad (4)$$

where assoc(X,X) are total weights of edges connecting nodes of X. It can be seen this is also an unbiased measure which shows the nodes that are group connected.
Last property that takes place on this method is:

$$Ncut(A,B) = \frac{cut(A,B)}{assoc(A,V)} + \frac{cut(A,B)}{assoc(B,V)} = \frac{assoc(A,V)-assoc(A,A)}{assoc(A,V)} + \frac{assoc(B,V)-assoc(B,B)}{assoc(B,V)} =$$

$$2 - \left(\frac{assoc(A,A)}{assoc(A,V)} + \frac{assoc(B,B)}{assoc(B,V)}\right) = 2 - Nassoc(A,B) \quad (5)$$

Hence, using last properties two criteria for seeking best group algorithm can be defined, minimizing the disassociation between groups and maximizing the association within groups which both can be satisfied simultaneously.

The only problem that rests is the computing issue as the minimal cut problem is NP-complete. Nevertheless we can assume small discrete problems that have been solved in an efficient way [7].

The next two segmentation methods have been implemented relying on other articles.

2.3 IMPROVED WATERSHED ALGORITHM

We decided to implement an improved watershed algorithm based on the previous work of Zhao et al. [16], naming it IW.

It consists of an algorithm of watershed transformation based on opening-closing operation and distance transform. It improved the classical watershed segmentation algorithm based on distance transform, overcoming over-segmentation. This method for segmentation inherits the advantage of watershed algorithm based on distance transform that it successfully segments out each dowel in the image bringing convenience to computer vision and auto-counting of dowels. It also overcame over-segmentation existed in traditional watershed segmentation preserving the original edges of each dowel in the image completely.

The steps followed to implement this method were:

1. Apply an opening – closing filter over the image, with a structural 3x3 element.
2. Select the proper threshold for the filtered image and obtain a binary image.
3. Implement a distance transform and negate the resultant image.
4. Calculate again the distance transform to produce a new greyscale image.
5. Segment the resultant image using a negate Watershed algorithm and add the resultant image to the one obtained in Step 2.
6. Detect the edges of the image obtained in Step 5 and add the edges to the original image to obtain the final result of the segmentation.

After implementing the previous steps, we realised that in order the method was able to properly segment spermatozoon images, some modifications had to been fulfilled.

As it can be seen in Figure 1, intact spermatozoa are correctly segmentate directly using this method. However, when dealing with damaged spermatozoa the method fails when it tries to locate the damaged part.

2.4 K-MEANS WITH AN IMPROVED WATERSHED ALGORITHM

Finally, the last method we implemented is based on [7] and consists of a k-Means with an improved watershed algorithm (KMWS).

The original paper incorporated k-Means and improved watershed (IW) segmentation algorithm for medical image segmentation. Watershed algorithm for medical image analysis is widespread because of its advantages, such as always being able to produce a complete division of the image but its main drawbacks include over-segmentation and sensitivity to false edges. The method described in this paper addresses the drawbacks of the watershed algorithm when it is applied to medical images by using k-Means clustering to produce a primary segmentation of the image before applying an improved watershed segmentation algorithm to it.

The steps followed to implement this method are represented in Figure 1.

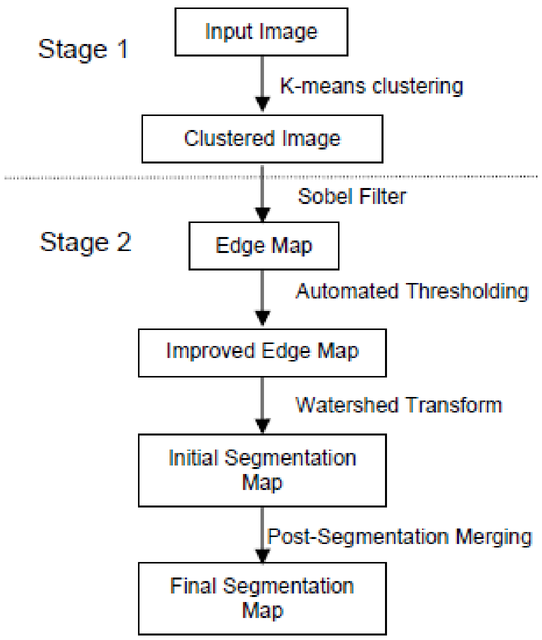

Figure 1: Steps to implement the method presented in [7] that uses k-Means with an improved Watershed Algorithm

Like the previous method, some modifications were performed to the proposed steps in order the method was able to properly segment spermatozoon images.

As Figure 1 shows, the result of this algorithm is not clear enough in order to recognise even an intact spermatozoon. Some improvements have been made to achieve better results in the segmentation. The fact that in the obtained images the spermatozoon is barely visible will have consequences in the evaluation, by having greater errors than the rest of the methods.

3 HAUSSDORF METHOD

The proposed evaluation method to compare the already explained methods is the Haussdorf distance [9].

Haussdorf distance measures how far two subsets of a metric space are from each other. It turns the set of non-empty compact subsets of a metric space into a metric space in its own right.

Informally, two sets are close in the Haussdorf distance if every point of either set is close to some point of the other set. The Haussdorf distance is the longest distance that can be forced to travel by an adversary who chooses a point in one of the two sets, from where you then must travel to the other set. In other words, it is the farthest point of a set that can be to the closest point of a different set.

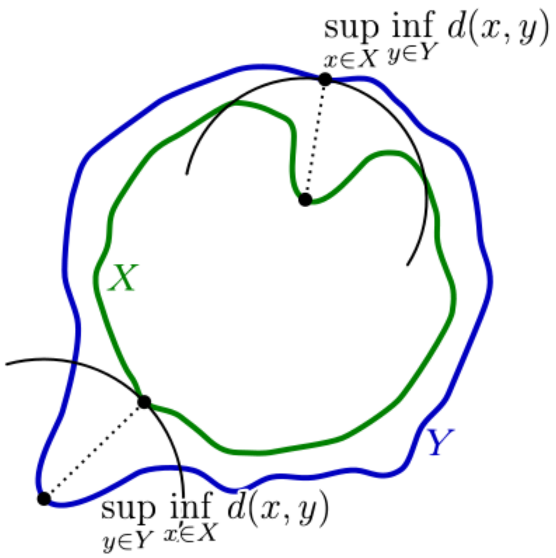

Figure 2: Components of the calculation of the Haussdorf distance between the green line X and the blue line Y.

Let X and Y be two non-empty subsets of a metric space (M, d). We define their Haussdorf distance $d_H(X,Y)$ by

$$d_H(X,Y) = \max\{\sup_{x\in X} \inf_{y\in Y} d(x,y), \sup_{y\in Y} \inf_{x\in X} d(x,y)\} \quad (6)$$

4 COMPARATIVE BETWEEN METHODS

4.1 ORIGINAL AND EXPERT IMAGES

To evaluate the methods described in Section 2 by using the Haussdorf distance explained in the previous section, 200 images were analyzed with the mentioned methods:

- 100 images of intact spermatozoa.
- 100 images of damaged spermatozoa.

Figure 2 shows two samples of these images.

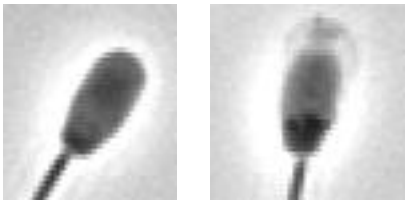

Figure 3: Intact (left) and Damaged (right) spermatozoon

These images have been extracted from larger images containing dozens of spermatozoa taken at 40X. Basler Scout scA780-54fc linked to a computer had been used to acquire the 40X images. All these spermatozoa have been cropped as the way the images showed. It can be distinguished between intact (left) and damaged (right) spermatozoon because damaged spermatozoon head structure is not continuous and a leakage of the internal material can be seen in the upper part of the head.

The evaluation method requires two inputs to calculate the Haussdorf distance. One is going to be the output of the previous methods. The other one will be an expert segmentation of the corresponding original images. By doing this, a measurement of the error of the method that is being used will be computed after it has been applied to an image.

Expert segmentation has been performed over the 200 images. The purpose of this segmentation was to determinate what the zone that represents the head of the spermatozoon is. In case the spermatozoon is damaged, expert segmentation will include the damaged zone.

Figure 4 shows the expert segmentation applied over the same images in the Figure 3.

Figure 4: Expert segmentation of an Intact (left) and Damaged (right) spermatozoon head.

Since all the methods are going to be applied over the same set of images, with the same expert segmentation and the same evaluating method, a quick comparison among the methods have been achieved.

4.2 EVALUATION OF THE METHODS

As it was establish in Section 2, the outputs of the different methods are not uniform in terms of having the same type of image as an input of the Haussdorf distance. Some pre-processing operations have been done on each case in order to have uniform input images to Haussdorf method.

For all the images, two basic operations were performed:

1. Conversion to binary images. Since the methods did not produce similar results in terms of image homogeneity (for example, all the resultant images are binary), all the images are converted to binary.
2. Check input size. All the crops size is checked. If one of the crops do not fit the size of 73x73 pixels (standard size internally accepted to crop a spermatozoon from an image of 40X), the cropped image will be resize to the mentioned size. One of the main causes of a “non-standard” size can be that the spermatozoon has been cropped close to the edge of the original image.
3. The standard inputs to the Haussdorf distance will be two binary images where the spermatozoon will be white (1’s) and the background black (0’s).

Figure 5: Sample of desired input image to the Haussdorf distance method (Evaluation Method).

4.2.1 K-Means and Normalized Cuts.

The only operation to get the proper input to Haussdorf method on the images obtained by k-Means method is an inversion of the resulting image (Figure 6). In contrast, NC method requires a special mention since their resultant images do not need to be pre-processed; they are perfectly valid as inputs for the Haussdorf distance (Figure 7). However, it has been detected that some of the images are not correctly labelled and hence they are not valid as inputs for the Haussdorf method. Therefore, a small operation to detect these images has been developed in order to invert them when necessary.

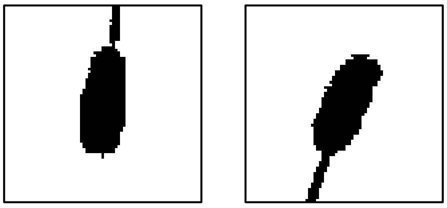

Figure 6: Result of K-Means applied to an Intact (left) and Damaged (right) spermatozoon. An inversion is necessary.

Figure 7: Result of Normalized Cuts applied to an Intact (left) and Damaged (right) spermatozoon.

4.2.2 Improved Watershed.

The images obtained from this method are only edges (Figure 8).

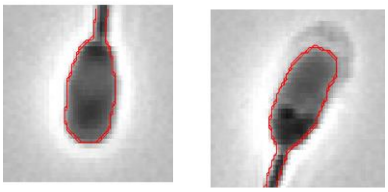

Figure 8: Result of Improved Watershed applied to an Intact (left) and Damaged (right) spermatozoon.

These spermatozoon edges have been filled in order they will be appropriate to be the input of the proposed evaluation method.

4.2.3 K-Means with improved Watershed.

As it can be seen in Figure 9, the images procured by this method are not close to be the inputs of the proposed evaluation method in the way it has been decided.

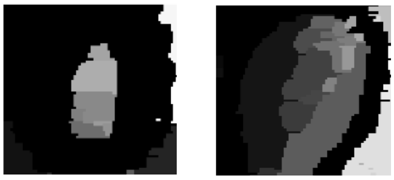

Figure 9: Result of K-Means with IW applied to an Intact (left) and Damaged (right) spermatozoon.

Despite of that, it has been performed a quick conversion from gray-scale to binary images. The results will be discussed in the next section.

5 RESULTS

After applying the proposed evaluation method (Section 3) for each method, a table similar to Table 1 has been obtained.

Table 2: Sample of the data obtained per image in all the methods.

Image_Name	Error (pixels)	Time invested
20110325_749_03.tif	3,742	0,020
20110325_752_02.tif	4,243	0,016
20110325_753_01.tif	3,873	0,014
20110325_754_02.tif	2,646	0,015
20110325_755_02.tif	4,000	0,015

It contains the Image Name, the Error (measured in pixels) obtained per image and the time invested in analyzing the image (with the corresponding pre-processing stage to adapt the images to the evaluation method).

Table 2 and 3 present a summary for all the methods by computing the Maximum and Mean error obtained after analyzing all the images with the Standard deviation.

Table 3: Summary for Intact Spermatozoons.

Intact Spermatozoon			
Method	Max Error	Mean Error	Std deviation
NC	5,196	3,359	0,820
Kmeans	5,292	3,406	0,783
IW	5,000	3,584	0,700
KMWS	7,616	5,440	0,950

Table 4: Summary for Damaged Spermatozoons.

Damaged Spermatoon			
Method	Max Error	Mean Error	Std deviation
IW	6,164	3,729	0,754
k-Means	7,000	3,739	0,786
NC	8,485	3,877	1,180
KMWS	7,348	5,745	0,750

A lower error value means a higher similarity between the resulting and the expert segmentation images. On the opposite term, higher values of the error involve bigger differences between the expert image and the result of the method. Figures 10 and 11 explain better these two statements.

Figure 10 presents the minimum error after applying the Haussdorf distance on the 100 intact spermatozoa by using the IWPSAK method. It can be seen that the result (centre) is very similar to the expert segmentation of the head (right).

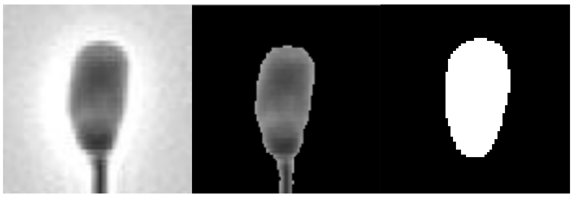

Figure 10: Original Intact Spermatozoon (left), Result of PS method (centre) and corresponding expert image (right).

Figure 11 shows the maximum error after applying the Haussdorf distance on the 100 damaged spermatozoa by using k-Means algorithm. It can be seen that the result (centre) is not even close to be a little similar to the expert segmentation of the head (right). In this case, the method has failed in segmentating the expert image and it is immediately identified after applying the evaluation method.

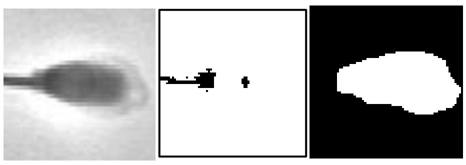

Figure 11: Original Damaged Spermatozoon (left), Result of k-Means method (centre) and corresponding expert image (right).

In Tables 2 and 3, it can be quickly checked that the Normalized Cuts has obtain the minimum error for intact spermatozoon and Improved Watershed for damaged spermatozoa segmentation. Best overall

Mean results were yielded by NC when segmenting intact heads (3,359 pixels) and by IW in case damaged heads were considered (3,729).

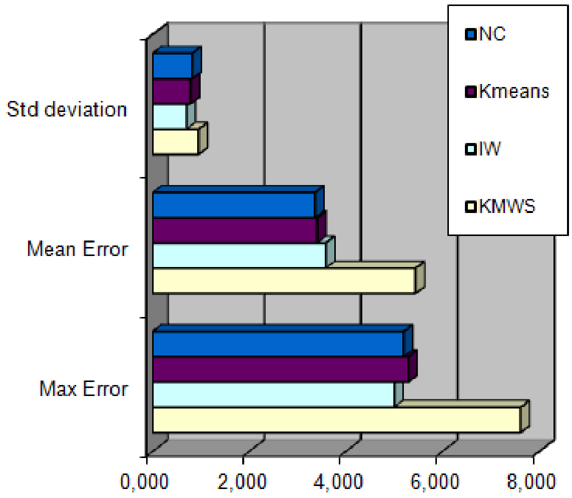

Figure 12: Graphic summary for Intact Spermatozoon.

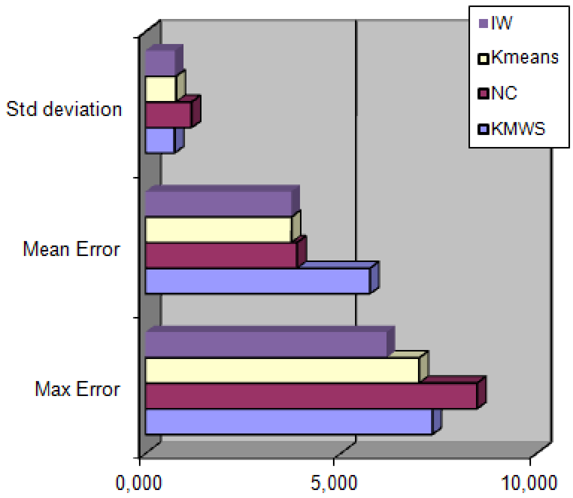

Figure 13: Graphic summary for Damaged Spermatozoon.

Nevertheless, an important note should be added. By comparing the two tables it can be seen that the errors obtained for damaged spermatozoa are always bigger that the ones obtained for intact spermatozoa. The reason of this difference is that all the methods fail when segmentating the damaged part of the spermatozoon. Then, when the resultant image is compared with the expert one, the error difference between intact and damaged spermatozoa detection is the non-segmented part in the damaged spermatozoa (see Figure 14).

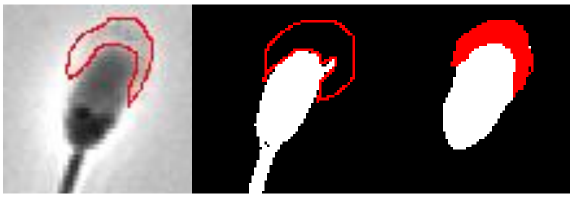

Figure 14: Damaged zone that all the methods **did not detect,** causing the increase in the error value after evaluation.

Among other data in the graphic / tables it can be seen that the KMWS method gets the worse errors. Remembering the results of this method (Figure 9), it can be seen that several regions compose a spermatozoon. Fusion applied to these regions will produce more accurate images as inputs of the evaluation method.

6 CONCLUSIONS

This paper presents an evaluation between four different segmentation methods applied over the same image dataset (intact / damaged spermatozoa). Values obtained after the evaluation represent the quality of the segmented image by comparing it with the corresponding expert segmentation image. Despite of that, a remark has been made. It has been seen that all of the methods segmentate in a good way intact spermatozoa but also all the methods fail when a damaged spermatozoon is segmented by not recognising the damaged zone.

Future works will include additional methods to the evaluation, improving the outputs of the methods with the biggest errors (like KMWS), getting an expert dataset that includes the tails in order to decrease the errors got with the Haussdorf distance and the modification of all the methods in order damaged spermatozoa can be correctly segmented.

Acknowledgements

This work has been supported by grants DPI2009-08424 and PR2009-0280 from the Spanish Government. The authors would like to thank CENTROTEC for providing us the semen samples and for their collaboration in the acquisition of the images.

References

[1] G. Cleuziou, "*An extended version of the k-means method for overlapping clustering*" ICPR, 2008, pp 1-4.

[2] Vance Faber, "*Clustering and the continuous k-means algorithm*", Los Alamos Sciende, 1994, vol. 22, pp 138-144.

[3] V. González Castro. “*Descriptores de textura adaptativos y técnicas de estimación de proporciones basadas en clasificadores*”, Trabajo de Investigación. Universidad de León. (2009).

[4] Zhexue Huang, “*Extensions to the k-Means Algorithm for Clustering Large Data Sets with Categorical Values*”, Data Mining and Knowledge Discovry, Vol. 2 Issue 3, September 1998, pp 283-304

[5] D. Levis. “*Liquid boar semen production: Current extender technology and where do w ego from here!*” Boar Semen Preservation IV. L. A. Johnson and H. D. Guthrie, ed. Allen Press Inc., Lawrence, KS. pp. 121-128.

[6] S. Martín, E. Martínez, C. García, and C. D. Alba, “*Semen de verraco: evaluación práctica*”, Archivos de reproducción animal, Vol. 1, pp. 12-23, 1996.

[7] H.P. Ng; S.H. Ong; K.W.C. Foong; P.S. Goh; W.L. Nowinski. "*Medical Image Segmentation using k-means clustering and improved Watershed 105 Algorithm*". *IEEE Southwest Symposium on Image Analysis and Interpretation*, 2006, pp. 61-65.

[8] E. Sellés. “*Evaluación de la capacidad fecundante de espermatozoides porcinos refrigerados y congelados*”, Universidad de Murcia. (2008).

[9] Jianbo Shi, Jitendra Malik, "*Normalized Cuts and Image Segmentation*.", IEEE Transactions on Pattern Analysis and Machine Intelligence, vol. 22, no. 8, pp 1-18.

[10] Suman Tatiraju, Avi Mehta, “*Image Segmentation using k-means clustering, EM and Normalized Cuts*”, ICIAP’11 Proceedings of the 16th international conference on Image analysis and processing, Vol. 2, pp 229-240.

[11] J. Verstegen, M. Iguer-Ouada, and K.Onclin, “*Computer assisted semen analyzers in andrology research and veterinary practice*.” Theriogenology, vol.57, nº 1, January 2002, pp.149-179.

[12] *Wikipedia The free Encyclopedia* [online]. <http://en.wikipedia.org/wiki/Haussdorf_distance> [12 May 2012].

[13] J. Shi and J. Malik, "*Normalized Cuts and Image Segmentation*," Technical Report CSD-97-940, UC Berkley, 1997.

[14] Z. Wu and R. Leahy “*An Optimal Graph Theoretic Approach to Data Clustering: Theory and Its Application to Image Segmentation*.” IEEE Transactions on Pattern Analysis and Machine Intelligence 15:11 (1993), 1101—1113.

[15] Linli Xu, Wenye Li and Dale Schuurmans. “*Fast Normalized Cut with Linear Constraints.*”, Proceedings of the IEEE Computer Society Conference on Computer Vision and Pattern Recognition, CVPR, 2009.

[16] Y. Zhao; J. Liu; H. Li; G. Li. “*Improved Watershed Algorithm for Dowels Image Segmentation*”. Proceedings of the 7th World Congress on Intelligent Control and Automation. Chongqing, China, 2008, pp. 7644-7648.