Vitality assessment of boar sperm using NCSR texture descriptor in digital images

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Abstract. Two new textural descriptor, named N Concentric Squares Resized (NCSR) and N Concentric Squares Resized (NCSH), have been proposed. These descriptors were used to classify 472 images of alive spermatozoa heads and 376 images of dead spermatozoa heads. The results obtained with these two novel descriptors have been compared with a number of classical descriptors such as Haralick, Pattern Spectrum, WSF, Zernike, Flusser and Hu. The feature vectors computed have been classified using kNN and a backpropagation Neural Network. The error rate obtained for NCSR with $N = 11$ was of 23.20% outperforms the rest of descriptors. Also, the area under the ROC curve (AUC) and the values observed in the ROC curve indicates the performance of the proposed descriptor is better than the others texture description methods.

Keywords: Descriptors, classification, boar, sperm

1 Introduction

In this work we have assessed the vitality of boar sperm cells using a new texture descriptor that allow us to classify each spermatozoon head as dead or alive. Currently, this assessment is carried out using fluorescence microscopy and stains and is not possible to perform it with phase contrast microscopes without stains. It means the assessment is a time consuming process and also requires the laboratory to have access to expensive equipment.

The sperm assessment is a very important problem for the porcine industry. In most of the countries there is a big demand of alimentary products obtained from pig's meat so there is lots of companies trying to obtain as better as possible pork flesh and at the same time at the lower price available. The way to do it is by selecting the semen used in artificial insemination i.e. to assess the semen of the donor boars, picking the best specimens up and finally use only the best ones.

For several decades the CASA (Computer-Assisted Semen Analysis) systems have been used for assessing the seminal quality. Currently these systems analyse the mobility, concentration and provide some simple geometric measures of the spermatozoa's head to characterize abnormal head shapes, obtaining an assessment of the studied sample based on that values. But there are three valuable criteria, used by veterinary experts, that theses systems are not yet able to analyse automatically, as are the integrity of the acrosomal membrane, the number and presence of proximal and distal droplets and the vitality of the sample based on the presence of dead or alive spermatozoa.

A number of works have addressed some of the problems related to the semen analysis using digital image processing. Most of them use CASA systems for evaluating the sperm motility [1] or for studying the relationship among sperm cell motility patterns, morphology and boar fertility [2, 3]. Others researches have developed new methods to characterize the sperm shape by using spectral approaches [4, 6], or they have been looking for subpopulations [7] using shape descriptors of the spermatozoa head. There is only little work addressing the evaluation of the membrane integrity, in this case using texture descriptors [9] and, as far as we know, there is not any work published that assess the vitality of a sample classifying the spermatozoa heads as dead or alive.

The rest of the paper is organized as follows: the section 2 explains how the images have been captured and then how they have been segmented and preprocessed. In section 3 the composition of features vectors of classical texture descriptors used is detailed and the new proposed descriptors are explained. Section 4 indicates what classifiers we have used. In section 5 the results obtained with the proposed and classical descriptors are shown and, finally, the main conclusions of this work are presented in section 6.

2 Image dataset and preprocessing

2.1 Image acquisition

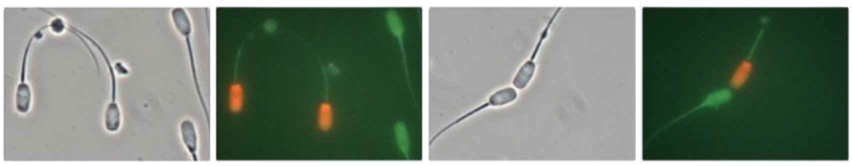

Fig. 1. Figures shown images in positive phase contrast and images with fluorescent stains respectively. Alive spermatozoa are coloured in red and dead ones in green.

The images used have been captured in CENTROTEC, an Artificial Insemination Centre that is an University of Leon spin-off. The semen were obtained from boars of three different races- Piyorker, Large White and Landrace. 450 pairs of images have been obtained using a Nikon Eclipse microscope and a Baster A312f camera of progressive scan. Each pair contains an image in positive phase contrast and a fluorescent image obtained using two different stains, propidium iodide (PI) that dyes dead spermatozoa as red and dichlorofluorescein (DCF) for turning green the alive spermatozoa 1. Further information about the sample preparation can been found in [11].

We have captured these pairs of images because we have used the phase contrast images for developing and testing the texture descriptors evaluated on the proposed method. For this purpose, first we need a ground truth that we have obtained using the red and green colours of the fluorescent images for labelling the grey level ones.

2.2 Segmentation

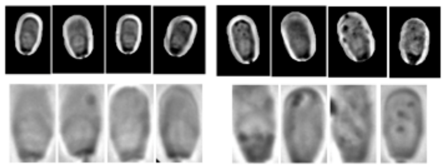

Fig. 2. Masked images. Upper images represents masked images, and grey scale spermatozoa are shown in the lower images (alive in the left side and dead in the right).

Every spermatozoa head from the phase contrast images have been automatically segmented. First, the image regions containing the heads have been detected by thresholding and later head regions are cropped. Then, the heads have been segmented using the method presented in [10]. The binary image obtained have been used for masking the region cropped previously so the images that are used in the description step have a black background. The original grey level of the spermatozoa heads and the masked images can be seen in figure 2. Later, all the spermatozoa heads have been cropped using its bounding box, then resized to 108×63 pixels, rotated placing the longest side in vertical. Using the location of the tail, the apical part have been placed at the upper side and the tail's insertion at the lower side (figure 2). Finally, 472 images of alive's heads and 376 images of dead's heads have been obtained.

3 Texture description of the spermatozoa heads

3.1 Texture oriented descriptors

Our first approach has been to try a number of descriptors for classifying the spermatozoa as dead or alive using the different texture distributions present in their heads. We also have proposed two new texture descriptors, called NCSR (N Concentric Squares Resized) and NCSH (N Concentric Squares Histogram) that we will explain in next section. The descriptors considered have been Hu, Flusser, Zernike, Haralick, some statistical descriptors, Wavelet Statistical Features and several variations of the Patter Spectrum.

The following feature vectors have been used. The Hu descriptors used have been the seven normalized moments defined for Hu [14]. Likewise, we have computed the six invariant affine moments proposed by Flusser [13]. The Zernike moments vector contains the nine first Zernike moments until order four [16]. In the Haralick's feature vector we have include the value of the first thirteen values computed over the grey level co-ocurrence matrix, with distance 1 and the average of the directions 0, 45, 90 and 135 degrees. The thirteen descriptors [15] are: energy, contrast, correlation, variance, inverse difference moment, sum average, sum variance, sum entropy, entropy, difference variance, difference entropy, first information measure of correlation and second information measure of correlation. From the histogram of each cropped and masked region we have computed four statistical values: average grey level, average contrast, measure of uniformity, entropy that make up the statistical feature vector used.The WSF feature vector is the proposed by Arizazhagan and Ganesan [12]. It is a 24-D features vector containing the mean and the standard deviation of the twelve images from the three first sub-bands of the wavelet decomposition. Finally, three different Patter Spectrum vectors have been computed using the Maragos's proposal [17], with 10 elements both without normalization and normalizing the vectors.

3.2 Proposed descriptors: N Concentric Squares (NCS)

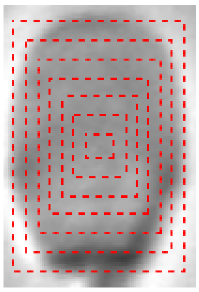

Fig. 3. Spermatozoon image is divided in N squares. In this case, N=7

The N Concentric Squares (NCS) descriptor gathers the grey levels along N equidistant squares that are concentric to the bounding box of the interest image. The value assigned to N sets the number of squares obtained. As N increases, the squares will get closer. All the N squares are concatenated making up one vector whose longitude depends on the size of the image and the number of squares extracted. As we have been working with registered images, all the images are vertical and have the same size. The first part of the vector is the horizontal segment closer to the left upper corner of the image, the second part is the vertical segment of the outer NCS that is closer to the right side of the image, the third part is the horizontal segment at the bottom and the last part of the outer square is the vertical segment closer to the left side of the image. The four segments are concatenated constituting the vector that comes from the outer square. Later, the same process is followed with the rest of the inner squares concatenating all the resulting vector into one. The grey level gathered along this vector are not the value placed in the position indicated by the square but the maximum value in its 3×3 neighbourhood. The distances between squares are given by the expression:

$$NpbHs = \frac{\frac{nRows}{2}}{N+1}, N = 1, 2, 3, \ldots \tag{1}$$

$$NpbVs = \frac{\frac{nCols}{2}}{N+1}, N = 1, 2, 3, \ldots \tag{2}$$

where NpbHs and NpbVs are the number of pixels between Horizontal and Vertical segments, respectively; and nRows, nCols are the number of rows and columns of the image.

The first descriptor proposed, called NCS Resized (NCSR), takes the previous vector and resizes it to a new vector of 100 elements by interpolation using a method based on the Fourier transform. The second descriptor proposed, figure 4, called NCS Histograms (NCSH), computes a histogram with 20 bins for each of the concentric squares and then it concatenates all the N vectors yielding a new vector of length $20 \times N$.

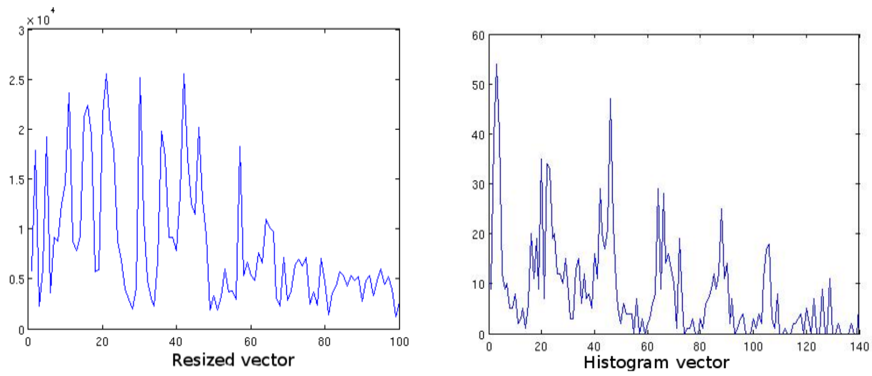

Fig. 4. The vector of the squares values histogram, and the original vector interpolated by Fourier method

4 Classification

First, we have carried out a kNN classification with k values $1, 3, 5, 7, 9, 11$ and 13. In most cases the best hit rate have been obtained with $k = 9$ and $k = 11$.

Later, we have also classified the data using a Neural Network looking for robust classification. The NN used was trained using backpropagation. It has one hidden layer and a logistic sigmoid transfer function for the hidden and the output layer. Learning was carried out with a momentum and adaptive learning rate algorithm. Data were normalized with zero mean and standard deviation equal to one. Classification was carried out by means of 10-fold cross validation with several different combinations of neurons in the hidden layer and training cycles in order to find out the optimal configuration in terms of accuracy. As there are some authors [8] from the machine learning community who claim that the hit rate is not the most suitable option for illustrating the performance of a classifier, we have also obtained ROC curves and we present in the results section the area under that curve (AUC) as a more robust tool for comparing the computed descriptors.

5 Results

5.1 Best N value for NCSR and NCSH

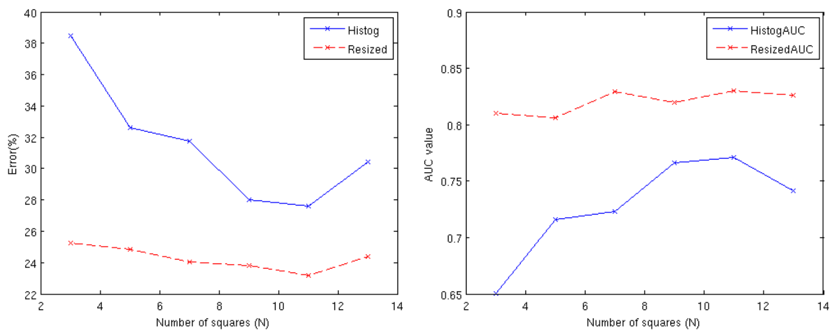

Fig. 5. Descriptor error and AUC values for different values of N

The errors obtained classifying with NCSR and NCSH when different values of N used can be seen in figure 5. In this case the classification was carried out with NN. It is possible to appreciate that the error decreases with higher values of N, from $N = 1$ to $N = 11$. The behavior for the AUC is the opposite, also obtaining the best values for $N = 11$. In this figure is clear the performance of NCSR is much higher than NCSH so our choice for this problem has been CSR with $N = 11$.

5.2 Errors and ROC curves

Descriptors	KnnError	KnnError_0	KnnError_1	AUC	NNError	NNError_0	NNError_1	Std	Std_0	Std_1
NCSR	25.22%	43.30%	11.11%	0.8299	**23.20**%	26.81%	20.38%	0.78	1.70	1.25
Haralick13	31.30%	34.35%	28.92%	0.8297	**24.07**%	35.01%	15.53%	0.80	1.38	0.64
PS10Norm	28.09%	36.47%	21.56%	0.8092	**24.93**%	41.17%	12.27%	0.43	1.17	1.34
PS20Norm	30.84%	38.46%	24.89%	0.8053	**25.14**%	41.48%	12.40%	0.72	1.17	1.44
PS10	28.46%	40.74%	18.89%	0.8032	**25.24**%	38.90%	14.60%	0.63	1.47	0.60
NCSH	27.09%	24.79%	28.89%	0.7707	**27.63**%	31.88%	24.31%	1.38	1.90	1.74
FlusserGray	44.57%	46.72%	42.89%	0.7751	**28.63**%	44.04%	16.60%	1.17	3.08	1.97
Zernike	32.21%	46.44%	21.11%	0.7453	**30.55**%	39.51%	23.56%	0.98	1.79	1.29
HuGray	31.59%	40.74%	24.44%	0.7318	**32.44**%	50.66%	18.22%	0.83	3.14	2.02
HuBW	43.82%	51.00%	38.22%	0.6096	**39.50**%	68.82%	16.62%	1.06	3.41	3.06
FlusserBW	44.44%	47.29%	42.22%	0.5872	**40.47**%	79.60%	9.96%	0.54	2.21	1.49

Table 1. Errors using kNN and NN classifiers arranged by NNError column. Class 0 stands for dead spermatozoa and class 1 for alive ones.

Table 1 summarize the errors obtained using kNN, Neural Networks and the AUC value for each descriptor. The table is arranged in ascending order from the global error rate yield by the NN point of view. This order is almost the same, but descending, when the AUC criteria is used. It is possible to observe the errors provide by kNN do not correspond exactly with the yield by the NN classifier but, in both cases, one of the proposal descriptors, NCSR, outperforms to the rest of descriptors. The same behaviour can be seen in the Receiver Operating Characteristic (ROC) curve 6. Whereas there are several descriptors with a high accuracy, as the Haralck13, the WSF or the NCBR, the proposed descriptor with $N = 11$ surpasses the rest methods in the interval of specificity $[0.1, 0.4]$, been similar or slightly lower to Haralick13 in the other parts of the curve. That values jointly with the error rates obtained allow us to say that the proposed descriptor has a better performance than the other ones in this context.

6 Conclusions

We have proposed two new texture descriptors named NCSR (N Concentric Squares Resized) and NCSH (N Concentric Squares Histogram) for describing the texture present in the images taken of boar spermatozoa heads. A first evaluation of which one was the best value of N for this problem was carried out. Later, we have computed these descriptors and we have use them for classify the spermatozoa heads as dead or alive. The results obtained have been compared with a number of classical texture descriptors as Haralick, Pattern Spectrum, WSF, Zernike, Flusser and Hu, using both kNN and a backpropagation Neural Network. The results illustrate that one of the proposed descriptors, NSCR, outperforms the others using both the error rate criteria and the accuracy seen at the ROC curve.

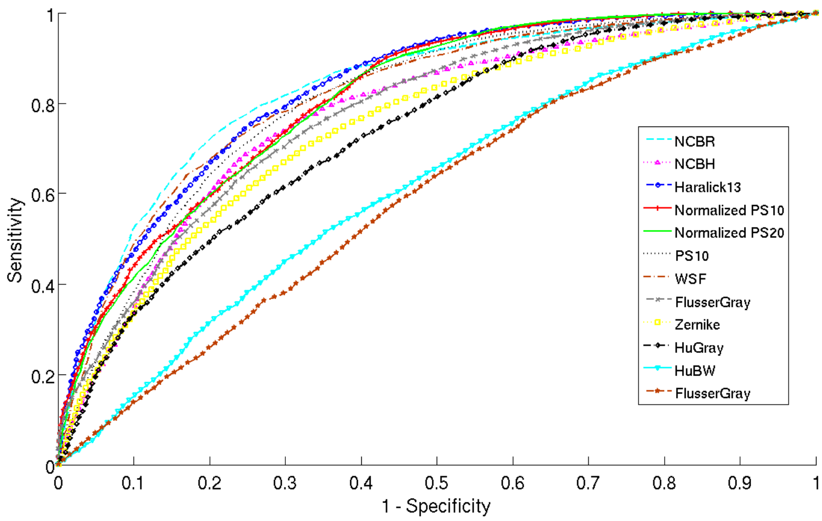

Fig. 6. ROC Curve for descriptors comparison

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