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Algorithms for automatic segmentation of bovine embryos produced *in vitro*

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Abstract. In vitro production has been employed in bovine embryos and quantification of lipids is fundamental to understand the metabolism of these embryos. This paper presents a unsupervised segmentation method for histological images of bovine embryos. In this method, the anisotropic filter was used in the different RGB components. After pre-processing step, the thresholding technique based on maximum entropy was applied to separate lipid droplets in the histological slides in different stages: early cleavage, morula and blastocyst. In the post-processing step, false positives are removed using the connected components technique that identify regions with excess of dye near pellucid zone. The proposed segmentation method was applied in 30 histological images of bovine embryos. Experiments were performed with the images and statistical measures of sensitivity, specificity and accuracy were calculated based on reference images (gold standard). The value of accuracy of the proposed method was 96% with standard deviation of 3%.

1. Introduction

In vitro production (IVP) has been employed to improved features genetics of bovine embryos [1]. However, the increase in the levels of cytoplasmic lipid droplets in the IVP of bovine embryos are relationship reduction of embryos after cryopreservation [2]. Therefore quantify the lipids is fundamental to understand the metabolism of these embryos.

Some methods are applied to evaluate and measure the features of lipids in the investigation of IVP of bovine embryos. Among the techniques applied, the Sudan black B staining is a method that consists of a lipophilic dye, which reacts with lipids. Regions with lipids are represented by darker colours compared to the rest of the embryo without lipid droplets. After Sudan black B staining of bovine embryos, a microscope equipped with digital camera is used to acquire images [3].

Some computational techniques have been applied to quantification of lipid droplets in images of bovine embryos [3, 4]. However, the accuracy rate is low and these methods are supervised. Thus, quantification of lipid by Sudan black B staining becomes one of the difficulties for the specialists.



This paper presents a unsupervised segmentation method for histological images of bovine embryos. In this method, the anisotropic filter was used in the RGB colour components. After pre-processing step, the thresholding technique based on maximum entropy was applied to separate lipid droplets in the histological slides in different stages: early cleavage, morula and blastocyst. In the post-processing step, false positives are removed using the connected components technique that identify regions with excess of dye near pellucid zone. The proposed segmentation method was applied in 30 histological images of bovine embryos.

2. Materials and Methods

The method was organized in three steps: (1) Pre-processing, (2) Segmentation and (3) Post-Processing. Each step is described in detail in the following subsections.

2.1. Image Database

The database image used in the present study was made up of 30 histological slides in different stages: early cleavage, morula and blastocyst. The images were acquired in the Laboratory of Cellular and Molecular Biology of the Federal University of ABC. The microscopic images were acquired using an inverted light microscope Olympus IX71 with digital camera coupled (Lumenera Innity 1-1). Each slide was photographed at a magnification of 100x, using 10x objective lens and ocular lens and image was saved in .png using the RGB model, 2048x1536 pixels, with 24 quantization bits.

2.2. Pre-processing

In the mounting process of each slice, fragments, dyes and other parts of the embryo can leak out of the zona pellucida. The algorithm considers these problems by calculating the center of the image and considering only the embryo centered. In this step, the pixels of the image were compared with the coordinate of the center and the values were used to calculate the hypotenuse. Regions with value 22% greater than the image area were removed.

Anisotropic diffusion filter can be used to smooth the outer regions of the edge giving a blur effect and highlight the edges with a sharp effect keeping unchanged the inner regions of the edges [5]. In this work, we applied this filter using the equation 1:

$$I(s, t + 1) = I(s, t) + \frac{\lambda}{|\eta_s|} \sum_{p \in \eta_s} g(|\nabla I_{s,p}(t)|) \nabla I_{s,p}(t) \quad (1)$$

where $I(s, t)$ is the image, s is the pixel position, t is the number of interactions, η_s is the set of neighbors space, λ is the diffusion speed, $I_{s,p}(t)$ is the gradient magnitude of the image I at the point s in the direction (s, p) in iteration: $t \nabla I_{s,p}(t) = I(p, t) - I(s, t), p \in \eta_s$. In this paper, we used parameter g defined by equation 2 .

$$g_2(x) = \exp \left[\frac{-x^2}{2\sigma^2} \right] . \quad (2)$$

In equation 1, $t = 20$, $\lambda = 0.2$ and in equation 2, $\sigma = 15$.

2.3. Segmentation

In this step, a global thresholding was used, considering the maximum entropy technique. This technique maximize entropy of the image dividing the histogram into two probability distributions [6]. Each distribution represents one of the classes: background and object. The entropy $H_b(T)$ and $H_w(T)$ associated to the pixels of the background and objects, respectively, are expressed by

$$H_b(T) = - \sum_{i=0}^T p_i \log p_i \quad (3)$$

$$H_w(T) = - \sum_{i=T+1}^{L-1} p_i \log p_i \quad (4)$$

where n_i is the number of pixels with gray scale i , p_i is the probability of gray scale i found in the image, n is the number of pixels of the image and L is the number of gray scales of image.

The gray scale is analyzed in order to find a threshold value T , such that $T = H_b(T) + H_w(T)$ is maximum. This technique defines a good separation between the object and the background of the image, $T = \arg \max[H_b(T) + H_w(T)]$

2.4. Post-Processing

The connected components technique was used to identify all segmented regions and remove the regions that do not represent lipids: the 4-adjacency technique was used to remove regions false-positives [7].

2.5. Quantitative Evaluation

The quantitative evaluation was performed by calculating the overlap between the image regions segmented by the proposed method and the regions of a binary reference demarcated by a specialist. This calculation was performed using statistical measurements of sensitivity (SE), specificity (ES) and accuracy (ACC) ([8]).

3. Results and Discussion

The proposed segmentation method was applied to an image bank with 30 samples and measures of sensitivity (SE), specificity (SP) and accuracy (ACC) were calculated. Figure 1 respectively shows an image of embryo which was manually segmented by a specialist (gold standard) and with the proposed method. Table 1 shows the measures considering sensitivity (SE), specificity (SP) and accuracy (ACC).

The best ACC results (proportion of pixels defined as correctly segmented, both true positives and true negatives) were obtained with the R colour component for the Early cleavage, Morula and Blastocyst stages. Also, the proposed method provided segmented images with high rates of SE and SP . In this study, sensitivity relates to the method's ability to identify positive areas or belong to a specific stage such as Early cleavage (SE of 94%), Morula (SE of 96%) and Blastocyst (SE of 92%). These values were obtained with the B colour component. The method's ability to identify negative results (specificity) also is important: Early cleavage with 87%, Morula with 78% and Blastocyst with 88%, where these values were obtained with R colour component. These measures suggest that textural features from bovine embryos images are sufficient to separate lipids on different stages. Segmentation methods have been proposed for investigation on different histological images but are not yet applied to bovine embryos. This is a limitation for comparisons of our results. So, the proposal presented here provides a significant contribution to studies focusing on IVP of bovine embryos.

4. Conclusion

This paper presented an unsupervised method for the automatic segmentation of bovine embryos images considering different stages: early cleavage, morula and blastocyst. The proposed method is effective for the segmentation of embryos images, considering the quantitative results calculated. Also, the method offers the advantage of making automatically the segmentation.

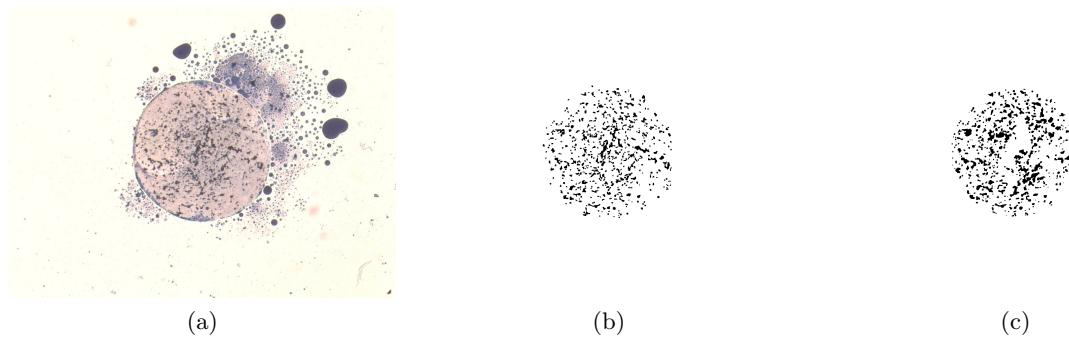


Figure 1: Sample of an embryo is shown (a), the segmentation provided by a specialist is shown in (b) and the result obtained with the proposed method is illustrated in (c).

Table 1: Measures of sensitivity (SE), specificity (SP) and accuracy (ACC) obtained for each stage and colour component.

Stages	Colour Channel	ACC	SE	SP
Early cleavage	Red	0.92 ± 0.04	0.92 ± 0.04	0.87 ± 0.12
	Green	0.91 ± 0.03	0.91 ± 0.04	0.83 ± 0.22
	Blue	0.93 ± 0.04	0.94 ± 0.04	0.68 ± 0.32
Morula	Red	0.93 ± 0.04	0.93 ± 0.04	0.78 ± 0.30
	Green	0.94 ± 0.04	0.95 ± 0.04	0.66 ± 0.33
	Blue	0.96 ± 0.04	0.96 ± 0.04	0.56 ± 0.32
Blastocyst	Red	0.92 ± 0.04	0.92 ± 0.04	0.88 ± 0.14
	Green	0.92 ± 0.04	0.92 ± 0.04	0.84 ± 0.22
	Blue	0.92 ± 0.04	0.92 ± 0.04	0.83 ± 0.24

In future studies, performance tests will be performed and the results compared to others segmentation methods.

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