Conference Paper

Counting the Amount of Spermatozoa Active Perframe Video Using Morphology and Local Adaptive Threshold

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Abstract

Sperm analysis is a preliminary examination performed on male infertility cases, one of which is determining normal and abnormal motility, performed by the expert. This sperm analysis can also be done automatically with computer-assisted, that is by taking per-frame sperm video then done segmentation process. In the process of segmentation there are some constraints such as video data taken has different intensity, so it takes some segmentation method. This study compares the process of Morphology and Local Adaptive Threshold (LAT) process, with the same preprosessing process using Otsu segmentation, so that from some segmented sperm video frame data will be known which segmentation should be used. From the result of the research, it is found that the intensity of light in the sperm frame is analyzed evenly, morphology can be used and if the sperm image in uneven intensity analysis will be more effective using Local Adaptive Threshold (LAT), thus to perform better feature extraction, but from some frames of sperm that were randomly tested obtained more accuracy using morphology process that is 82% compared to using local adaptive threshold process that is only 52%.

Keywords: Local Adaptive Threshold, morphology, motility, otsu, segmentation, sperm

INTRODUCTION

The process of sperm analysis automatically one of them is to do the process of sperm video segmentation obtained from the microscope through the camera. Camera video capture results do not completely get a picture that the intensity of its light evenly, sometimes between the background and sperm images have the same intensity. Spermmatozoa segmentation can be done using the Marker-Controlled Watershed Segmentation method (Abbiramy, 2010), the use of the segmentation method because the image used is data derived from public data, so any segmentation method used will produce a good segmented image.

Similarly, in the image binarization process, the process of separating pixel values into two groups, white as background and black as foreground (Singh et al., 2011). So Thresholding plays a major in image binarization. compared to global thresholding and local thresholding, the image data used there are some of them are document images, with a uniform contrast distribution of background and foreground, so it is said more precise global

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thresholding. Then again segmentation of degraded document images, where sufficient background intensity or variations in contrast and exposure exist, there are many pixels that can not be easily classified as foreground or background. In such cases, the binarization with local thresholding is more appropriate. Then again segmentation of degraded document images, where sufficient background intensity or variations in contrast and exposure exist, there are many pixels that can not be easily classified as foreground or background. In such cases, the binarization with local thresholding is more appropriate then the Gray Scale Image process using automatic contrast stretching (Adjust Image Intensity and Histogram Equalization), the next process is arithmetic operation, filtering process and global threshold technique so as to be able to segment the image of blood cells and count them.

Hand geometry is one of the most commonly used biometric types in authentication systems . Binary image binary process, determination of threshold value for binary process using Otsu method. This method determines the threshold value by using the dithrriminant analysis. The input image is a gray-level 256 gray color image with a black background. Based on the results of experiments performed, Otsu method is able to provide binary hand image results are very satisfactory. To see the success, in this paper also included comparison of binary process results by using image processing application.

From several previous studies, several segmentation processes using various methods have been done, with the conclusion that the method of global segmentation threshold (Otsu Method) with Local Adaptive Threshold method can be used in accordance with the needs of the image or image to be segmented. In this paper we will discuss the application of local threshold otsu and adaptive methods to the segmentation of sperm morphology, in order to obtain the best results from sperm form or morphology, since data obtained from digital microscopes are not all good.

METHODS

The stages of the proposed method of sperm segmentation are shown in Fig. 1.

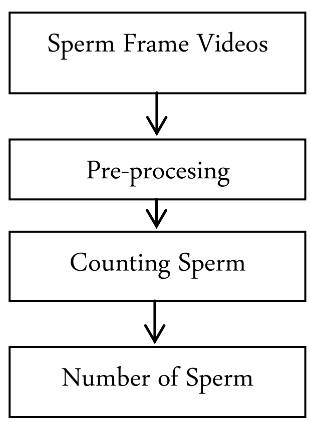


Figure 1. System design to calculate the number of sperm per video frame

Sperm frame videos

Sperm data used is data from one of the patients who perform fertility tests in the laboratory Poltekes Surabaya. In accordance with the WHO standard sperm test procedure 2010, sperm taken directly from the patient after ejaculation 10-20 minutes. Then the sperm that have been lyufeksi, processed, as Figure 2, so obtained sperm video.

Pre-procesing

Preprocessing is the original data processing before the data (sperm video frame) is processed (labeling process and sperm count). The purpose of this process is to eliminate noise, clarify data features, minimize / enlarge data size, convert original data to obtain data as needed. In Figure 3, the process of pre-processing of sperm analysis is shown.

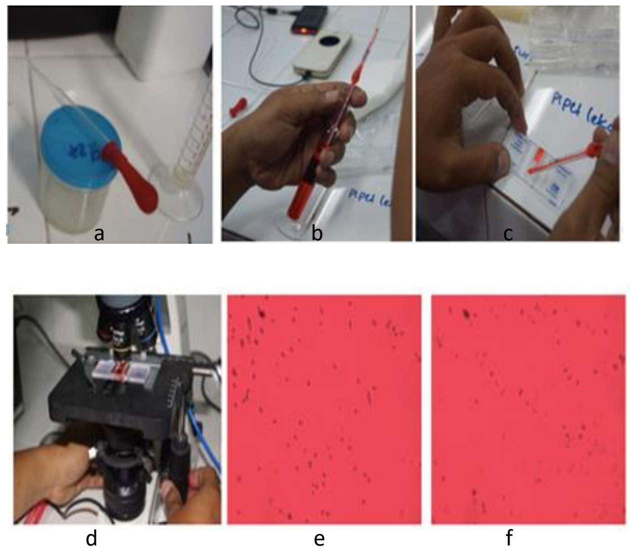


Figure 2. a. Sperm that be tested, b. the mixing process with 0.5% eosin solution and stirred evenly, c. Dripped on count room neubauer, d. viewed on microscope, e and f, result of sperm capture on camera

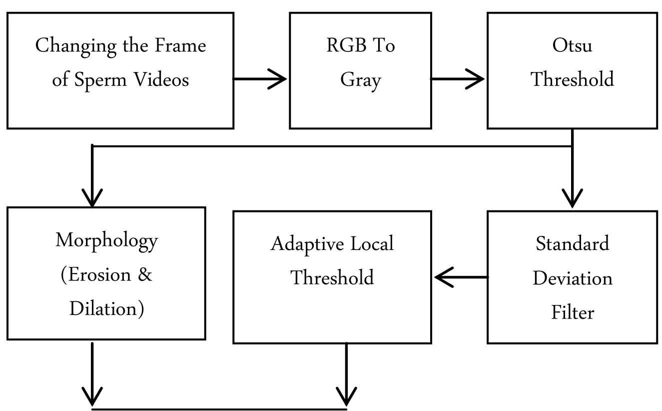


Figure 3. The process of pre-processing sperm analysis

RGB 2 Gray

It is a digital image that has only one channel value on each pixel, meaning the value of Red = Green = Blue. These values are used to indicate the intensity of color (Otsu, 1979). The images displayed from this type of image are of a gray color, varying in black on the weakest intensity and white in the strongest intensity.

The grayscale image is different from the "black-and-white" image, which in the computer context, the black-and-white image consists of only two colors: "black" and "white" only. In the grayscale image the color varies between black and white, but the color variations are very numerous. Grayscale images are often a calculation of the intensity of light on each pixel on a single band's electromagnetic spectrum.

Grayscale images are stored in 8 bit format for each pixel sample, allowing as many as 256 intensities. To change the colored image having the matrix value of each R, G and B be grayscale image with X value, then the conversion can be done by taking the mean of R, G and B values so that it can be written to:

$$X = \frac{(R+G+B)}{3} \quad (1)$$

$$\text{Color} = \text{RGB}(X, X, X) \quad (2)$$

Binary Image

Binary image is a digital image that has only 2 possible colors, namely black and white. Binary imagery is also called W & B (White & Black) image or monochrome image. It takes only 1 bit to represent the value of each pixel from a binary image. Binary image formation requires a gray boundary value to be used as a benchmark value. Pixels with grayscale greater than boundary values will be assigned a value of 1 and otherwise pixels with a gray degree smaller than the boundary value will be assigned a value of 0.

Binary imagery often arises as a result of processing, such as segmentation, mining, morphology or dithering. The function of binaryization itself is to facilitate the pattern recognition process, because the pattern will be more easily detected on images containing less color. The equations for binaryization can be seen in the following formula:

$$\int(x, y)^1 = \begin{cases} a_1, f(x, y) < T \\ a_2, f(x, y) \geq T \end{cases} \quad (3)$$

The value of a_1 is usually 0 dan the value a_{12} = 1

Otsu Threshold

Image thresholding is a widely used technique for segmentation, which divides gray level images into segments that are appropriate for some classes based on gray level values. Most thresholding approaches are proposed for two classes, and are based on the gray level histogram of the image (Hidayatullah and Zuhdi, 2014). In this research, thresholding is not used for image segmentation as the final destination, but as a reference point in maximizing contrast for maximum background and foreground distinctions. Threshold divides the area into two classes as follows:

$$g(x, y) = \begin{Bmatrix} 0 & f(x, y) < T \\ 1 & f(x, y) >= T \end{Bmatrix} \quad (4)$$

The purpose of the Otsu method is to find the threshold point that divides the gray level graphic histogram into two different regions automatically, where the selected point is such that the inter-class variance is as large as possible. This goal is achieved by minimizing the weight of within-class variance, which is actually the same as: maximizing inter-class variance. The weights of within-class variance are:

$$\sigma_w^2(t) = q_1(t)\sigma_1^2(t) + q_2(t)\sigma_2^2(t) \quad (5)$$

With: $\sigma_w^2(t)$ is the total within-class variance, $\sigma_1^2(t)$ is within-class variance of the first class, is $\sigma_2^2(t)$ within-class variance of the second class, $q_1(t)$ is class probabilities of the first class, $q_2(t)$ is the class probabilities of the second class t is the value of the gray level ie the threshold value. The class probablilites of the first and second classes are (where represents the number of pixel occurrences for a given gray-level, whereas the gray level). Within-class variance of each class are:

$$\sigma_1^2(t) = \sum_{i=1}^{t}[i - \mu_1(t)]^2 \frac{P(i)}{q_1(t)} \quad (6)$$

$$\sigma_2^2(t) = \sum_{i=1}^{t}[i - \mu_2(t)]^2 \frac{P(i)}{q_2(t)} \quad (7)$$

If we calculate all values of $\sigma_1^2(t)$ and $\sigma_2^2(t)$ on the gray scale image we will get the minimum within-class variance value. The t value that makes $\sigma_1^2(t)$ and $\sigma_2^2(t)$ be the minimum is the threshold value sought. However, the computation process can be saved by utilizing the fact that the total variance of an image is always constant, independent of the threshold value. So for any threshold t, the total variance is the sum of within-class variance and inter-class variance:

$$\sigma^2 = \underbrace{\sigma_w^2(t)}_{\text{withinclas s variance}} + \underbrace{q_1(t)[1-q_1(t][\mu_1(t)-\mu_2(t)]^2}_{\text{interclass variance} = \sigma_B^2(t)} \quad (8)$$

Here is a Pseudo code to calculate the $\sigma_B^2(t)$ value in Equation 5 to perform the threshold process in the sperm video frame is as follows:

1. Calculates the histogram and probability of each intensity level (ie normalization of the histogram so that the total is equal to one).
2. Set initial value $q_1(t)$, $q_2(t)$ and $\mu_1(t)$, $\mu_2(t)$
3. Explores all possible threshold values $t = 1 \ldots$ maximum intensity
   a. Update the value $q_1(t)$, $q_2(t)$ and $\mu_1(t)$, $\mu_2(t)$
   b. Count $\sigma_B^2(t)$
4. The searched threshold is the one that gives the maximum $\sigma_B^2(t)$.

Morphology (Delation & Erosi)

Morphology is a technique of digital image processing using shape (shape) as a guide in processing. The value of each pixel in the resulting digital image is obtained by comparing the corresponding pixel to the input digital image with its neighboring pixel. Morphological operations depend on the order of occurrences of pixels, not taking into account the numerical value of pixels so that morphological techniques are appropriate when used to perform binary image and grayscale image processing (Kaula et al., 2009). By adjusting or selecting the size and shape of the structuring element used, we can adjust the sensitivity of the morphological operation to a particular (specific) shape to the input digital image. Standard morphological surgery is a process of erosion and dilatation. Dilatation is the process of adding pixels to the boundary of an object in the digital image of the input, while the erosion is the process of removal / pixel reduction at the boundary of an object (Nemane et al., 2013). The number of pixels added or omitted from the object boundary in the input digital image depends on the size and shape of the structuring element used (Maini and Aggarwal, 2010)

Dilation is a morphological operation that will add pixels to the boundaries between objects in a digital image. This operation uses the following rule: "For a grayscale image then the result value of the operation (pixel output) is the maximum value obtained from the neighboring pixel set. In binary image, if there is a neighboring pixel of 1 then the pixel output will be set to 1 ".

Erosion is a morphological operation that will reduce the pixels at the boundaries between objects in a digital image. This operation uses the following rule: "For a grayscale image then the result value of the operation (pixel output) is the minimal value obtained from the set of pixels. In binary image, if there is a neighboring pixel of 0 then the pixel output will be set to 0".

RESULT AND DISCUSSION

The application results from this sperm analysis can be described as follows;

1) Sperm data used sperm video one of the real patients. Sperm video data is processed and taken frames per frame as much as 100 frames sperm. Examples of sperm used can be seen in Figure 4.

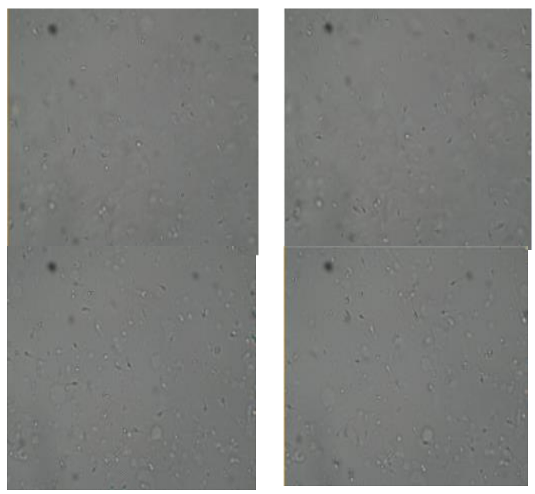
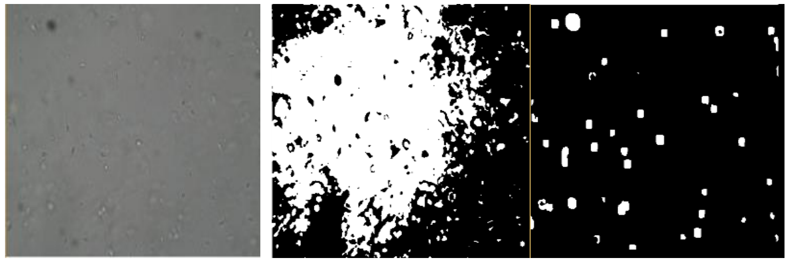


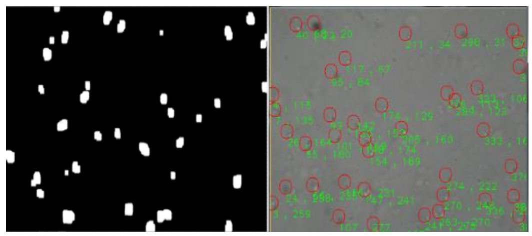
Figure 4. Sampel Frame of processed sperm

2) Then do the process of pre-processing. In this process is done frame conversion into grayscale image, process continued by removing noise. In this process the mean filter is used, as it gives better results than the median filter.
3) The next step is to change the frame into binary images, and segmented using the otsu threshold.
4) The next process tries to apply some methods to do this process. From process (3) above will be compared result using Local Adaptive Threshold by using morphology. The results of the comparison can be seen in Figure 5.

Figure 5 (a) is a sperm video frame data taken and to be tested, in the original image it is seen that not only sperm cells are caught, but also leukocytes or others other than sperm, in the form of white or black clumps in the frame, it can mask the original sperm image and sperm can not be caught in some parts, so the data can sometimes be said to be sperm but sometimes unreadable as sperm, and this is one of the system deficiencies.



a b c



d e

Figure 5. (a) Sperm frames (b) otsu threshold results (c) local adaptive threshold results (d & e) result with morphological process

The result of segmentation by using otsu threshold, not suitable to be used in image which have same color spread. In Figure 5. (a) The image is dominated by a gray color that has the same value as the color of sperm cells, making it more difficult to recognize sperm. From the results of segmentation using the method otsu threshold can be seen in Figure 5 (b). The Otsu method is good for finding a single boundary and separating the color of a single sperm from the background. However, the background and sperm cells have almost the same color. The color of semen also has a high variant, so the process of otsu threshold is continued by comparing between using morphological process and by using Local Adaptive Threshold, the result can be seen in Table 1.

5) Adaptive local threshold gives better results (Figure 5 (c)) because it is adjusted in every kernel m x m for each region. Nevertheless, this method provides a thick sperm appearance that is useful for separating sperm cells against the background. The method used has adaptive properties that include local average, local standard deviation and global deviation standard. The result of segmentation by using Local Adaptive Threshold can be seen in Table 1, it can be seen that the average of tested video sperm frame, the reading result manually and system still many mismatch, so the average error is 52%.
6) As for the results of the process by using the morphological process of the otsu threshold results in Fig. 5 (d), it appears that the average of sperm video frames tested, the readings manually and the system are still much corresponding, so the average error is 82%.
7) The above average results (52% and 82%) are obtained systemally after a separate image then calculated the contours of (Fig. 5 (c) and 5 (d)). Look for a value of a set of pixels greater than 25 pixels. From the search 25 pixels obtained sperm position and sperm count as (Figure 5 (e)). Finding the error threshold by testing is less effective because of the results do not represent the character of the sperm.

Tabel 1. Experimental results, calculating manual sperm and system

Video/Frame	Total quantification manual of spermatozoa (M)	Morphology					Local Adaptive Threshold				
		TP	*FP*	*FN*	*TN*	$\frac{TP}{M}$ (100%)	*TP*	*FP*	*FN*	*FN*	$\frac{TP}{M}$ (100%)
Real1-Frame1	49	41	2	10	*N/A*	84	45	22	4	*N/A*	92
Real1-Frame4	58	48	0	10	*N/A*	83	2	1	56	*N/A*	3
Real1-Frame7	53	45	3	11	*N/A*	85	3	3	50	*N/A*	6
Real1-Frame15	64	58	3	9	*N/A*	91	18	4	46	*N/A*	28
Real1-Frame28	52	41	1	12	*N/A*	79	5	3	47	*N/A*	10
Real2-Frame1	63	39	2	26	*N/A*	62	35	62	28	*N/A*	56
Real2-Frame2	71	62	0	9	*N/A*	87	60	33	11	*N/A*	85
Real2-Frame7	67	58	1	10	*N/A*	87	53	20	14	*N/A*	79
Real2-Frame15	37	26	2	13	*N/A*	70	25	57	12	*N/A*	68
Real2-Frame28	53	50	1	4	*N/A*	94	47	57	6	*N/A*	89
		Rata-rata				82					52

CONCLUSION

This research shows several results, among others:

1) To segment the sperm image using otsu method, do not get good segmentation result. Because the sperm image with the background has almost the same color value. So the otsu method that calculates the spread of the whole image, is not suitable for use
2) Local Adaptive Threshold method is suitable to be used as a threshold on images that have almost the same value density. From the results obtained sperm image has been separated from the background even though the color values are almost the same. Yet another image like dust is also caught
3) Of the few frames (frames) sperm tested at random obtained more accuracy using morphology process that is 82% compared with using Local Adaptive Threshold process that is only 52%.
4) From the segmentation results can already be determined sperm position and the number of sperm that can be calculated by the method of contour but still a lot of noise caught like dust or leukemia cells in the microscope

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