TELKOMNIKA, Vol.16, No.4, August 2018, pp. 1642~1649
ISSN: 1693-6930, accredited First Grade by Kemenristekdikti, Decree No: 21/E/KPT/2018
DOI: 10.12928/TELKOMNIKA.v16i4.8685

Bull Sperm Motility Measurement Improvement using Sperm Head Direction Angle

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Abstract

It is well known that sperm motility is the most important parameter in measuring sperm quality. Sperm motility can be measured manually by a medical veterinarian or using Computer Aided Sperm Analysis (CASA) system. Measuring bull sperm motility manually is the most popular method nevertheless it has major drawbacks which are subjective and high variability. CASA gives consistent results, hence it is an expensive system. Therefore, an alternative method is required. In this paper, a method to measure bull sperm motility was proposed, It was using ellipse detection and sperm head direction angle. The video samples were taken using a digital microscope. Compared with the manual assessment by medical veterinarians, the proposed method gave the average absolute margin of 5.16% which surpassed the previous method which gave 10.69%.

Keywords*: Bull sperm quality measurement; Sperm motility; Ellipse detection; Computer aided sperm analysis*

1. Introduction

Indonesia is a high population country. Therefore, it has enormous food consumption including beef meat. Indonesia's government took necessary steps to ensure that Indonesia will be able to meet its own food su:lies [1]. One of the steps taken by the government for food self-sufficiency is initiating food research and development agency such as Artificial Insemination Center (*Balai Inseminasi Buatan*/BIB).

Important activities in the processing of frozen semen (sperm and plasma) include analysis of sperm quality. The Analysis must be carried out before semen is distributed to local insemination centers. There are several parameters need to be analyzed such as semen volume, sperm quantity, sperm morphology, sperm motility, and sperm viability. In this research, sperm motility is chosen as it is the main parameter in measuring sperm quality.

The process of measuring bull sperm motility is as follows: The first step is to read the video file and divide it into a number of digital image frames. From each frame, sperm will be identified based on its physical characteristics. After the sperm is identified then the location of the sperm is stored. However sperm location is not sufficient to more precisely track sperm in video frames. Therefore we need more parameters to increase tracking precision. This information is important to predict the location of the sperm in the next frame.

Sperm analysis can be su:orted by Computer Aided Sperm Analysis (CASA). However, the cost of commercial CASA is relatively high. In Indonesia, only big BIB has a commercial CASA. Therefore a CASA alternative is required. The developed CASA followed WHO categories [2] and Indonesia nasional standard for bull semen evaluation [3]. It was able to measure: Progressive Motility (PR), Non-Progressive Motility (NP), and Immotility (IM).

Sample videos was acquired from the existing system in BIB Lembang. A microscope was equi:ed with high definition camera to provide a video stream to a monitor. The video stream was ta:ed by video grabber which then processed with digital image processing

Received January 21, 2018; Revised April 3, 2018; Accepted June 1, 2018

program. Sperms were detected from the video by utilizing ellipse detection algorithm. The motility of detected sperms is recorded by using matching based algorithm. A modification of matching based algorithm based on sperm head direction angle was proposed to improve the performance. The motility of the detected sperms was measured based on their trajectory which was the result of the improved matching based algorithm

2. Previous Work

In the previous work [4], digital video was acquired directly from a digital camera or video grabber. It produced a video file that will later be split into several frames. For each frame, sperm detection algorithm was a:lied. This algorithm included image conversion into a grayscale image. A threshold value was used to get a binary image from a grayscale image. Shapes in the obtained binary image was opened or closed to form clear shapes. These shapes were then detected to find their edge by using canny edge detection algorithm. Adaptive local threshold and ellipse detection algorithms were then used to find sperms. The location of these sperms was then recorded to calculate sperm quality parameters.

The detection was quite successful. Nevertheless, location recording was a big obstacle [4]. It was not always successful due to the following reasons:

a. Adjacent frames do not always provide the continuous a:earance of sperm.
b. Collided sperms were not recorded correctly since there is no guarantee of the direction of sperm movement.

This work motility measurement result differs about 7% with medical veterinarian's judgment [4]. In some studies, one of the problems that remains the center of attention is to find a reliable method for knowing the sperm motion path [5]. Especially if there are some sperms whose positions are close together or collide each other. Actually, dilution of semen has the effect of reducing the probability of a collision between sperm. Nevertheless, in real cases in BIB Lembang, high concentration semen is often used in measuring sperm quality.

There were several a:roaches to solve this collision problem which were the center-weighted a:roach, the correspondence a:roach, the predictive a:roach, and probabilistic a:roach based on Bayesian theory [6]. Nevertheless, in order to improve the sperm quality measurement, Imani et.al. [7] proposed a new method to track multiple human sperms. It is called Matching-Based algorithm which was optimized with Hungarian Algorithm to find the correct track of sperm including when the sperm is missing in the adjacent frames. The algorithm a:lies weighting based on sperm movement distance for adjacent frames. The result was encouraging with 96.76% accuracy. Nevertheless, it was effective in case studies with not more than 10 sperms in each frame.

Jian Shi et.al. [8] used a different method to a:roach the measurement, that is Fireworks algorithm. This method triggers an initial location of an explosion and eliminates it with the specific border condition, Zhang et.al. [9] increased the robustness of tracking by failure recovery which handled changes in scale, illumination, a:earance, and occlusions.

3. Proposed Method

The proposed method consists of several stages that generally perform sperm detection using modified ellipse detection, then tracks sperm motion using matching based algorithm by adding sperm head motion direction angle parameter. The proposed method can be illustrated in Figure1 [10].

3.1. Binarization Modification and Ellipse Detection

By the binary processing of image, the global standard deviation was modified, due to the samples were much poorer image clarity than the samples in [4] and [11]. From the experiments, the best result is obtained by reducing the global standard deviation’s value with the average of 35%. Then the minimum and maximum length of the major axis is set by 6 and 13 pixels in the ellipse detection method based on the experiments performed.

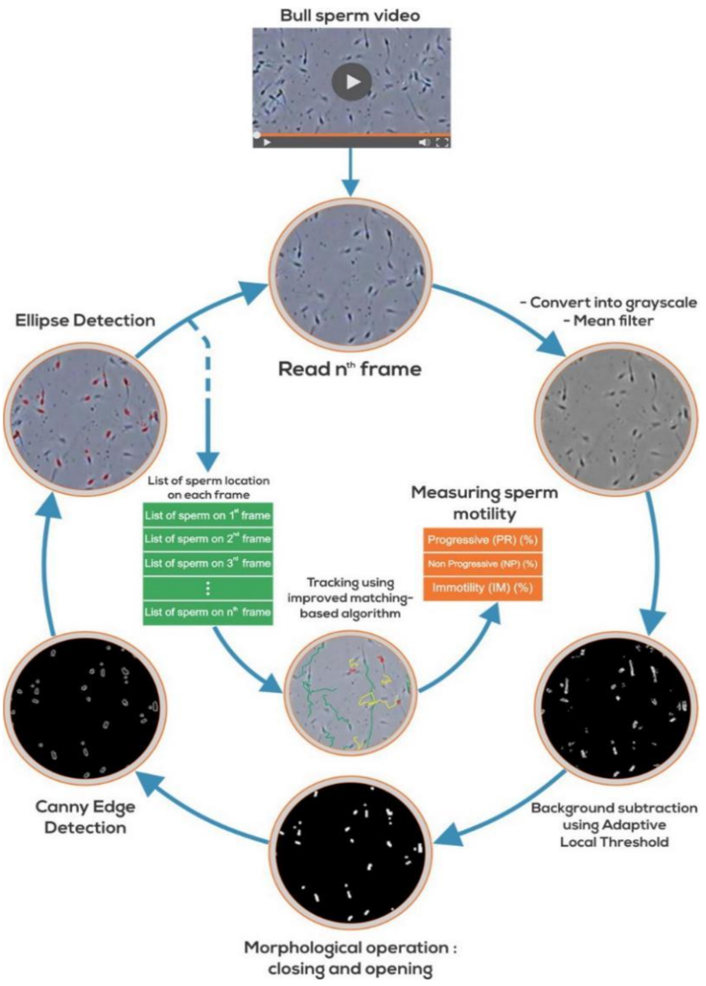


Figure 1. Overview of the proposed method [10]

3.2. Determination of Sperm Head Directions Angle

Determination of the sperm head direction angle was performed by gaining orientation angle and edge of the sperm head based on ellipse detection step [12]. The process of determining the direction of the sperm head can be written in the following steps:

1) Detected elliptical objects was rotated parallel to the x-axis (horizontal) based on the orientation angle,
2) The elliptical objects were divided into 3 equal parts (Figure 2) and both ends of the parts were taken (right as area-1, left as area-2).

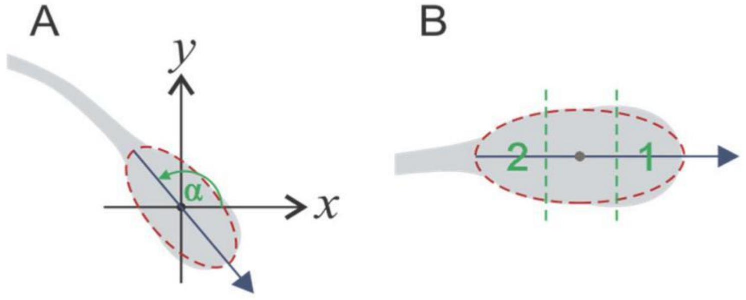


Figure 2. Sperm head division [10]

3) Calculating the fitness value (*fitness$_k$*) on both ends of the section to find out which part a:roaches the ellipse shape, by using equation of horizontal elliptic (1) and comparing the value of expected y with the actual value of y (*y'*).

$$\frac{(x-h)^2}{a^2}+\frac{(y-k)^2}{b^2}=1 \quad (1)$$

$$y=\pm\left(\sqrt{b^2\left(1-\frac{(x'-h)^2}{a^2}\right)}\right)+k \quad (2)$$

$$fitness_k=\left(\sum_{i=0}^{M_k} 0^{\left\lfloor\frac{\left|y'^{i}_{k}-y^{i}_{k}\right|}{y_{tolerance}}\right\rfloor}\right)^{\frac{1}{M_k}} \quad (3)$$

4) The direction of the sperm head (*direction*) is determined by using (4) based on the fitness value. Direction to which the sperm head towards is indicated by the smallest fitness value[10].

$$direction=\begin{cases}0, & fitness_2>fitness_1\\ 1, & else\end{cases} \quad (4)$$

5) The direction angle (θ) of the sperm head motion is determined based on the orientation angle and direction of the sperm head using (5).

$$\theta=\alpha-(180^\circ\times direction) \quad (5)$$

3.3. Improved Matching-Based Algorithm

Matching between the sperm cells to the entire sequence of frames is required for tracing the multiple sperm cells in a semen observation video. These were conducted by using the improved matching based algorithm [7]. The improvement of the algorithm was performed by adding sperm head direction angle in sperm weighting and matching with the frames respectively.

$$w_{d_{p,q}}^{i,j}=\begin{cases}1-\frac{\left|d_p^i-d_q^j\right|}{d_{max}}, & \left|d_p^i-d_q^j\right|<d_{max}\\ 0, & else\end{cases} \quad (6)$$

$$w_{\theta_{p,q}}^{i,j}=\begin{cases}1-\frac{\Delta\theta_{p,q}^{i,j}}{\theta_{max}^{d}}, & \Delta\theta_{p,q}^{i,j}<\theta_{max}^{d}\\ 0, & else\end{cases} \quad (7)$$

$$where\ \Delta\theta_{p,q}^{i,j}=\left|\left(\left(\left(\theta_q^j-\theta_p^i\right)+180\right)\ mod\ 360\right)-180\right|$$

For minimizing possible errors in determining sperm head direction angle, the angle correction (θ_c) is done by looking at the corner of the future path of sperm from time *t-2* to time *t-1* where θ^t_{max} is the maximum angle of sperm head direction angle correction.

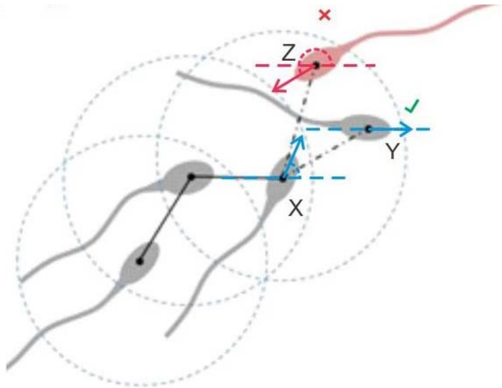


Figure 3. Using sperm head direction angle for improving sperm tracking accuracy [13]

$$\theta_p^i = \theta_p^i + \begin{cases} \theta_c, & \frac{\Delta\theta_{t-1,t;t}^i}{\theta_{max}^t} > 1 \\ 0, & else \end{cases} \tag{8}$$

$$where\ \theta_c = \begin{cases} 180, & \theta_p^i < 180 \\ -180, & else \end{cases}$$

After obtaining the weight of the sperm movement distance and the weight of sperm direction angle, the sperm tracking was performed by calculating the maximum matching value (*M*) of both weights using (9). Parameter γ is the dependent value between sperm movement distance and the sperm head direction angle. The best results were given by = 0.6. The data association between two sperms from frame to frame can be measured by finding the maximum value based on a bipartite graph matching using the Hungarian algorithm [7].

$$M = arg \max_{M \in H} \sum \gamma w_{d}{}_{p,q}^{i,j} + (1-\gamma) w_{\theta}{}_{p,q}^{i,j} \tag{9}$$

In the case of rea:earing sperm, prediction based on sperm location and sperm head direction angle were performed. The angle prediction was calculated using (10) which was averaging between sperm angle at time *t-1* with the sperm displacement angle from time *t-2* to time *t-1*. Sperm location predictions were carried out by averaging the sperm movement based on head sperm direction angle as mentioned in (11) and (12).

$$\theta_t^i = \bar{\beta} = atan2\left(\sum_{i=1}^{n} \sin(\beta_i), \sum_{i=1}^{n} \cos(\beta_i)\right) \quad where\ \beta = \{\theta_{t-1}^i, \theta_{t-2,t-1}^i\} \tag{10}$$

$$x_t^i = x_{t-1}^i + \left(\bar{d}_x \times \cos(\theta_t^i \times \pi/180)\right) \tag{11}$$

$$y_t^i = y_{t-1}^i + \left(\bar{d}_y \times sin(\theta_t^i \times \pi/180)\right) \tag{12}$$

3.4. Sperm Motility Parameter Calculation

The sperm motility measurement was performed using some CASA parameters which are curvilinear velocity (VCL), straight-line velocity (VSL), and linearity (LIN). These parameters were calculated using equation (13), (14), and (15) [4], [14]. Based on 2010 WHO laboratory manual [2], sperm motility analysis using CASA parameters can be done with a minimum of 1-second video.

$$VCL_i = \frac{\sum_{j=1}^{M} \sqrt{\left(x_{j+1} - x_j\right)^2 + \left(y_{j+1} - y_j\right)^2}}{(M-1)\Delta t} \tag{13}$$

$$VSL_i = \frac{\sqrt{(x_M - x_1)^2 + (y_M - y_1)^2}}{(M-1)\Delta t} \tag{14}$$
$$where, \quad \Delta t = 1/fps$$

$$LIN_i = VSL_i / VCL_i \tag{15}$$

All sperm motility parameters were measured in μm or μm/s unit. Therefore, it was necessary to do units conversion from pixel into μm. Image sampling using a Neubauer improved chamber were performed to know the scale of the samples. Based on observations, it was known that the scale was 1: 0.943 (1 pixel = 0.943 μm).

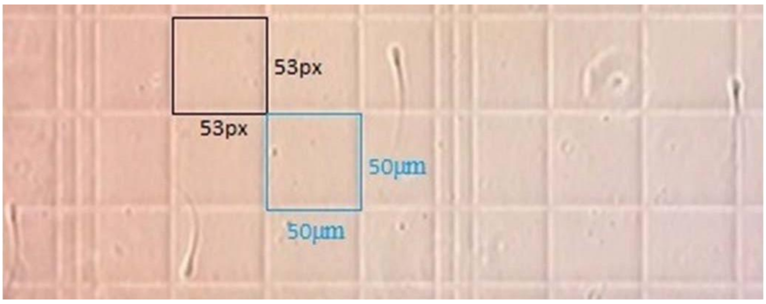


Figure 4. Unit comparison between pixel and μm [10]

4. Results and Analysis

This section elaborates the experiment and discusses its result. In this research, the experiment in [10] was extended with more video samples. Nevertheless, there is no difference in the sample's video frame rate as it has been found that higher frame rate gave better tracking accuracy [10].

4.1. Sample

The observed sample was fresh bull semen sample. The videos were recorded using a digital microscope with 200x total magnification and Ph1 lighting conducted by a medical veterinarian. There were total 5 sample videos taken from 5 bull semen samples. The videos were then cut into several 1 second video clips which made them a total of 120 video clips. The resolution of the videos was 640 x 480 px with 30 fps frame rate.

4.2. Development and Test Environment

The a:lication was developed and tested using a laptop with an Intel Core i5 1.7 GHz and 4 GB of RAM with Windows 10. The a:lication was developed using C++ / CLI programming language with Visual Studio 2015, OpenCV 3.1.0 libraries [15] and a Hungarian algorithm implementation [16].

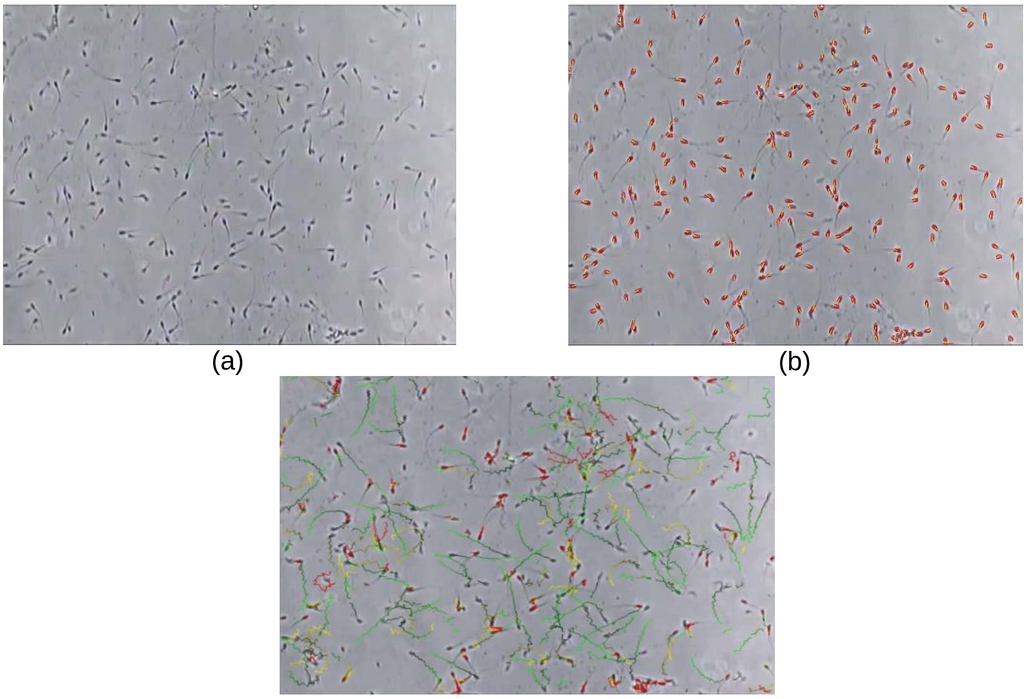
4.3. Results

We carried out the experiment on all video samples. Table I contains the experiment results. For benchmarking purpose, we compared our results with previous method[4] results. Manual measurement results were used as the reference. The manual measurement was done by two medical veterinarians. Based on the experiment, the results of the proposed method were better because of the smaller difference to the manual measurement. The result illustrations can be found in Figure 5.

Table 1. Experiment results

Samples	Manual	Proposed Method Average Result	Average difference	Absolut	Previous Method[4] Average Result	Average difference	Absolut
#1 (25 clips)	40%	36.68%	5.40%		36.68%	5.40%	
#2 (17 clips)	45%	50.05%	5.41%		34.88%	10.82%	
#3 (28 clips)	30%	31.68%	5.61%		50.36%	23.71%	
#4 (14 clips)	75%	69.57%	5.43%		64.93%	10.07%	
#5 (36 clips)	70%	66.14%	4.67%		65.65%	5.26%	
Average			5.16%			10.69%	

The results of bull sperm motility measurement gave the average absolut margin of 5.16% compared to the manual measurement by medical veterinarians. In the other hand, the previous method gave 10.69% margin. In general, both methods gave an encouraging results. However the proposed method, which added sperm head direction angle parameter, gave a better result.



(a) (b) (c)

Figure 5. (a) Video sample example (b) Ellipse detection result (c) Sperm trajectory result[10]

5. Conclusion

In this research, a bull sperm motility measurement a:lication has been developed. The method to detect sperm was modified as well as the method to track the amount of sperm in the video. Sperm motility was measured using the CASA parameters that are VCL, VSL, and LIN. LIN was used for classifying the sperm into three motility classes.

The proposed method achieved an average absolute difference of 5.16% to manual measurement which surpasses the previous method result (average absolute difference 10.69%). This indicates that the addition of sperm head direction angle gives improvement to the accuracy of bull sperm motility measurement.

Currently, the software a:lications are being developed in order to get a better accuracy. The developed software is believed to provide the better calculation results. For future works, we will test the a:lication and hardware that has been developed using fresh bull semen samples from livestock around Lembang as it is one of the most bull populous region in Indonesia.

Acknowledgement

We would like to thank DIKTI for funding this research by *Penelitian Produk Terapan* scheme. Our greatest gratitude to Mr. Oloan Parlindungan, Mr. Supraptono, Mrs. Emi Rochmiati, Mrs. Nurjannah Syakrani, and Mr. Yudi Widhiyasana for meaningful help and feedbacks.

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