A New Method To Improve Movement Tracking Of Human Sperms

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Abstract—One of the determinants of sperm quality is the motility of spermatozoa. The motility of spermatozoa is measured by microscopic sperm test. Conventionally; the determination of sperm motility is performed by experts, in which the judgment tends to be subjective. The existence of Computer-Assisted Sperm Analysis (CASA) is beneficial in solving problems related to the emergence of subjectivity in the determination of sperm motility. Generally, CASA and researchers in this field use phase contrast microscopes to obtain images with higher contrast. In this study, the position and motility determinations of spermatozoa in the video were performed using video records taken from a bright field microscope with low contrast, along with various other deficiencies. With a combination of several stages of works, namely frame difference background subtraction, contrast-setting with Otsu threshold as an indicator, filtering process using mathematical morphology to determine the position of objects, as well as linear regression and root mean square value (RMS) calculations. From the results of experimental tests performed on human spermatozoa video data, the above method indicated that the positions of sperm motility from tracking results had recognizable trajectories based on the average distance position to the linear regression line, with an RMS threshold of 10. There were ten progressive spermatozoa and four non-progressive spermatozoa. The method used successfully determined 14 human spermatozoa. There were 71% progressive spermatozoa, while the remaining 29% were non-progressive. Under the WHO 2010 guidelines, a 71% percentage indicates normal sperm motility.

***Index Terms*— Spermatozoa, Linear Regression, Root Mean Square, Background Subtraction, Mathematical Morphology**

I. Introduction

A Sperm examination is one of the easiest ways to find out the fertility or infertility of a male. The level of fertility is generally associated with a male's ability to produce offspring. A test of spermatozoa analysis to measure infertility on the husband is performed in a laboratory. One of the objectives of this test is to determine the imperfect shape and motility of sperm. The sperm must be perfectly shaped and able to move quickly and accurately to the ovum to trigger the fertilization process. When the form and structure (morphology) are not standard, or the motility is not perfect, then the sperm cannot reach or penetrate the egg. Generally, normal sperm can swim at a speed of 2.5 cm every 15 minutes [1].

Spermatozoa analysis procedures commonly practiced today have constraints when associated with low-quality acquisition devices and limited magnification modes. The manual method has difficulties in determining the level of abnormalities of sperm motility because the motility of each spermatozoon need to be tracked and their speed to be calculated. The use of computers has been employed to address the issues related to experts, on a system known as the Computer-Aided Sperm Analysis (CASA) or sperm analyzer [2]. With this system, microscopic images of semen are taken, which are then analyzed regarding concentration (sperm count), mobility, and morphology. The parameters tested using CASA include the calculation of spermatozoa, determination of movement agility (motility), and determination of spermatozoa morphology. However, commercial CASA products are still too expensive. These products are also imperfect. The accuracy of the analysis is highly dependent on the quality of the supply of semen. The low quality of semen supply decreases the accuracy of spermatozoa calculations and the determination of motility levels. Also, these commercial products still cannot perform automatic spermatozoa classification based on the estimate and judgment of motility into the normal, less than normal, or abnormal classes.

Several studies relating to spermatozoa analysis had begun in 1988. The early generation was called CASMA (Computer Aided Sperm Motility Analysis), which used a contrast phase lighting microscope [3] [4]. In Donald T. S., et al. (1988), the early generation sperm analyzer used analog recording, segmentation, and tracking between two frames. The monitoring of spermatozoa in the next frame compares the frame of tracking by taking into account the maximum speed of motility of spermatozoa between frames. The separation of spermatozoa is done based on the position of each spermatozoa and marker contained by each spermatozoon. During the tracking process, video recording is displayed on the screen. The spermatozoa head missing during tracking will be marked (+) at the last position so that the laboratory attendant can evaluate the tracking accuracy. This system can track with an accuracy of 85%. There are several disadvantages to this system, namely: the number of spermatozoa during the process is limited, no real-time results, and there are escaping spermatozoa during the tracking process. This study was continued by J. L. Yániza, et al., who developed CASMA using digitally recorded spermatozoa video [3].

A few years later, a system called RSTS (Real Time Spermatozoa Tracing System) [5] by S. T. Young, et al. (1998) was introduced to overcome the constraints in the sperm analyzer system. RSTS, as the name implies, was developed with a real-time observation method when the spermatozoa enter the microscope's field of view and its

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tracks are tracked for a period of time. The tracking of the spermatozoa paths provides information on the results of measurements of kinematic values that can be used for the analysis of sperm motility. The system uses a contrast phase lighting microscope to record spermatozoa in glowing sphere-like shapes. The spermatozoa are segmented using the gray-level threshold method. Periodically, the system performs scanning in the microscope viewport in the direction of vertical lines and horizontal lines. If the system finds spermatozoa, then the system will store the space of view of the size (n × n) pixels predetermined by the user. Then, the spermatozoa will be moved to the center point of the box. The process continues until the spermatozoa disappear from the microscope's field of view or until the maximum number of boxes corresponds to user input. The total number of observation frames could reach 100-150 pieces, far more than most CASA systems at the time. The system also has some disadvantages: (1) the number of spermatozoa in the observation box potentially disrupts the determination of kinematics of spermatozoa; (2) box size determines the speed and accuracy of spermatozoa tracking. If the box is large, there is the possibility of some spermatozoa collecting in one box, making it difficult to calculate the spermatozoa trajectory with a decrease in recording ability down to 12 frames/second. If the box used is small, then there is the possibility of spermatozoa disappearing from the tracking process.

Tamboli, et al. (2003) introduced the detection phase of the movement of moving microscopic objects [6]. As a first step in tracking spermatozoa, the study used several preprocessing steps: the growing region method for spermatozoa segmentation, increasing contrast values to clarify differences of spermatozoa from the background, median filter to reduce noise, and equalization filter for seed generation.

Qiaoliang Li, et al. (2012) [7], introduced an application for detecting and counting the number of human spermatozoa in a video streaming using Open CV. The adopted method combined Gaussian-Modeling and Morphology Method. The algorithm of Gaussian-modeling was used to filter non-target bright objects, while the image processing method was adopted to enhance the spermatozoa video image quality using combined methods/algorithms and real-time video streaming.

Jiaqian Li, et al. (2014) [8], conducted research to identify the morphology of spermatozoa cells to conclude whether or not they were healthy. The adopted method was a combination between Principal Component Analysis (PCA) to extract image features and K-Nearest Neighbor (KNN) algorithm to diagnose the spermatozoa health. The pictures of the analyzed spermatozoa cells were taken from microscopic images with very tiny sizes. The spermatozoa health diagnosis comprised three parts: spermatozoa image segmentation, extraction of features using PCA and spermatozoa classification using the KNN algorithm.

The final results identified from the research: PCA features and SHIFT features, KKN classifier, and BPPN classifier were then compared. The result was that the accuracy of the diagnosis depended on training sets, as the results of tests with specific training sets proved to generate excellent performance with level of accuracy by 87.53% when compared to the ones with other feature extraction methods, such as Scale-Invariant Feature Transform (SIFT) and another classifier, such as Back Propagation Neural Network (BPNN). To cope with the weaknesses of the previous researches, we propose a new method to have better spermatozoa tracking. The proposed method consists of 4 phases: back reconstruction, background subtraction, Otsu thresholding and mathematical morphology. The spermatozoa movement identification using the proposed method was helpful to experts in identifying the number of sperms. The whole processes are targeted to suggest highly efficient computation

Ravanfar et al. [9] used the adaptive temporal median filter algorithm to separate the background of the video frame by a sampling rate of ±30 fps, with sperm motility not being recorded smoothly. A proposed solution is to use the temporary Gaussian filter for the tracking process. Vladimir conducted another research related to object tracking, et al., 2013 [10], the application for which can be seen in the audience analysis system to detect faces, face tracking, and gender recognition and classification. This system can be applied to security systems that observe human movement through video surveillance. If compared with the research conducted, the flow of objects tracked can go to the reverse direction, while the flow of spermatozoa in the progressive category is a forward movement with a certain speed.

From several studies above, the semen video recording data used on average has a sampling rate of ± 30 fps (frames per second). Meanwhile, to be able to produce data that can be adequately observed, it takes a sperm video sampling rate of ≥ 50 fps. In this research, sperm video was recorded using sampling rates above 50 fps. Also, previous studies were not conducted on popular sperm video, and the tracking performed using linear regression analysis, and RMS (Root Mean Square) curbs had not been applied to identify progressive or non-progressive spermatozoa movements.

Thus, this research proposed a new approach to detect and identify spermatozoa movements by modifying several algorithms for labeling and numbering processes on spermatozoa, so as to obtain a path of sperm motion, which would be tested by the linear regression analysis method to identify whether a spermatozoon is progressive or non-progressive, then comparing it with manual calculations by experts. The identification of sperm motility tracking used TABI (Time-Averaged Background Image), background subtraction, Otsu Threshold, and mathematical morphology. Also, the analyzed sperm video was recorded using a bright field microscope device and a high-speed Point Gray camera paired in an ocular position and an objective lens with 40 times magnification, connected to a laptop as a video processor.

II. Material and Methods

The research to identify sperm motility began with the design of the hardware system. The type of microscope used is bright field microscope. This system produced spermatozoa motility recording video that comprised the input on the spermatozoa-tracking step utilizing the Time-Averaged Background Image, background subtraction, Otsu Threshold, and mathematical morphology methods. The tracking of spermatozoa yielded a two-dimensional position values and the RMS (root mean square) variable required to identify sperm motility, calculated from the position of a spermatozoon during movement in a particular trajectory, as shown in Fig. 1.

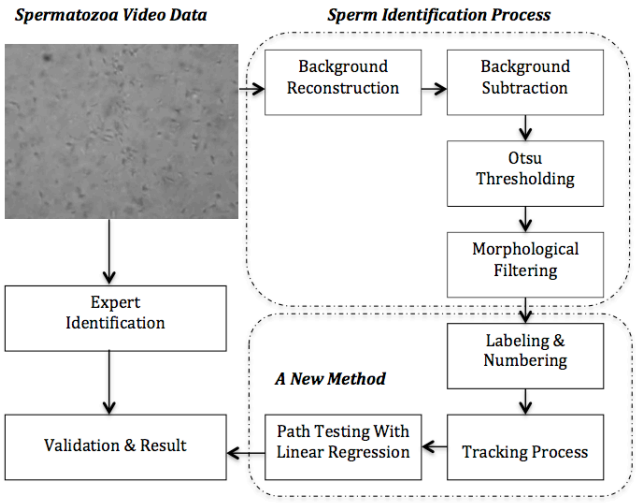

Fig 1. Tracking Process and Identification of Spermatozoa Abnormalities.

A. Data Acquisition

All the video capturing process was performed in the Integrated Laboratory of Microbiology of Health Polytechnic of Surabaya. The data used in this study was in the form of sperm video obtained from the results of sperm fluid scans of some volunteers. Before being observed, the sample was first left aside for about 20-30 minutes [11] under room temperature. It is necessary because, at the time of ejaculation, the sperm fluid was still thick and needed dilution so that spermatozoa observed would not be too concentrated, was able to move more actively, and could be distinguished.

The sperm video was captured using a bright field microscope. The Point Gray type FL3-U3-13S2C-CS camera was placed as a replacement for the ocular lens with USB 3.0 cable connection. The researchers used a laptop with Core i5 processor, 4 GB RAM, and 500 GB of hard drive capacity as well. Video recording with a microscope was set using an objective lens of 40 times magnification so that the motility of spermatozoa was visible. The camera and program settings were set so that the video had the same contrast, brightness, and white balance. Also, the microscope field of view was set to remain immobile during the recording process. The resulting video was saved and then converted to then be processed. Because the computer could not match in performance with the frame rate of the video, sometimes there was slowness to up to nine frames in a row. The result was stuttering video image, where the movement of the objects becomes jumpy (not smooth). An illustration of this process is in Fig. 2.

B. Background Reconstruction

The system's ability to separate the background and foreground in the form of moving spermatozoa is needed to identify moving sperm. Background retrieval was done by scanning when no sperm was running. Background retrieval in this way creates 2 (two) significant difficulties. The first difficulty is the process of retrieving the background because it is challenging to obtain the condition without moving sperm or no sperm at all. It is not possible to stop all sperm from getting the desired background. The second difficulty is the inaccuracy of the background obtained with real-time conditions at that time. If the background is found in one location of observation, then the background is used to detect sperm in other areas, hence triggering problems caused by the discrepancy between the background and real-time condition at that time caused by the difference of location conditions.

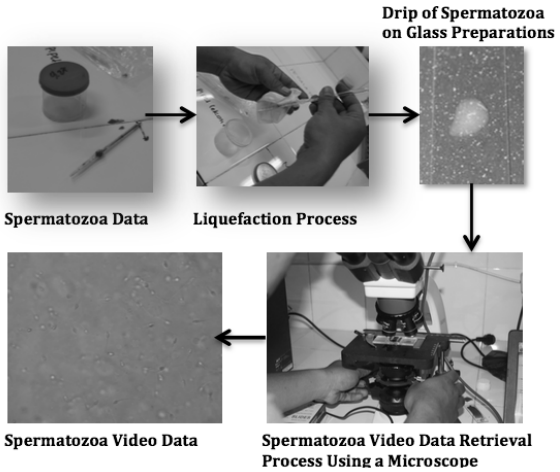

Fig 2. Real-time process of spermatozoa video data collection

Some researchers had researched background reconstruction or preprocessing using a series of motion pictures, such as Akara S. et al. (2011), that confirmed the need for preprocessing in the detection of diabetic retinopathy disease by observing retinal image textures [12]. The image data used had an uneven contract, so the Contrast Limited Adaptive Histogram Equalization method and shade correction algorithm was applied. Similarly, Kornprobst et al., (1999), stated that the background is the most frequently seen image of a series of moving images. In other words, background formers have the most frequent frequency of occurrences [13]. Meanwhile, Long et al. (1990) stated that the background has a stable intensity for a long time [14], as well as Gloyer et al. (1995), who stated that the background would at least be seen more than 50% of a series of moving images [15]

From several assumptions above, it can be concluded that the background can be obtained by taking the average value of a series of images. The average value will be close to the desired background image. Thus, this research used the TABI (Time-Averaged Background Image) method [16] to conduct preprocessing or background reconstruction.

The steps to retrieve background by separating it from foreground based on sperm video can be explained as follows. Step 1 was Video Extraction (the video was extracted to obtain frames of the video). Suppose (l_1, l_2, ..., l_m) is a series of moving images, then some frames with the N number of frames of the total m of frames is selected, symbolized by (f_1, f_2, f_3, ... f_n). Step 2 was to obtain the frames of the average video frame extract results. $f_i(x, y)$ represents the pixel value of i-th frame with i = 0, 1, 2, ... n. Then, the average value per pixel was taken using equation 1. The selected frame can be any frame or frame based on the desired interval.

$$\mu(x,y) = \frac{1}{n}\sum_{i=1}^{n} f(x,y) \quad (1)$$

C. Background Subtraction

The background subtraction process was used to detect moving objects on the video [17]. In a study of sperm infertility, the sperm that will eventually fertilize the egg is the sperm that keeps moving [18]. Therefore, the background subtraction process is necessary for detecting moving sperm. In the case of sperm detection, the advantages of doing the background subtraction process are that the data used has of unimodal characteristics, the distance between frames is short, and the effect of light changes is absent [19].

On the other hand, the challenge faced is the presence of background objects that move and the existence of new objects considered as background. The input of the background subtraction process was the preprocessed video frames, and the output were binary images that represented the objects (sperm) moving in the video. In this research, the Frame Difference background subtraction algorithm was applied [20].

In this algorithm, the background model was taken from the frame (f) a moment before the current frame. The equations used to model the background (B) image on the Frame Difference algorithm was defined by [21]:

$$B = f_{t-1} \tag{2}$$

Foreground (F) image was obtained by calculating the difference between the background image value with the current frame:

$$F = |f_t - \mathrm{B}| \tag{3}$$

This algorithm was able to detect pixels that move quickly and precisely but would fail to recognize if the moving object stopped instantaneously

D. Otsu Thresholding

At this stage, a segmentation process was performed to distinguish or separate the detected object against its background as a result of segmentation in the form of binary images. Input for this process was the image extracted frames from the video and average frame result of the TABI (time-averaged background image) procedure [22]. Furthermore, a BS process with different frame method was performed, resulting in the difference in the pixel value of its corresponding coordinates for all pixels. Then, this stage was followed by a thresholding process using the Otsu threshold method [23], where the difference value was less than the threshold value. Thus, the color values were changed to 0. If the value was greater than or equal to the threshold value, then the color value was changed to 1, as in the following equation [24]:

$$g(a,b) = \begin{cases} 0 & , f(a,b) < T \\ 1 & , f(a,b) \geq T \end{cases} \tag{4}$$

with:

$g(a,b)$ = Pixel image values from thresholding, which contains 0 or 1

T = Threshold value

Thus, binary imagery would be obtained as a reference point in maximizing contrast for maximum background and foreground differentiation.

E. Morphological Filtering

Features of the obtained image from the segmentation process usually still contained noise, due to unwanted objects being segmented. The morphological filtering method was used to eliminate the noise [25]. The first process was to remove objects that had less than 400 pixels, thus retain objects that had more than 400 pixels. The second process was to make the object smoother by closing the small gaps of the objects. This process used image morphology operation techniques, where the edges of the images obtained still needed to smoothened using the closing method. Closing is a morphological operation that can be categorized as a second-level operation, meaning that the closing is defined as a dilation operation, followed by erosion operation. The closing operation tends to refine the object on the image by connecting fragments and eliminating small holes in the object.

F. Labeling Objects and Calculating the Coordinate Points of Objects Centers

After the filtering process improved the image, the image was then searched for boundaries using the boundary detection method [26]. Each of the found objects was also labeled. From each object found, the central point coordinates of each such object (x_b, y_b) were searched using equation 5.

$$x_b = \frac{\sum_{i=1}^{n} A_i x_i}{\sum_{i=1}^{n} A_i} \qquad y_b = \frac{\sum_{i=1}^{n} A_i y_i}{\sum_{i=1}^{n} A_i} \tag{5}$$

With:

A_i being the pixel area at point (x_i, y_i)

n being the number of pixels

In this research, the area of A pixel is 1 unit, so equation 6 becomes:

$$x_b = \frac{\sum_{i=1}^{n} x_i}{n} \qquad y_b = \frac{\sum_{i=1}^{n} y_i}{n} \tag{6}$$

G. Spermatozoa Trajectory Linear Regression

The process of tracking spermatozoa for one sequence in a static view space would result in two-dimensional position values of motility representation. To be able to determine the shape of spermatozoa motility, a test line taken from linear regression [27] of a set of spermatozoa position values was required.

$$y = a + bx \tag{7}$$

$$b = \frac{n\sum_{i=1}^{n} x_i y_i - \sum_{i=1}^{n} x_i \sum_{i=1}^{n} y_i}{n\sum_{i=1}^{n} x_i^2 - \left(\sum_{i=1}^{n} x_i\right)^2} \tag{8}$$

$$a = \frac{\sum_{i=1}^{n} y_i - b\sum_{i=1}^{n} x_i}{n} \quad (9)$$

With n being the amount of data, x being the x-axis position, y being the y-axis position. The linear regression line $y = a + bx$ passes through a set of motility spermatozoa position values, which measure the distance to sperm motility position for one sequence.

H. Root Mean Sequence

The RMS value should be sought to determine whether the mean sperm motility is in the straight line, which compares the prediction value (linear regression line) and motility position of a spermatozoon during one sequence. If given a set of data of n, i.e., $\{X_1, X_2, ..., X_n\}$, then the RMS value woud be [28]:

$$RMS = \sqrt{\frac{1}{n}\sum_{i=1}^{n} r_i^2} \quad (10)$$

with:

RMS = average value of spermatozoa distance to the linear regression line

r = spermatozoa position distance from the linear regression line

n = total data.

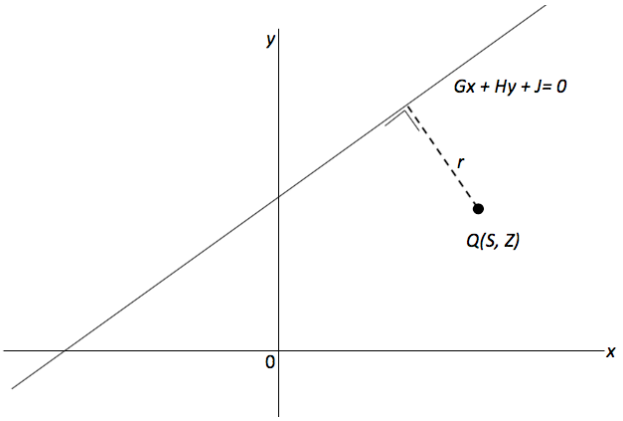

Fig 3. Illustration of determination of the distance between point and line equation

Fig. 3 shows the illustration for calculating the value of r, which is the distance between point $Q(s, z)$ and a line equation. The following equation is used to obtain the equation of line $Gx + Hy + J = 0$:

$$Gx + Hy + J = 0 \quad (11)$$

$$G = n\sum_{i=1}^{n} h_i k_i - \sum_{i=1}^{n} h_i \sum_{i=1}^{n} k_i \quad (12)$$

$$H = \left(\sum_{i=1}^{n} h_i\right)^2 - n\sum_{i=1}^{n} k_i^2 \quad (13)$$

$$J = \sum_{i=1}^{n} h_i^2 \sum_{i=1}^{n} k_i - \sum_{i=1}^{n} h_i \sum_{i=1}^{n} h_i k_i \quad (14)$$

To calculate the distance value r between point $Q(s, z)$ with line Gx + Hy + J = 0 the following equation is used

$$r = \frac{|Gs + Hz + J|}{\sqrt{G^2 + H^2}} \quad (15)$$

III. EXPERIMENTAL RESULTS

The testing of spermatozoa video movement tracking in this research used two kinds of data: video recording of human spermatozoa from the UNSW embryology collection and video of human spermatozoa motility/movement self-recorded from the sperm of some volunteers. In the spermatozoa recording process, sperm samples were dropped on object glasses without cover glasses, then each was placed under the objective lens, and the high-speed camera was connected to the laptop using a USB 3.0 cable connection, acting as a replacement for the ocular lens.

A. Acquisition of Test Data

The test data were used to view tracking performance. The test data in question were the video of human spermatozoa motility obtained from the UNSW Embryology collection (Fig. 4). The video featured the normal human spermatozoa motility and its response to progesterone photo-release.

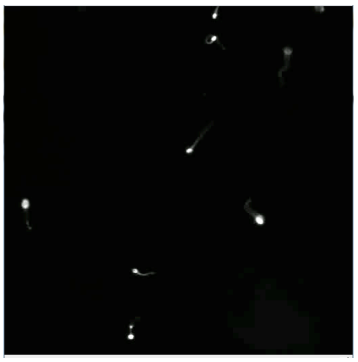

Fig 4. The frame of motion video motility spermatozoa collection UNSW Embryology

B. Human Spermatozoa Data Acquisition

The recording of human spermatozoa motility was prepared by varying the viscosity, objective lens magnification, and recording frame rate (fps) being performed to observe the best results of traceable spermatozoa. The speed of sperm motion reaching 35 μm/s can be appropriately captured by the camera when set at 60 fps. Although the camera's frame rate capability for recording can reach 120 fps, the specification of the computer device was only able to process 60 fps.

Fig. 5.a shows the field of view of the system recorded through the camera after 10 minutes from sperm fluid ejaculation with 10x objective lens magnification. It can be seen spermatozoa population were not able to move naturally because it encountered obstacles in its path (in the form of other spermatozoa or other objects). Also, the sizes of visible sperm cells were tiny, making it difficult to conduct observations.

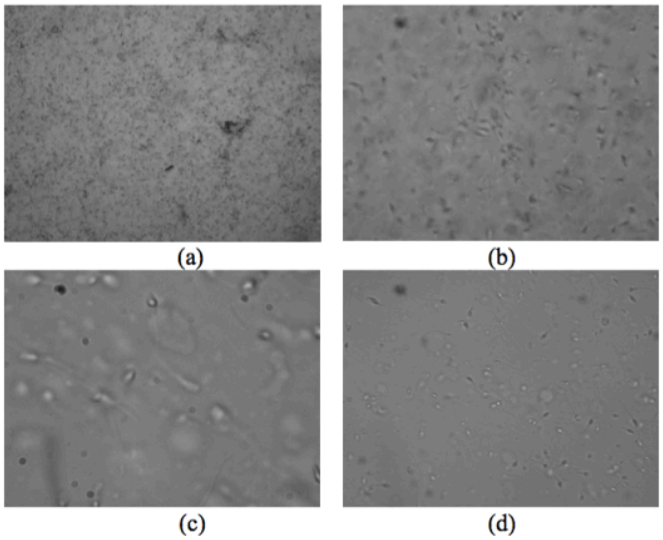

Fig. 5. a. Frames of spermatozoa motility video 10 minutes after ejaculation, 10x objective lens magnification (b) 40x objective lens magnification(c) 100x objective lens magnification and (d) Frames of spermatozoa motility video 30 minutes after ejaculation, 40x objective lens magnification

Fig. 5.b shows the field of view of the system recorded through the camera after 10 minutes from sperm fluid ejaculation with 40x objective lens magnification. It can be seen that the spermatozoa population were still not able to move naturally because it encountered obstacles in its path (in the form of other spermatozoa or other objects). Also, the sizes and shapes of visible sperm cells could be clearly observed. In Fig. 5.c, the camera was less focused, so the sperm morphology was less evident. The head to tail shapes could not be adequately recognized. The recording of the image used 100x objective lens magnification. With the duration of stirring at room temperature for 30 minutes after the sperm fluid was ejaculated, observations were made using a microscope with 40x objective lens magnification. From this observation, the sperm morphology and motility could be seen clearly. The shape of the head and tail, as well as the movement of sperm, could be observed well, as in Fig. 5.d.

C. *Test On Sperm Identification Process*

This experiment was performed on spermatozoa video data after 30 min of leaving with a 40x objective lens. The spermatozoa video was a video with 1280×960 pixel resolution without video compression in AVI format. This video represents the actual sperm motility condition and has 120 frames.

In Fig. 6, (a) is the frame of the video with (a1) being the 15th frame image, (a2) being the 45th frame, and (a3) being the 105-th frame. (b) is the result of the background reconstruction by taking 50 frames out from a total of 120 frames; the frame was selected with the 1-frame interval between frames. (b2) is the result of the detection and segmentation of the vehicle movement, where (b2) corresponds to the 15th frame, (b3) corresponds to the 45th frame, and (b4) corresponds to the 105th frame. Image (c1) is the result of background reconstruction by taking 100 frames with inter-frame intervals of 1 frame. Image (d1) is the result of background reconstruction by taking all frames, i.e., 100 frames. The results of the background and its relation to the frames in the video are shown in the order as in Figure 6.

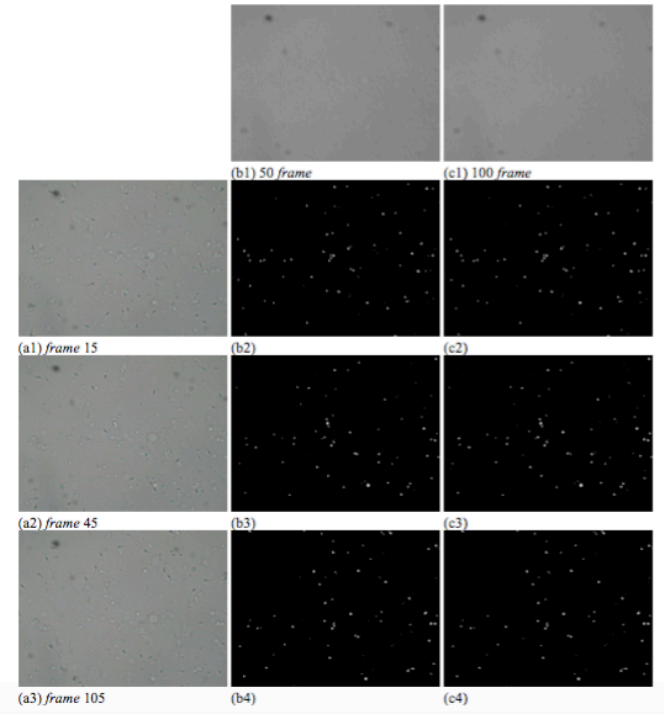

Fig. 6 The motility images of spermatozoa: (a) is the original video extracted image, where (a1) is the 15th frame image, (a2) is the 45th frame and (a3) is the 105th frame. Figures (b) and (c) are the resulting background images from reconstruction

From Fig. 5, it can be seen that the number of frames involved in the background reconstruction process does not significantly affect the background results obtained. The background results obtained used only 50 frames or 41.7% of all frames, resulting in a background similar to the reconstruction using 87.5% of all frames.

D. *Results of Spermatozoa Tracking Test*

Tracking was done on two kinds of data, namely human sperm data and test data. Test data was an ideal video for monitoring using the Frame Difference Background Subtraction and Mathematical Morphology. The video had good contrast, sufficient amount of spermatozoa, and no impurities. Tracking on test data aimed to test the methods used before being used in human sperm data.

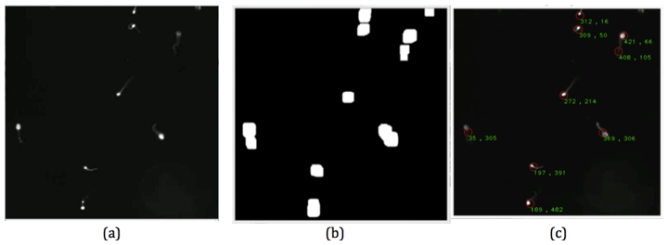

Fig 7. (a) Image of the 120th video frame of the test data, (b) Binary image of the 120th video frame of test data after the process, (c) Tracking of the 120th frame of test data spermatozoa video

The human spermatozoa motility test data has a duration of approximately 13 seconds with dimensions of 512 x 512. This video is ideal for testing because spermatozoa stand out with high contrast and no impurities. There were various motilities of spermatozoa in the field of view, such as linear, stopping and then bending, bumping, and colliding. Then, this video was cut to 120 frames only with duration of 4 seconds.

After background subtraction, thresholding, and morphology operations, we obtained a binary image as in Fig. 7.b, which is a binary image captured from the 120th frame. The spermatozoa tracking process can be performed for all visible objects in the field of view, which was done from one frame to the next. In Fig. 7.c., sperm tracking appeared on the 120th frame

E. Results of Human Spermatozoa Video Tracking

The human spermatozoa motility test data had a duration of approximately 2 seconds with dimensions of 1280 x 960. The spermatozoa video data was obtained after 30 minutes of incubation with a 40x objective lens. As in the test data, in this video the background subtraction, thresholding, and morphology operation processes were conducted, which produced a binary image as shown in Fig. 8.a., displaying the binary image obtained from the 120th frame.

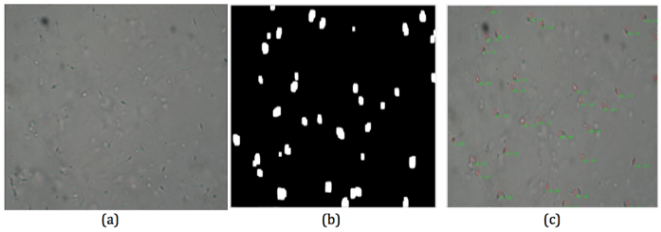

Fig 8. (a) The 120th frame of the human sperm video, (b) Binary image of the 120th frame of the human sperm video, (c) Tracking of the 1120th frame of human sperm video

The movement of the marker represents the sperm motility tracking during the duration of the video observation. The center position of this marker was recorded regarding x position and y position, then the linear regression and RMS were calculated. The motility of the spermatozoa during the 120 frames was reviewed without colliding obstacles in the form of impurities. Thus, the tracking performed was the natural movement of spermatozoa and produced a path form that can be searched for its linear regression value, and its root means square from the motility path of spermatozoa.

F. Linear Regression Calculation Analysis

The position variables recorded during the sperm motion tracking motility were the depictions of the path forms. The RMS value was determined from the spermatozoa position during the duration of the observational video on its straight line. The more spermatozoa position that lied from the direct line, the RMS value would be higher. From here, the threshold value that distinguishes spermatozoa with straight or bent paths was obtained.

From the spermatozoa tracking process, using both human sperm data and test data, the spermatozoa motility position values were obtained during the observations in the x-axis and y-axis coordinates shown in Table 1 and Table 2. Where *m* in both tables are the number of frames per sperm, with the number of sperm test data are 8 and the number of human sperm data are 14. The number of frames for each sperm is different depending on the video of sperm movement per frame.

From the table of the spermatozoa position motility, the researchers obtained a plot that describes the shapes of the paths of spermatozoa as in Fig. 9 and Fig.10.

The next process was to determine the linear regression for each spermatozoon using equations (7), (8), and (9). The linear regression equation for spermatozoa was shown in Table 3.

TABLE 1
POSITIONS OF TEST SPERMATOZOA DURING TRACKING

	Sperm-1		*Sperm-2*		*Sperm-3*		*…*	*Sperm-8*	
Frame To	*x*	*y*	*x*	*y*	*x*	*y*	*…*	*x*	*y*
1	62	251	251	184	62	225	…	482	218
2	63	251	251	188	63	225	…	482	218
3	65	251	251	190	63	225	…	482	219
4	67	253	253	189	66	225	…	482	224
5	70	253	253	189	71	225	…	482	224
6	71	254	254	193	71	221	…	484	225
7	71	254	254	195	82	225	…	485	226
8	75	254	254	195	94	223	…	485	228
…	…	…	…	…	…	…	…	…	…
123	287	300	300	290	184	276	…	472	461

TABLE 2
POSITIONS OF HUMAN SPERMATOZOA DURING TRACKING

	H-Sperm1		*H-Sperm2*		*H-Sperm3*		*…*	*H-Sperm14*	
Frame To	*x*	*y*	*x*	*y*	*x*	*y*	*…*	*x*	*y*
1	398	261	197	265	391	269	…	486	305
2	396	261	190	261	394	265	…	474	310
3	396	261	189	258	396	260	…	473	299
4	400	261	183	256	396	264	…	442	326
5	401	263	178	253	397	263	…	442	326
6	400	267	173	263	398	261	…	441	324
7	406	269	167	263	396	261	…	437	322
8	411	265	165	257	396	261	…	436	321
9	409	279	165	257	400	261	…	434	320
10	411	274	168	249	401	263	…	433	318
11	405	278			400	267	…	429	315
…	…	…			…	…	…	…	…
n	599	349			413	263	…	405	293

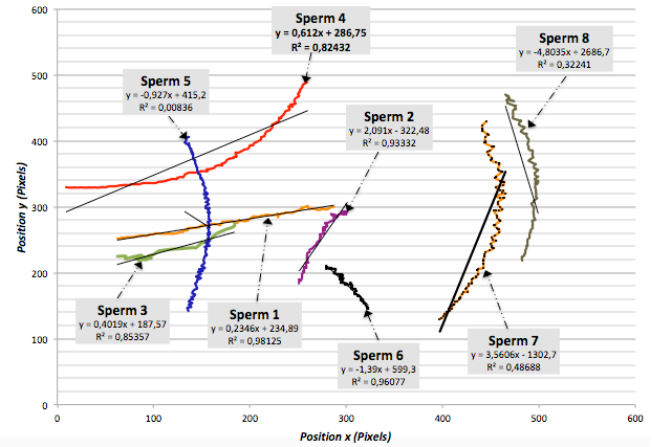

Fig 9. The plot of test data spermatozoa

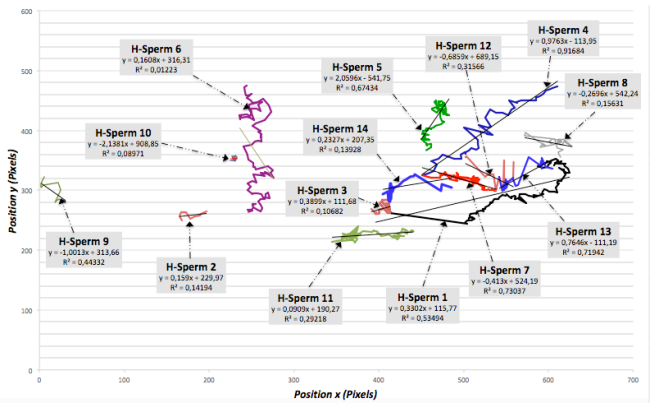

Fig 10. The plot of human spermatozoa motility

G. RMS Calculation

After obtaining a linear regression value for each spermatozoon, the next step was to find the RMS value representing the average distance between the spermatozoa position value and the linear regression line. From this RMS value, it would seem that if the position of spermatozoa that was far from the regression line added up, then the RMS value would be higher. The RMS value would give an average picture of the spermatozoa position during its motility, which would determine whether the location of the movement was far from the straight line or not. The RMS value was calculated using equation (9) and (10). The RMS value of each spermatozoon could be seen in Table 3 and Table 4.

TABLE 3
LINEAR REGRESSION AND RMS FOR EACH TEST DATA SPERMATOZOON

Order of Sperm	Linear Regression	*RMSValue*
1	$y = 0{,}23\,x + 234{,}8$	2,14
2	$y = 2{,}09\,x - 322{,}4$	8,68
3	$y = 0{,}40\,x + 187{,}5$	3,90
4	$y = 0{,}61\,x + 286{,}7$	21,43
5	$y = -0{,}93\,x + 415{,}2$	77,03
6	$y = -1{,}39\,x + 599{,}3$	3,91
7	$y = 3{,}56\,x - 1302{,}0$	62,75
8	$y = -4{,}80\,x + 2686{,}0$	63,56

TABLE 4
LINEAR REGRESSION AND RMS FOR EACH HUMAN SPERMATOZOON

Order of Sperm	Linear Regression	*RMSValue*
1	$y = 115{,}77 + 0{,}33\,x$	21,03
2	$y = 229{,}97 + 0{,}16\,x$	4,29
3	$y = 111{,}68 + 0{,}39\,x$	6,96
4	$y = -113{,}95 + 0{,}98\,x$	9,17
5	$y = -541{,}75 + 2{,}06\,x$	5,68
6	$y = 908{,}85 - 2{,}14\,x$	26,46
7	$y = 524{,}2 - 0{,}41\,x$	4,61
8	$y = 542{,}24 - 0{,}27\,x$	7,25
9	$y = 313{,}66 - 1{,}00\,x$	7,74
10	$y = 316{,}32 + 0{,}16\,x$	2,64
11	$y = 190{,}27 + 0{,}09\,x$	4,39
12	$y = 689{,}16 - 0{,}69\,x$	10,78
13	$y = -111{,}19 + 0{,}77\,x$	6,74
14	$y = 207{,}35 + 0{,}23\,x$	10,86

By comparing the plot of the spermatozoa position to the RMS value, it can be concluded that the RMS threshold value for the spermatozoa path is 10. Progressive spermatozoa that move straightforward or forward but not straight, sometimes twisted, and slow movement has RMS value below 10. While spermatozoa nonprogressive whose tail runs, but it does not move forward, sometimes it appears to be spinning or just running in place has an RMS value above 10. Based on the value of the threshold then for the test data, there are four progressive spermatozoa and four non-progressive spermatozoa, while for human spermatozoa data there are ten progressive spermatozoa and four non-progressive spermatozoa. Table 5 shows the number and percentage of the spermatozoa group.

TABLE 5
NUMBER AND PERCENTAGE OF SPERMATOZOA GROUP

Types of sperm data	Test Data		H-Sperm	
Groups	Prog.	Non-Prog.	Prog.	Non-Prog
Number	4	4	10	4
Percentage	50%	50%	71%	29%

Note: Prog = Progressive

Of the 8 tested spermatozoa tested data, there were 50% progressive and 50% nonprogressive. As for 14 human spermatozoa in tracking, there were 71% progressive and 29% nonprogressive

IV. Conclusion

With the device being developed, the determination of human motion spermatozoa abnormalities in video files can be done.

The path shape identifies the position of the spermatozoa movement of the tracking results based on the average distance of its position on the linear regression line. With threshold ten there are four progressive spermatozoa and four non-progressive spermatozoa for the test data, while for human spermatozoa data there are ten progressive spermatozoa and four non-progressive spermatozoa. The methods used successfully determine 8 (eight) spermatozoa data UNSW Embryology, and 14 human spermatozoa. Of the eight tested spermatozoa tested data, there were 50% progressive and 50% non-progressive. As for the 14 man-trained spermatozoa, there were 71% progressive and 29% non-progressive. According to the WHO laboratory manual for the examination and processing of human cement in 2010, a value of 71% progressive means the movement of normal human spermatozoa.

The offered system algorithms were perceived to be representative for sperm calculation, although there was sperm traffics during observation.

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