A Survey on Multisperm Tracking for Sperm Motility Measurement

Priyanto Hidayatullah, Tati L. E. R. Mengko, and Rinaldi Munir

***Abstract*—Sperm motility is the main criterion in evaluating the quality of semen. Sperm motility measurements can be done in many ways. But the most effective way is to simultaneously track all sperm and calculate the motility parameters of Computer Aided Sperm Analysis (CASA). Based on those parameters, the sperm motility was categorized and the percentage of motile sperm was calculated. This paper presents the analysis of the currently available multisperm tracking methods for sperm motility measurement. In this paper, we discuss why sperm motility is an important parameter for assessing sperm quality and compare several multisperm tracking methods along with an analysis of their advantages and disadvantages. It can be concluded that the main problem in sperm motility measurement is having a good multisperm tracking to obtain precise sperm paths with an efficient computation on semen with high sperm concentrations. If the generated path precision is high, then the CASA parameters calculation results will better describe the actual sperm motility conditions. None of the existing methods can produce precise trajectories in complex cases yet. The complex case is, especially, when sperms collide or cover each other in the situation of large sperm counts appears in one microscope field of view.**

***Index Terms*—Multisperm tracking, sperm motility, object tracking, CASA.**

I. Introduction

To increase cattle production, artificial insemination (AI) technique is one of the proper alternative solutions. Artificial insemination is a technique of cow breeding by injecting sperm into the cow's womb. This technique is also used in many exporting countries of cattle such as India, France, and Australia. In Australia, for example, about 1.5 million cows are inseminated each year [1]. To support an artificial insemination program, the Indonesian government established Artificial Insemination Centers (*Balai Inseminasi Buatan*/*BIB*). This center provides cattle and other good quality livestock semen with a total of 12 centers throughout Indonesia.

Fertilization is largely determined by the quality of semen [2]. Therefore, routine activities undertaken by the AI Center is to evaluate the health of semen before the semen is preserved or inseminated. In addition to evaluating and preserving semen, AI Center also distributes the frozen semen to farming centers.

Semen examination is divided into two groups, namely examination in macroscopic and microscopic. The macroscopic examinations are general semen inspections without the need for complicated tools. The inspections include the volume, semen color, viscosity, and the pH of the semen. The microscopic examinations are performed to see more detail conditions of the semen where a sophisticated tool needed in the process. These include sperm mass movements, motility, sperm concentration, viability, and sperm morphology [3].

As a case study, in Lembang AI Center, which is the biggest AI Center in Indonesia, the percentage of fresh semen discarded for not passing the macroscopic examinations is at 0.136% which is very small. While the percentage of fresh semen removed for not passing microscopic examinations is about 30%. As for the frozen semen, 100% semen passes the microscopic examinations that shows the preservation is well performed. These data suggest that microscopic examinations are more important because in most cases, semen examination is problematic on the microscopic side.

In addition, this data also indicate that the examination of fresh semen requires more attention because semen is more frequent discarded in fresh semen inspections. It is important to note also that the fresh semen concentration is so high that a large number of sperms appear in one field of view. In this situation the occurrence of sperm occlusion and collision is very high. Of the five microscopic examinations, there are two main examinations that are always performed on every examination i.e. motility and sperm mass motion.

The macroscopic examinations can be done easily and quickly manually by a veterinarian or a laboratory technician with good results. Microscopic examination can be done manually, however it takes a long inspection time to get more objective results because we must observe up to 10 fields of view per examination task [3]. In addition, the manual examination has other shortcomings: depending on the experience of the veterinarian [4], the occurrence of human error [4], subjective [5], [6], intra and inter observer variability [7], [8], and causing exhaustion to the observer.

This paper discusses more primarily about the examination of motility. Although sperm mass motion has a close relationship with fertility [5], the related papers are not numerous. As for the measurement of motility, in addition to having a significant role in fertility [4], [9], [10], there are many papers discussing it. Motility measurement is also still a big challenge because the cases listed above have not been handled well.

There are various methods for measuring sperm motility.

Manuscript received August 16, 2017; revised October 10, 2017. This work was supported by Indonesia Endowment Fund for Education (Lembaga Pengelola Dana Pendidikan RI).

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Of the many methods, multisperm tracking simultaneously is the most trusted to deliver results as expected. All motility parameters come from the sperm trajectory formed by the method. If the trajectory can be formed with high precision, then the resulting parameters will have high precision as well. Therefore, the main problem is multisperm tracking to obtain a high precision sperm trajectory [8], [11].

Multisperm tracking sperm has specificity than multiobject tracking in general. Its specificity lies in sperm objects that are very similar to each other both from color, shape, texture, and size. Therefore, a truly reliable tracking method is required to obtain a precise sperm trajectory. In addition to trajectory precision, computational speed is important as motility measurement results are required immediately by the evaluating veterinarian to decide whether the semen being evaluated deserves to be preserved or not.

This paper is arranged by the following arrangement. Section I discusses the background and the objective of the paper. Section II explores in general about CASA. Section III explains more detail about parameters which are used for measuring sperm motility using CASA which called CASA parameters. Section IV analyzes the available multisperm tracking methods along with their summary of advantages and disadvantages. Section V concludes the analysis and give insights for future works.

II. Computer Aided Sperm Analysis

CASA is a system consisting of hardware and software. Since the development of the 1980s, CASA has given promising results [12]. It is estimated that in the future CASA will greatly assist the process of sperm analysis digitally [13]. The expected help keyword from CASA is automatic processing. In addition to being used for human sperm analysis, CASA has been used for the analysis of bull sperm [14] and pig sperm [15].

In general, the CASA system consists of microscope that is given an additional digital camera. This camera will replace the function of the veterinarian eye to record video of the sperm being analyzed. This digital video is then sent to the computer for processing.

The process of digital image processing of sperm is performed in several stages [16]-[18]. First of all, it must be ensured the video taken from the microscope has good quality. To get a good video quality required lighting settings and possibly adding contrast enhancer. In addition, focus settings of the microscope should also be done so that the video is taken with good contrast.

The recording results are stored in the form of a digital video file. In order to be processed, this video must be extracted into image frames. The analysis is done on each frame to identify sperm. After the sperm can be identified, comparison between sequential frames is carried out to obtain sperm motility parameters.

The process of sperm identification is described in [16] with some preprocessing techniques and identification of sperm features. In more detail, the process of sperm image is usually obtained in the form of an RGB image. This image cannot be directly used to recognize sperm. It must, therefore, be converted into binary (black and white) image. The binary image is obtained by applying a threshold on the grayscale version of the RGB image. After having the binary image, edge detection is performed. The edge is required to find the elliptical shape that characterizes the sperm features.

The next stage is sperm tracking. Sperm tracking is performed by mapping between identified sperm on one frame with the identified sperm on the next frame. If the mapping can be well performed, then the trajectory of each sperm can be well drawn which makes it much easier to calculate its CASA motility parameters.

III. CASA Parameters

Observation of sperm motion itself is very difficult to do manually because of fast sperm motion. It takes expertise gained from experience long enough to be able to determine the sperm motility. Therefore the existence of CASA is very helpful. In CASA there are parameters that describe sperm motion.

World Health Organization (WHO) defines 9 parameters for measuring the quality of sperm using CASA [19]. These nine parameters are internationally recognized standard parameters. In [9], it was said that sperm concentration and VCL are the most significant parameters in predicting male fertility. While Nagy *et al*. [10] stated that the Average Path Velocity (VAP) is the most clinically relevant motility parameter of fertility in bull sperm. In short conclusion, there is diversity among researchers considering this issue. For more details, here is a more detailed explanation of the 9 parameters [19]-[21]:

1) Curvilinear velocity (VCL) is the velocity of the sperm along its curve-shaped lane.
2) Straight-line velocity (VSL) is the sperm velocity along the line connecting the starting position and the sperm end position.
3) Average path velocity (VAP), is the average time velocity of the sperm head along the average path. The path is calculated by smoothing the curved trajectory based on the algorithm at CASA.
4) Amplitude of lateral head displacement (ALH) is the farthest distance from the average sperm trajectory
5) Linearity (LIN) is how straight sperm motion.
6) Wobble (WOB) is the actual path oscillation size of the average path, VAP / VCL.
7) Straightness (STR) is linearity to the average path, VSL / VAP.
8) Beat-cross frequency (BCF) is the average value at which the curved path crosses the average path.
9) Mean angular displacement (MAD), is the absolute value of the average time of the rotation angle of the sperm head along the curved path.

IV. Multisperm Tracking Methods

A. Sperm Trajectory Multidimensional Visualization Methods

Corkidi *et al.* [22] built a system to capture the movement

of sperm in 3 dimensions. This system was a complete system consisting of several components. This system provides video output with dimensions of 512×512 pixels, frame rate 4200 fps, and a depth of 100 μm. The semen sample used is sea urchin semen observed in a duration of 1 second.

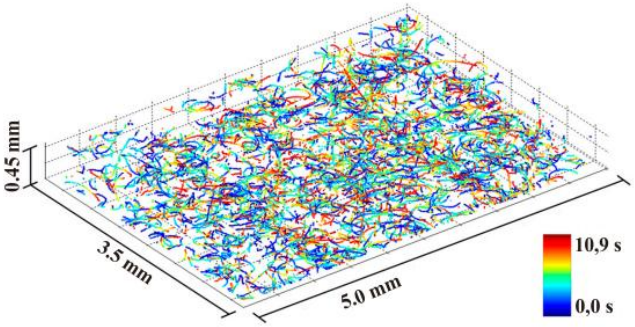

Fig. 1. The reconstructed 3D human sperm trajectories (adapted from [24]).

The trajectory of the sperm contained in the semen can be displayed in 3 dimensions. But there was no explanation about the utilization of this trajectory for measuring the quality of semen. There was no explanation either of the total sperm that exists and how many sperms that are successfully tracked.

Su *et al.* [23] tackled the problem of sperm dynamic movement visualization on a large volume of semen. They used a lens-free on-chip device which tracked the individual movement of human sperms in 3D. The volume was about 8-17 mm^3 with more than 1500 sperms contained. In the previous year, they also published a paper which findings is human sperm's movement formed helical trajectories [24] which illustration is given in Fig. 1. They also quantified some parameters such as VCL, VSL, ALH, BCF, and LIN.

B. Tracking Methods

Sorensen *et al.* [25] divided tracking into several stages. The first stage was detection and followed by an estimation of movement and handling of sperm labeling. Sperm detection was carried out using the scale space blob detection method. The basis of using this method was because, in the image of the contrast phase microscope results, 2/3 sperm head looked like a blob (light blob) with an almost circular shape. The position of this bright blob was tracked.

The operator that is commonly used for doing scale space blob detection is Laplacian of Gaussian operator (LoG). At each frame in the video, a scale space blob detector at a certain scale was operated to obtain a filter response on each pixel. This response was converted to the probability of the pixel as the midpoint of the sperm head using the un-normalized Gaussian distribution. Based on the pixel probability map, the sperm position was extracted from the image using a combination of thresholding and connected component analysis.

The process was continued by estimating the sperm movement with two popular methods which are particle filter and Kalman filter. Labeling was done using Hungarian and Hidden Markov Model (HMM) algorithms.

There were three human sperm videos from observation of contrast phase microscope used as samples. The tracking results were measured with Mean Square Error (MSE) and the failure percentage of tracking. The following was the quantitative result of tracking

- Video 1: MSE = 2.0; Failed track = 10%,
- Video 2: MSE = 1.2; Failed track = 4%,
- Video 3: MSE = 1.6; Failed track = 16%.

With this method, the majority of sperm can be tracked properly. However, this method was suitable when the concentration of sperm was not too high. When the sperm concentration was high, the system had difficulty dealing with occlusion. In some cases, objects in the background were considered as sperms. Tracking failure was still considered quite high.

Tomlinson *et al*. in 2010 [8] used a multitarget algorithm to validate a CASA system. The CASA system features a PC with Windows XP, Fire-I 400 Firewire camera, Firewire capture card, Olympus microscope, and a 37°C warm-up table set. Each observation was made on the 20-mm Leja sliding board. In the algorithm, sperm detection was automatically detected by assigning a threshold to the frame so that the frame converted into a binary image. The objects in the image were then subjected to erosion and dilation operations. Objects whose size was not close to the size of the sperm head were removed. If there were still undesirable artifacts, they were removed manually. The detected sperm objects were then tracked simultaneously using the Markov Chain Monte Carlo (MCMC) method. Based on the trajectory and movement parameters, each sperm was then categorized by its motility based on 1999 WHO laboratory manual [26].

The result was that the system has proximity to manual measurement results, especially for sperm with motility categories a, b, and d. The sperm with motility c was difficult to assess.

In addition to the various advantages, the system ought to still be given manual intervention for the results to be good. The categorization of motility still used the old WHO guidelines [26] so that it needs to be adjusted again with the new WHO guidelines [19].

In 2014, Nurhadiyatna *et al*. [27] compared several human sperm detection and tracking methods. In fact, this paper more focused to sperm detection comparison. The experiment result itself showed that Gaussian Mixture Model with Hole Filling Algorithm obtains the highest accuracy for human sperm detection compared to other sperm detection methods. For tracking, they used only Kalman filter without comparing to any other tracking method.

Similarly to Nurhadiyatna, Imani *et al.* [28] in 2014 started the multisperm tracking process with background modeling on the video and subtracted it from the frame so that objects can be extracted. The method used for the background subtraction is 2D-Non-linear Diffusion Filtering. The process continued with morphological operations to get a clearer object. Its multisperm tracking was done by comparing the results of system detection on each frame and calculating its proximity using the Hungarian algorithm.

To measure its accuracy, system results were compared with ground truth data. This method was good for low-density semen (about 10 sperm in one microscope field of view). The

results showed an accuracy of 96.76%. However, some limitations of this method were the difficulty of handling the following cases:

1. Sperm coming out of the microscope field of view,
2. The missing sperm and reappear in the microscope field of view,
3. The passing sperm,
4. Partial occlusion.

Beya *et al.* [29] in 2015 performed sperm tracking more gradually. Beginning with a preprocessing stage in which consists of thresholding and contrast stretching. In the preprocessing stage, there was also a potential region detection using the thresholds obtained from entropy maximization and morphological operations with a 10x10 kernel. The region was then represented as a histogram. The histogram was then inputted into Support Vector Machine (SVM).

At the detection stage, all sperm were detected by the bag of words and SVM. While the literature for the bag of words itself used several features, namely: interest points resulted from Speeded Up Robust Features (SURF), Histogram of Oriented Gradients (HOG), and Local Binary Patterns (LBP).

In the tracking phase, potential regions were detected in the first frame. Afterwards, SURF, HOG, and LBP were extracted in the candidate region, which is followed by Mean Shift Tracking.

The system was tested on 3 samples of sheep sperm observation video that already had ground truth data. Experimental results showed the precision of sperm detection of 0.94, 0.93 and 0.96, and recall of 0.96, 0.92, and 0.97. Root mean square error (RMSE) in sperm tracking results of 8.06, 9.01, and 7.09 pixels.

Hidayatullah *et al.* [17] in 2015 proposed a multisperm tracking for automatic motility measurement. In the first phase, they performed sperm detection using adaptive local threshold and an ellipse detection algorithm which was adjusted according to sperm shape[16]. At the tracking phase, they used Hungarian algorithm based on sperm locations from k^{th} frame to k+1th frame. The detection result was encouraging which is useful for tracking phase. Nevertheless, the tracking result was not satisfying enough because position parameter was not enough to predict sperm movement in semen with highly densely populated sperm.

Mahapatra *et al.* [30] in 2016 proposed a combination method between background subtraction using mixture of Gaussian with a synchronized frame difference. The wavelet was used for reducing noise in advance.

The background subtracted image was then converted into a binary image by making the inter-frame difference. Binary image $B_1(x, y)$ was obtained by subtracting between the kth and the k-1th images while binary image $B_2(x, y)$ was obtained by subtracting between the k+1th and the k^{th} images as described in (1) [30].

$$B_1(x,y)=\begin{Bmatrix}1,|b_k(x,y)-b_{k-1}(x,y)\geq T\\0,|b_k(x,y)-b_{k-1}(x,y)<T\end{Bmatrix} \quad (1)$$

$$B_1(x,y)=\begin{Bmatrix}1,|b_k(x,y)-b_{k-1}(x,y)\geq T\\0,|b_k(x,y)-b_{k-1}(x,y)<T\end{Bmatrix}$$

$$B(x,y)=\begin{Bmatrix}1,|B_1(x,y)\cap B_2(x,y)|=1\\0,|B_1(x,y)\cap B_2(x,y)|=0\end{Bmatrix} \quad (2)$$

The final frame difference which called three-frame difference $B(x, y)$ was obtained by performing AND operation between $B_1(x, y)$ and $B_2(x, y)$ as formulated in (2). In the last step, the target was defined if $B(x, y)$ was equal to 1.

They performed the experiment using microscopic human semen videos with 40x to 400x total magnification. The method itself was implemented using MATLAB 8.0. They claimed that the detection and false alarm rate surpassed the classical mixture of Gaussian model. Unfortunately, this method was not compared to any other state of the art multisperm tracking methods which was already available at the time the paper was written. From the experimental result, false alarm rate was also still considered to be high (21.32%) and using visual assessment, there were still a lot of undetected sperms.

Jati *et al.* [31] in 2016 tackled the problem of multisperm tracking on low frame rate video. This was a challenging case as sperm moved very fast and unpredictable. Moreover, the sperm have similar shape and size.

They divided the process into two stages: multisperm detection and multisperm tracking. In the first stage itself, there were three steps which were background subtraction, 2D Gaussian Filter for noise reduction, and thresholding using Otsu's method. The position of the detected sperms was defined by the coordinate of the pixel which has maximal intensity value.

As an additional explanation, the 2D Gaussian Filter was described using (3) [31]

$$G(x,y)=\frac{1}{2\pi\sigma^2}e^{-\frac{x^2+y^2}{2\sigma^2}} \quad (3)$$

where σ was defined as the standard deviation of the distribution, while x and y were the coordinates of the pixel. The mean distribution was defined as zero. Laplacian of Gaussian $\Delta G(x, y)$ was designed based on Gaussian distribution for blob detection. The last step was performing Laplacian of Gaussian convolution to the input image.

In the case of colliding sperm, standard Kalman Filter performs poorly. To enhance its performance, they combined predicted sperm positions from Kalman Filter and detected positions from multisperm detection stage. Estimated positions were assigned using Hungarian algorithm. The Hungarian algorithm was used with distance (cost) matrices between all sperm pairs which rows were estimated positions and columns were detected positions.

The experiment used two datasets. The first dataset was acquired from Dr. Cipto Mangunkusumo Hospital Lab with frame rate about 20 fps [32] and the second dataset was retrieved from Kokopelli Technology [33]. The result was encouraging with 90% detection accuracy comparing to visual measurement. For multisperm tracking with three challenging cases: fast motion dataset, low frame rate dataset, and dataset with partial occlusion, they claimed to successfully track the sperms without mentioning the quantification.

Recently in 2016 Urbano *et al.* [34] proposed a more reliable and fully automated multisperm tracking method. At the detection stage, the Gaussian filter was used as much as n times to reduce noise. Afterwards, applying Laplacian of Gaussian (LoG) / Mexican Hat filter and Otsu thresholding to clarify the existing objects. Subsequently, small objects which size <5 pixels were discarded because sperm was unlikely to be that small. The remaining objects were considered sperms.

TABLE I: SUMMARY OF THE ADVANTAGES AND DISADVANTAGES OF MULTISPERM TRACKING METHODS

Research	Advantages and disadvantages
Corkidi *et al*., 2008[22]	Advantages: • The system was able to visualize trajectories in 3D with only 1 microscope and 1 camera • The track created can be used to analyze sperm motion based on its 3D motion. Disadvantages: • It is unclear exactly what benefits can be achieved by visualizing the 3D sperm motion trajectory • Sperm samples were sea urchins semen that were rarely used in everyday life which bring fewer research benefits. • There was no explanation either of the total sperm that existed and how many sperms that were successfully tracked. • Observation duration was only 1 second.
Sørensen *et al*., 2008[25]	Advantages: • Sperm trajectories can be well drawn (average MSE = 1.6 pixels). Disadvantages: • Difficulties occurred in handling occlusion when sperm concentration was high. • Sometimes the background objects were considered as sperms. • Tracking failure was still quite high. (4% – 16%).
Tomlinson *et al*., 2010[8]	Advantages: • Experiment settings were set to be as idealistic as possible for reliability. • The observation was performed by experienced observers. • The amount of specimens was considerably high (100). Disadvantages: • Manual intervention was still needed. • The assumption of hemocytometer results were always correct. • Manual motility measurement was relatively feasible for sperm categories a, b, and d. Unfortunately, it was difficult for category c. • Dead sperm that moved due to fluid motion was considered as motile sperm. • The results of motility calculations by the system were always smaller than manual calculations.
Ristic *et al*., 2011[36]	Advantages: • This measurement method can measure the performance of multisperm tracking methods with results consistent with expectations. Disadvantages: • In fact, there was already standard OSPA method previously. This paper added a few enhancements.
Su *et al.* 2012[24], 2013[23]	Advantages: • The system can handle numerous sperms (1500) at once. • The visualization result was very encouraging. Disadvantages: • The visualization capability had not been directly utilized for measuring sperm quality yet.
Nurhadiyatna *et al*.2014 [27]	Advantages: • The detection methods comparison were quite comprehensive. Disadvantages: • Despite its title for comparing both sperm detection and tracking methods, the paper factually more focused on comparing sperm detection methods. • The tracking method had not been utilized for measuring sperm quality yet.
Imani *et al*., 2014[28]	Advantages: • The method worked well for semen with low sperm concentration (1-10 sperms per viewing field, 120x, 25fps). • The research already used the new WHO guidance. There are limitations on the following cases: • High sperm concentration semen, • Sperm that comes out of the field of view, • Tracking reappear sperms, • Colliding sperm, • Partial occlusion.
Beya *et al.*, 2015[29]	Advantages: • The resulting trajectories have high precision and low RMSE. Disadvantages: • Over generalization: The title stated the research used animal samples while factually only sheep samples were used. • The number of samples were too small (3 videos.) • The number of sperm in one field of view was small (4-12 sperms).
Hidayatullah *et al*., 2015[17]	Advantages: • The method has high accuracy of sperm detection which helps tracking phase. • The tracking method has already been directly used for measuring sperm quality Disadvantages: • In the tracking phase, the sperm association accuracy between frames was still relatively low.
Arasteh and Vahdat, 2016[37]	Advantages: • The tools were helpful in the initial test with synthetic datasets • It has parameters for adding noise, blur. • The number of sperm generated and its type can be defined by the user. Disadvantages:

	• The synthesized dataset was not explicitly tested for its validity.
Jati *et al*., 2016 [31]	Advantages: • Overall performance showed encouraging result. • The tracker can successfully track the sperms in the whole sequence in low frame rate dataset where abrupt motion occurred frequently. Disadvantages: • There were still miss detections as some noises were detected as sperms. • In sperm tracking of fast motion datasets, the estimated position was occasionally not reliable and cause wrong sperm positions estimation from Hungarian assignment. • The method encountered the most complicated challenge in tracking with partially occlusion case. The tracker sometimes swapped from one sperm to another. • The tracking had not been utilized for measuring sperm quality yet.
Urbano *et al.*, 2016[34]	Advantages: • The method was fully automatic. • It was able to track hundreds of sperm at once. • It was robust on sample A. • The experiment used long video duration sample (15- 45 seconds). • The tracking speed was high (Real-time). • The tracking method was already utilized for measuring sperm motility. Disadvantages: • Number of samples was too small (2 videos). • Standards of examination protocol were not specified. • In specimen B, the tracking result was not good (24% difference with manual assessment).

In the tracking phase, the algorithm used was the modified Joint Probabilistic Data Association Filter (JPDAF). After tracking, eight CASA parameters were calculated which were VCL, VSL, VAP, LIN, WOB, STR, ALH, MAD. From that parameters, the sperm motility was measured.

The performance of the method was evaluated using a multiobject tracking algorithm metric called Optimal Sub-Pattern Assignment (OSPA). The experienced technician measures manually and states that there were 92% motile sperm in sample A and 97% in sample B. While the developed system showed there were 93% motile sperm in sample A and 73% in sample B. In sample A, the system measurement result was very close to the manual measurement result. However, in sample B, the measurement result was still too far from the result of manual measurement with 24% difference.

Experimental results also show that JPDAF and Gaussian Neural Network algorithms consistently outperformed Neural Network and PDAF algorithms. In general, this method has many advantages such as speed (real-time), fully automatic, able to track hundreds of sperm at once and reliable on sample A with a duration of long video samples (15-45 seconds). However, on sample B the accuracy was not encouraging. Fig. 2 shows the reconstructed trajectories.

C. Performance Measurements of Multisperm Tracking Algorithm Method

Some researchers focused on the method of measuring the performance of multiobject tracking methods. Of course, this research is very useful. Ristic *et al.* [36] in 2016 expanded OSPA metrics in measuring the performance of a multiobject tracking algorithm. In this measurement, it was given the mapping between ground truth value with the result of the tracking system and calculated the distance between them using the base distance formula.

Arasteh *et al.* [37] in 2016 built a system to test two multisperm tracking algorithms. The system was web-based with the purpose of being able to be used by many different platforms. The compared multisperm tracking algorithm was a Kalman filter-based algorithm and a particle filter one. The dataset used in the test was a synthetic dataset.

D. Summary of Multisperm Tracking Methods

The currently exist multisperm tracking methods have advantages and disadvantages. Based on the analysis above, here is the summary of the methods mentioned.

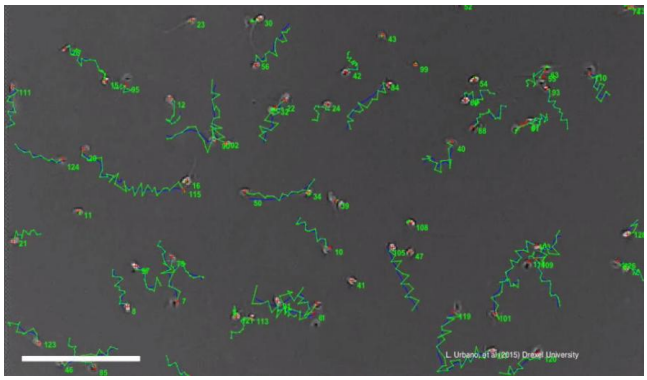

Fig. 2. The reconstructed 3D sperm trajectories [34], [35].

V. Conclusions

Since its introduction in the 1980s, CASA-based semen quality measurements have gained a place because of its urgent presence. But commercial CASA systems are so expensive that stimulate researchers to develop methods that are part of CASA. The major problem that remains unresolved is the multisperm tracking to obtain high precision sperm trajectories with efficient computation on semen with high sperm concentrations. With a high precision trajectory, the CASA parameter calculation results will better describe the actual sperm motility conditions. No existing methods that can actually produce precise trajectories in complex cases, especially colliding sperm and occluding sperm in the situation of large sperm counts appear in one microscope field of view. Even though these cases frequently occur in semen examination at Artificial Insemination Center. Several

methods of measuring the performance of multisperm tracking algorithms have been developed which include methods developed by Arasteh *et al*. [37] and Ristic *et al*. [36].

For future works, this review suggests that multisperm tracking for semen with highly densely populated sperm is very important for sperm motility measurement. Until now, this is still an open problem as none of the current methods could solve it robustly for high number of samples.

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