Automated Analysis of Human Sperm Number and Concentration (Oligospermia) Using Otsu Threshold Method and Labelling

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Abstract. Oligospermia is a male fertility issue defined as a low sperm concentration in the ejaculate. Normally the sperm concentration is 20-120 million/ml, while Oligospermia patients has sperm concentration less than 20 million/ml. Sperm test done in the fertility laboratory to determine oligospermia by checking fresh sperm according to WHO standards in 2010 [9]. The sperm seen in a microscope using a Neubauer improved counting chamber and manually count the number of sperm. In order to be counted automatically, this research made an automation system to analyse and count the sperm concentration called Automated Analysis of Sperm Concentration Counters (A2SC2) using Otsu threshold segmentation process and morphology. Data sperm used is the fresh sperm directly in the analysis in the laboratory from 10 people. The test results using A2SC2 method obtained an accuracy of 91%. Thus in this study, A2SC2 can be used to calculate the amount and concentration of sperm automatically

Keyword. Sperm, Oligospermia, Concentration, Threshold, Labelling

1. Introduction
Get married and have children is a dream for every couple. But for some couples sometimes very hard to get a descent it self though has been married in a long time. There are many factors that can affect pregnancy. Not only from the mothers who determine the success of a pregnancy but the fathers also have contribution. Sperm quality is also an important part. But sometimes the fathers do not realize have had disorders/disturbances in sperm that may affect the occurrence of pregnancy itself.

One of the sperm disorders that may interfere with male fertility is the Oligospermia. Oligospermia is a disorder in which during ejaculation, the sperm contains only low concentrations. Normally the concentration of sperm in the ejaculate is 20-120 million/ml, while in the Oligospermia case, the concentration of sperm less than 20 million/ml. Most of the causes of this problem are associated with

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lifestyle, such as less activity, testes or testicles often in a state of overheating, smoking, obesity, unhealthy diet, and stress, or illness experienced by people who could affect sperm production. Majority, the cause of oligospermia is a lifestyle that is not balanced like too many unhealthy foods and detrimental to health such as junk food, fast food, alcohol, smoking, and less exercise, which in turn cause problems in the body such as obesity or diseases. Another influence male hormone in the body and interfere with sperm production itself. Therefore for men who want to keep sperm healthy without any problems must keep with a healthy lifestyle.

To determine the occurrence of oligospermia can check the sperm in the fertility clinics laboratory. One way using haemocytometer, do as counting red blood cells (erythrocytes). By using a haemocytometer and microscope views, spermatozoa counted manually in five squares diagonally or four boxes in each corner and the middle of the box. 10 million equal to the concentration of spermatozoa multiplies the calculation result / ml.

Manual process conducted by experts, take a lot of time and energy [1]. In the laboratory, spermatozoa analysis is determined at random within each elevated view of the microscope. Each elevated view of a certain amount of information obtained normal and abnormal spermatozoa randomly. The results obtained in each pass of view then summed and averaged, in order to obtain information on the number and concentration of spermatozoa (either normal or abnormal) of each sample preparation. This conventional method will lead to differences in measurement results when performed by different experts, because the calculation of normal and abnormal spermatozoa done randomly. Therefore in this study created a computer-aided system to calculate the amount and concentration of spermatozoa in the semen. The data used were ejaculate data of 10 people and analysed directly in the laboratory of Microbiology Health Polytechnic of Surabaya. This procedure compared the number and concentration of sperm which are normal or not normal by using manual calculation according to the WHO standard [13] and calculations with a computer, that is by taking pictures of spermatozoa in parts from counting booths. The images taken were carried to initial processing stage that aimed to simplify further processing. At this stage, it will apply methods of image enhancement and noise removal. Next, the images will be subjected to the process of background removal, and then carried out to segmentation process to obtain sperm cells by comparing the use of Otsu threshold methods and the method of threshold local adaptive (deviation adaptive). The comparison of these two methods are based on the fact that data sperm images taken from the microscope were not all the same, it depends to the brightness of sperm shape and gray scale of the background. The next stage is the extraction of these features will be represented to other forms and being look for some of its features. The value of features will be labelled to determine the quantity per box of counting booth and are compared to the results of manual calculations. The While the previous research [11] focused on the methods and process of calculating the number of sperm per image, so that only known quantities or simply counting the number of sperm in one preparation, this research counts the number of sperm per ejaculate to obtain sperm quantity and concentration that will be used to determine whether a person is suffering from an oligospermia or not.

2. Literature Review

According to WHO, 2010 [13], normal sperm count is approximately 10-20 million/ml or about 2-6 ml. The characteristics include the amount of healthy sperm > 15 million/ml, have quick movements and straight, relatively normal shape, viability is good, no bacteria and leukospermia lot. When the sperm concentration <15 million/ml, it will be categorized as oligozoospermia. Furthermore if the sperm concentration less than 5 million/ml can be regarded as extreme/severe oligozoospermia which is the production of sperm produced minimal or included in the weight categories and if not handled immediately could lead to the possibility cryptozoospermia stage even to the stage of azoospermia. Here explained how to calculate the amount or concentration of
sperm in semen human clinical laboratory measurements and methods that will be used to analyse images from a digital microscope. Spermatozoa classification in humans with Fuzzy rules by Monali Khachane Y., et.al [8]. The classification based on the shape of the head, neck and tail of the sperm. The sperm segmentation using image processing techniques and classification of the sperm head. Research carried out on the digital image is sperm. Spermatozoa segmented consist of three parts: sperm head, neck, and tail. Sperm image is processed, the result is a segmented sperm feature, then the proposed method using Fuzzy for classifying normal and abnormal based on shapes and the sperm head area. The downside of this study when found flattened sperm head, it cannot be detected because the image is only based on two-dimensional images while the spermatozoa should be analysed in three-dimensional space in order to obtain more accurate information. Spermatozoa considered normal if the head, neck and tail have normal criteria, but in the study of normal and abnormal criteria only found on the head, so that cannot be used as a basic right.

Priyanto Hidayatullah and Miftahudin Zuhdi, [11] performs research detection and count the number of spermatozoa in certain areas (in a video taken from a microscope multimedia) using adaptive local threshold and Ellipse Detection to detect sperm head, the measures taken to detect and count the number of spermatozoa is to convert the input image into a grayscale image, noise filtering, converting a grayscale image into a binary image using a local adaptive threshold, perform Closing and Open on morphological operations, detecting the edges of objects, detect sperm using ellipse detection. Results from this study, the method used achieve 90.97% accuracy with only 6:09% false positive per frame which is better than the existing methods of detection of sperm. This study is only the limit counts the number of spermatozoa alone based head, so cannot be used as a basis for determining the quality of spermatozoa. Spermatozoa quality can be determined with more complex parameters such as spermatozoa shape, the velocity and trajectory of motion.

Jiaqian Li, Tseng Kuo-Kun, Haiting Dong, Yifan Li, Ming Zhao and Mingyue Ding, 2014 [4], their research is clicking identification of the morphology of the sperm cells, whether healthy or not. The method used is a combination of principal component analysis (PCA) to extract features of the image and the k-nearest neighbour (KNN) algorithm to diagnose Sperm Health. Sperm cells are analysed images taken from microscope images; the size of the spermatozoa is very small. Sperm health diagnosis method consists of three parts: the sperm image segmentation, feature extraction with PCA and classification with k-nearest neighbour (KNN) algorithm. At the end of the trial results will be compared between PCA and features SHIFT feature, KNN classifier and classifier BPNN. Accuracy of diagnosis is done turns out depends on the training set, one of the results with specific training set to obtain a good performance with an accuracy of 87.53% compared with other feature extraction methods such as Scale-invariant feature transform (SIFT) and other classifier such as back propagation neural network (BPNN). The downside of this study is the method compared to only two, and the paper does not explain the reason why accuracy is only obtained 87.53%.

2.1. Counting Sperm and Calculated Concentration

The concentration of spermatozoa is the number of spermatozoa in a millilitre of sperm. The concentration of spermatozoa can be calculated in several ways, one of which is by using a haemocytometer. Calculate the concentration of spermatozoa by using haemocytometer; do as counting red blood cells (erythrocytes). By using a haemocytometer, spermatozoa counted on five squares diagonally or four boxes in each corner and the middle of the box, the calculation result is multiplied by 10 million equal to the concentration of spermatozoa / ml, such as figure 1. [13]

To calculate the concentration and quantity of sperm ejaculated spermatozoa used new (<1 hour, standard WHO [9]), then the sperm has been shaken evenly and perfectly likwofeksi diluted,
with a ratio of 1:10, 1:20, 1:100 or 1 : 200, depending on the density of spermatozoa / LPB. For a 1:10 or 1:20 dilution can be used pipette leukocytes, to 1: 100 or 1: 200 can be used pipette erythrocytes.

Pipette leukocytes / erythrocytes shaken by the number 8 for 15-20 minutes. 3 drops first then discarded, before dripped into the count room, left 15-20 minutes so that all the cells settles evenly in the room count [9]. Then count the number of spermatozoa in the following manner:
- If the sample is less than 10 spermatozoa per LPB, then count all the big boxes that are 25
- If spermatozoa seen 10-40 per LPB, then simply counted 10 big boxes
- If the sample is> 40 spermatozoa seen LPB, then simply counted 5 large box.

Furthermore, when it has counted 25, 10 or 5 large box on haemocytometer then divided into appropriate conversion factor large box that has been calculated, the result is the concentration of spermatozoa in a million per millilitre. When normal spermatozoa concentration> = 20 million / ml (WHO, 1999) and> = 15 million / ml [13]

![Figure 1](image.png)

**Figure 1.** (a) Haemocytometer Counting booth, (b) Sperm Data to be analysed, (c) Process to Calculate Total Sperm Count In one booth

Besides, it can be used to calculate the number of spermatozoa that living or dead is done by making smears or staining differential. The fresh sperm (undiluted) given one drop coupled with 2-3 drops of colour eosin, then blended until homogeneous and then made thin smear alone and be heated over the fire for over a minute while moved about so dry evenly. Then check under the microscope with a magnification of 45 x, the spermatozoa that have died before made preparations means it will appear darker because it absorbs the dye, whereas spermatozoa were still alive when made preparations will be bright or brighter than the dead.

The percentage of living or dead spermatozoa can be calculated by taking a sample of 100-200 spermatozoa in some objects of observation. By smears can also be observed forms of spermatozoa abnormalities, both primary and secondary abnormalities. The primary is abnormal sperm shape abnormalities as a result of testicular disorder (seminiferous tubules), for example: small head, big head, piriformis heads, two heads, two tails, the middle and curled tails and linkage abaxial. Abnormal secondary is a form of abnormal spermatozoa occurring after spermatozoa leaving the seminiferous tubule epithelial sprouts or due to lack of maturation of spermatozoa in the epididymis can also be caused by the effect of cooling or heating. Examples: head and tail cut off, neck twisted, immature.

### 2.2. Threshold Segmentation Methods
Image thresholding is a technique widely used for segmentation, which divides the gray level image into segments suitable for some classes based on the gray level. Most thresholding approach is proposed for two classes, and based on the gray level histogram of the image. Two of the seven most popular approaches are the Otsu method [10] and Minimum Error Thresholding / MET method [5]. As has been surveyed in [2], there were no significant differences between the results of the two methods.

In this study, thresholding is not used for image segmentation as a final objective, but as a point of reference in maximizing the contrast to background and foreground as much as possible. Then the election between the two methods is not significant impact. Threshold divides the area into two classes as follows:

\[
g(x, y) = \begin{cases} 
0 & f(x, y) < T \\
1 & f(x, y) \geq T 
\end{cases}
\]  

(1)

The purpose of the Otsu method is to find the point threshold that divides the gray level histogram of the image into two different areas automatically, at which point chosen is such that inter-class variance is as large as possible. The object is achieved by minimizing the weight of within-class variance, which is actually equal to: maximize the inter-class variance. The weight of the within-class variance is:

\[
\sigma_w^2(t) = q_1(t)\sigma_1^2(t) + q_2(t)\sigma_2^2(t)
\]  

(2)

Class probabilities of first and second class are (where \(i\) represents the number of pixels for the emergence of a certain gray-level, while \(t\) is the gray level value):

\[
q_1(t) = \sum_{i=1}^{t} P(i)
\]  

(3)

\[
q_2(t) = \sum_{i=t+1}^{I} P(i)
\]  

(4)

Within-class variance of each class:

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

(5)

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

(6)

If counted all grades \(\sigma_1^2(t)\) and \(\sigma_2^2(t)\) the gray scale image will be obtained within-class variance value minimum. Value \(t\) what makes \(\sigma_1^2(t)\) and \(\sigma_2^2(t)\) becomes minimum is the threshold value sought. But utilizing the fact that the total variance in an image is always constant, not dependent on the threshold value can save the computing process. So for any threshold \(t\), total variance is the sum of the within-class variance and inter-class variance:

\[
\sigma^2 = \sigma_w^2(t) + q_1(t)\left[1 - q_1(t)\right]\left[\mu_1(t) - \mu_2(t)\right]^2
\]  

(7)

withinclass variance \hspace{1cm} interclass variance = \sigma_b^2(t)

From the equation above shows that minimize within class variance (\(\sigma_w^2(t)\) ) Is the same as maximizing the interclass variance (\(\sigma_b^2(t)\)). Value of \(\sigma_b^2(t)\) can be calculated recursively with one-way ranging from value \(t\) lowest to highest (0 to 255 for 8 bit gray scale). Pseudo code to calculate the value \(\sigma_b^2(t)\) are as follows:
1. Calculating the histogram and probability of each level of intensity (i.e. normalization of the histogram so that the total is equal to one)
2. Set the initial value $q_1(t), q_2(t)$ and $\mu_1(t), \mu_2(t)$
3. Exploring the entire value threshold may $t=1 ...$ maximum intensity
   a. Update value $q_1(t), q_2(t)$ and $\mu_1(t), \mu_2(t)$
   b. Calculate $\sigma_B^2(t)$
4. Threshold is sought is that gives the maximum $\sigma_B^2(t)$

3. Methodology
The researches conducted for Automated Analysis of Sperm Concentration Counters (A2SC2) are as in Figure 2.

![Figure 2. Methodology for Automated Analysis of Sperm Concentration Counters / A2SC2](image)

4. Result And Discussion
The research conducted for Automated Analysis of Sperm Concentration Counters (A2SC2) has following steps.

4.1. Pre-Processing
Pre-processing is also used to process data sperm image for further analysis. Pre-processing can purge noise in the image, the image colour format conversion and image edge detection process. Some existing processes include composite, cropping, equalization and the mosaic image. In a sperm analysis to determine the amount and concentration of sperm, pre-processing designed is as follows:

1. Mean filter is used for smoothing images having noise. Mean Filter replaces the pixel value at position $(x, y)$ with an average value of neighborhood pixels. The area of the number of neighboring pixels is determined as a masking / kernel / window size, for example $2x2, 3x3, 4x4$, and so on. In addition to the mean filter, this study have also tried other image processing techniques, to obtain the most effective technique as a pre-process prior to the process of segmentation, feature extraction and classification.
2. Gray scaling: The aim of gray scaling is to convert input RGB image into gray scale image. This process is done in order to simplify and clarify the image of the equalization process in the next step.
3. Equalization: In this equalization process will display the original image, then the original image is calculated the width $(w)$ and height $(h)$ image. Image of the equalization, image pixels that has not spread evenly match the color of the pixel will be spread evenly which the results will be more visible role in the image histogram equalization results

Results from this process as in the figure 3.b
4.2. Otsu Threshold Segmentation

Feature extraction process aims to take on the characteristics contained in an image. Feature extraction method used is the analysis of Otsu threshold. Results of the method of Otsu are a threshold value (threshold) whose value ranges between 0 to 255. Each of the input images will have a threshold value that is different. This is caused by differences in brightness and intensity of each gray degree on image of sperm obtained from a microscope. Then do the thresholding process is a continuation of the process of Otsu method for the purpose of executing the image corresponding to the threshold value. In this study, the image of the results of the pre-processing the image captured from the microscope sperm that equalization is then followed by determination of the threshold value will be at the threshold Otsu method so that the result will be like the figure 3.

The segmentation process was performed using locally adaptive thresholding based on block detection. In the block detection process, the input data is the result of the segmented images. This process requires initialization of the mean and standard deviation used as a threshold. The first step performed on the image that has been divided into several blocks, was to calculate the value of local mean and local standard deviation. Local mean and standard deviations were calculated for each block and used to determine whether a block should be processed with adaptive threshold or not. Then the value of local mean and local standard deviation of each block were compared to the mean and standard deviation threshold. If the local value of a standard deviation is lower than the standard deviation threshold and the local mean is higher than the mean threshold, then each pixel in the block is substituted with background pixels. Otherwise, each block is binarized with a global method (Otsu method). Otsu method is used because of its speed in performing the global thresholding.

Figure 3. Segmentation Result a. Original image b. Results Image of Pre-processing c. Result of Adaptive Threshold Image Segmentation d. Result of Otsu Threshold Image segmentation

4.3. Morphological Features Extraction

Improvement process of sperm image segmentation results performed using morphological features. The goal is for extracting the image of the sperm and then separated so that the shape of the sperm will be more obvious. Gray level image of microscopic sperm containing sperm forms segmented and then processed to separate the overlapping sperm. Segmentation procedure based threshold (threshold) and morphology filter, whereas sperm separation procedures based on a watershed approach. Microscopic image of sperm in gray level is read for processing (Figure
3.b.). Then do binary process with a threshold gray level and filter morphological opening operation to refine the shape of sperm from noise. The points of each cell pointer obtained by dilating the maximum area and the topography of the surface area that states the distance transformation. The boundary line between dot pointers gave sperm outside the boundary lines.

Dividing lines sperm contact form can be obtained by diminish filtered binary image with skiz geodesic lines. Likewise, to obtain sperm shape intact the removal of sperm clipped by the image boundaries is to reconstruct the image and the boundary cells that attached or truncated. Sperm barrier contour can be obtained by transforming dilatation and subtraction. Then the contour can be superimposed to the initial gray level image segmentation to obtain sperm.

4.4. Calculation Amount, Sperm Concentration and Labeling Sperm

The concentration of spermatozoa is the number of spermatozoa in a millilitre of sperm. The concentration of spermatozoa can be calculated in several ways, namely by: using a haemocytometer, Nefelmeter method (photoelectric calorimeter) and Methods Spermiodensimeter. In the shooting of the microscope used haemocytometer. Calculate the concentration of spermatozoa by using haemocytometer; do as counting red blood cells (erythrocytes). By using a haemocytometer, spermatozoa counted on five squares diagonally or four boxes in each corner and the middle of the box, the calculation result is multiplied by 10 million spermatozoa concentration equal to 1 ml.

To count the number of sperm from a sperm image per-booth taken from a microscope, the labelling process was performed. This process was performed after data segmentation, in which the image has been separated between the object and background. The labelling process served as the deciding point to mark an object. After a successful image labelling, then the amount of sperms can be determined, although it is still fused with the non-sperm image.

The algorithm applied to the process of labeling is Two-pass algorithm. This algorithm is relatively simple to understand and implement, where it will iterate twice in the binary image. In the first iteration (first pass), the two-pass algorithm serves to record the equivalence and establishes a temporary label on the detected object. The second iteration (second pass) serves to replace each temporary labels assigned in the first iteration. Thus, differences in the labels given to the pixels of the image are the exact number of objects.

4.5. Test Result

Sperm data analyzed were data from 10 people of various ages. The followings are the results of conventional calculations from a fertility clinic in Surabaya and calculation from Microbiology Laboratory of Health Surabaya Polytechnic. The result is on Table 1. The manual analysis of the sperm showed 16 million / ml spermatozoa in sperm-1 sample. Overall, a total of 15 million / ml can be identified automatically using Otsu segmentation and the other 1 million / ml were not identified. The 15 million / ml were identified automatically with adaptive local segmentation and 1 million / ml were not identified. The comparison between the automatically identified spermatozoa (IO) and manually identified spermatozoa (M) using each segmentation method is as follows:

\[
\frac{10o}{M} = \frac{15}{16} = 96\% \text{ and } \frac{10a}{M} = \frac{15}{16} = 96\%
\]

Description: IOo = Automatic Identification Otsu, IOA = Automatic Identification Adaptive.

Results of calculation 9 other sperm can be seen in Table 1.
Table 1. Test Results A2SC2 Compared To Manual Calculation Results

| Sperm Identification | Amount of Sperma / Expert Justice (M) / million / ml | Category | Otsu Threshold (IOo) Million/ml (IOo/M)% | Local Adaptive Threshold (IOa) Million/ml (IOa/M)% |
|----------------------|-----------------------------------------------------|----------|------------------------------------------|-----------------------------------------------|
| Sperm-01             | 16                                                  | Abnormal | 15                                       | 96                                           |
| Sperm-02             | 36                                                  | Normal   | 29                                       | 80                                           |
| Sperm-03             | 33                                                  | Normal   | 31                                       | 95                                           |
| Sperm-04             | 29                                                  | Normal   | 25                                       | 86                                           |
| Sperm-05             | 20                                                  | Abnormal | 17                                       | 84                                           |
| Sperm-06             | 30                                                  | Normal   | 29                                       | 98                                           |
| Sperm-07             | 184                                                 | Normal   | 168                                      | 91                                           |
| Sperm-08             | 12                                                  | Abnormal | 11                                       | 92                                           |
| Sperm-09             | 28                                                  | Normal   | 25                                       | 90                                           |
| Sperm-10             | 28                                                  | Normal   | 27                                       | 98                                           |
| **Average Accuracy (%)** | **91**                                              |          | **92**                                   |                                               |

Based on the results in Table 1, the comparison value between automatically identified and manually identified spermatozoa reached an average of approximately 91% using Otsu threshold method and an average of about 92% using local adaptive threshold. However, the figure does not describe the overall system accuracy, since there was an error factor (FP-False Positive) (which is non-spermatozoa identified as spermatozoa). However, if the input image was processed manually and contrast value was enhanced in such a way, sometimes it would seem that the point formerly being considered an FP in manual procedure, was, in fact, TP (True Positive).

5. Conclusion
Automatic spermatozoa calculations can be performed using the proposed method. A possible error only occurs if the sample images contain clumps or there is a case of a collision (coincide). The accuracy of the automatic calculation is approximately 91%. The best color space to analyze the calculation of spermatozoa is the red color space, because the base color of the shooting is red.

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