Assessment of Human Sperm Head Morphology for Assisted Reproduction Techniques using Open Source Software

Raghavendra. Maggavi[1], Sanjay. Pujari[2], Amar.Herekar[3]

Research Scholar [ECE], Visvesvaraya Technological University, Belagavi, India[1]

Professor, Dept. of ECE, Angadi Institute of Technology and Management, Belagavi, India[2]

Assistant Professor, Dept. of ECE, Maratha Mandal Engineering College, Belagavi, India[3]

ABSTRACT: Infertility affects about 15% of the population and going by the trends seen by infertility clinics worldwide, it is on the rise from its present about 15% of population to about 20% or even more. Head size and head shape defects are important criteria in the determination of a spermatozoon's morphological normality or abnormality and able to find out the sperm cell is alive or dead. In the present study, we have developed a freely available sperm head morphology analyzer plug-in for open source software. Described the systems functionality and confirmed its validity with respect to the commercial softwares.

KEYWORDS: Microscopic Image, Spermatozoon, WHO, IMAGEJ, Medea LAB CASA.

I.INTRODUCTION

When a couple had undergone the painful experience of miscarriage, and has been investigated thoroughly, have been placed under "Unexplained" category, the options for counselling, planning of treatment and prognosis all becomes troublesome for the treating consultants. Hence, increased research in to the factors governing pregnancy loss becomes validated. Over the years it has been noticed that we had paid relatively less attention to male partner or Sperm proper in contrast to the female factors. Now that we have known almost every possible female factor contributing to the pregnancy loss it's time we shift our serious attention on to the sperm. The set of four morphometric parameters was obtained. The definitions of these parameters are illustrated in the fig.1. Since the heads of human spermatozoa may not be bilaterally symmetric, head length (L) and width (W) must be carefully and objectively defined [1]. We measured length as the distance between the midpoint of the insertion of the flageller midpiece with the head (fig.1, point a) and the point farthest (b) from it. Head width was then defined as the length of the longest line perpendicular to the line ab and intersecting the sides of the sperm head (fig.1). Sperm head circumference (C) was directly obtained with the digitizer by tracing the border of the head, and in so doing projected area (A) was computed automatically. In characterizing the shape of the head the aspect ratio (length / width) also was determined. The definition of a morphologically normal head spermatozoon as proposed [2] is as given in the table 1.

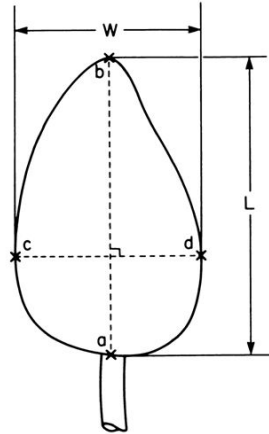| Parameter	Min	Max	Mean	Dev
Length	4.00	6.50	5.225	0.42
Width	2.50	4.50	3.50	0.33
Roundness	1.10	2.00	1.55	0.15

Table. 1 Normal Head spermotoza (WHO)

Fig1.Structure of Human Sperm Head

Though this criteria has been widely adopted by World Health Organization [3] for the Examination and Processing of Human Semen and recommended for human semen morphology assessment, this criteria is still of great scientific controversy, due to lack of accurate clinical validation. The sperm morphological analysis is through manual observe on at least 200 spermatozoa in a microscope. However, with the same criteria, we usually evaluators. In order to get more accurate and consisted morphological evaluation results, better methodology is needed, which is more objective, precise.

II.MATERIALS AND METHODS

Block diagram of the proposed system is shown in fig.2.1. Major steps are explained below.
- Image Collection:
  Papanicolaou stained [4, 13] sperm sample where imaged on to OLYMPUS BX41 [5] microscope attached with digital microscope camera ProgRes® CT3 [6] under 40x magnifications in bright field mode.
- Image Analysis:
  Sperm cells were displayed on the monitor at equivalent brightness and all the cells which did not present any overlap with debris or other cells were considered for analysis. From each sample heads were captured and analysed using the ImageJ open source software [7] using custom macros. After treatment of the images some of the cells had to be discarded because of defective binarization as observed by incorrect correspondence between the original image and its mask. Each sperm head was measured for four primary parameters [head area (A), head length (L), head width (W), head roundness (R).
  Procedure described below.
  1. If the image is in RGB form, it is transformed to a gray scale image.
  2. Then filtered using un sharp mask filter to increase the intensity of the sperm cells
  3. Thresholding is done on the image such that shape of the all sperm cells will be appeared.
  4. Heads of the sperm cells will be extracted using head extraction algorithm which is the part of the custom macro.
  5. Image is calibrated to measure the various parameters on sperm head.
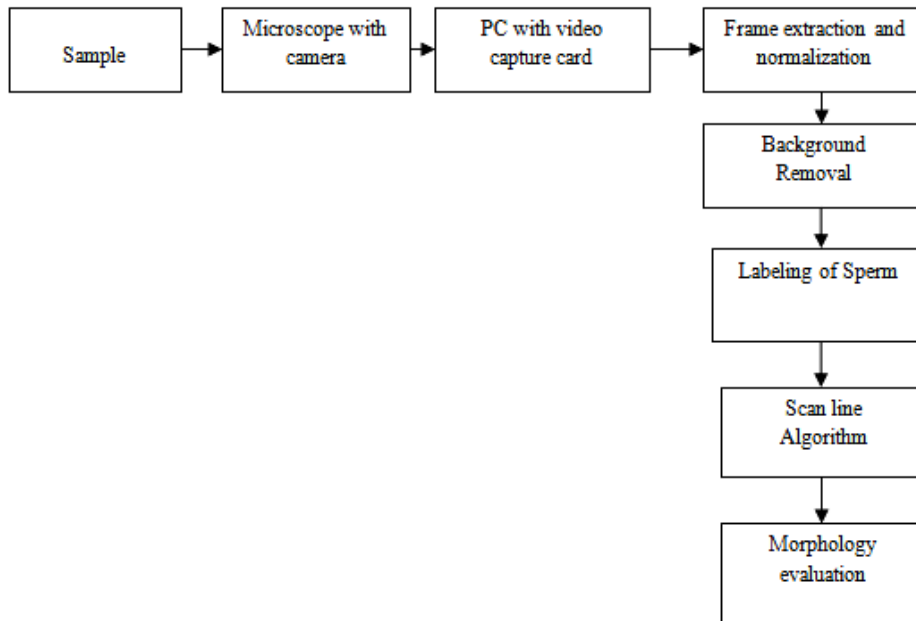
Fig. 2 Proposed System

III. RESULTS AND ANALYSIS

The figures [3-5] show analysis on sperm sample which is taken from system microscope first RGB image is converted to 8 bit gray scale. Then head extraction filter is applied on the gray image after that thresholding is made on the image to differentiate sperm cells with respect to background. Next particle analysis is achieved to remove the noise and produce a mask on all sperm cells available in the image. Finally using ROI manager labeling and available in the image. Finally using ROI manager labeling and Measurement is carried out on each sperm cell. Using ImageJ.
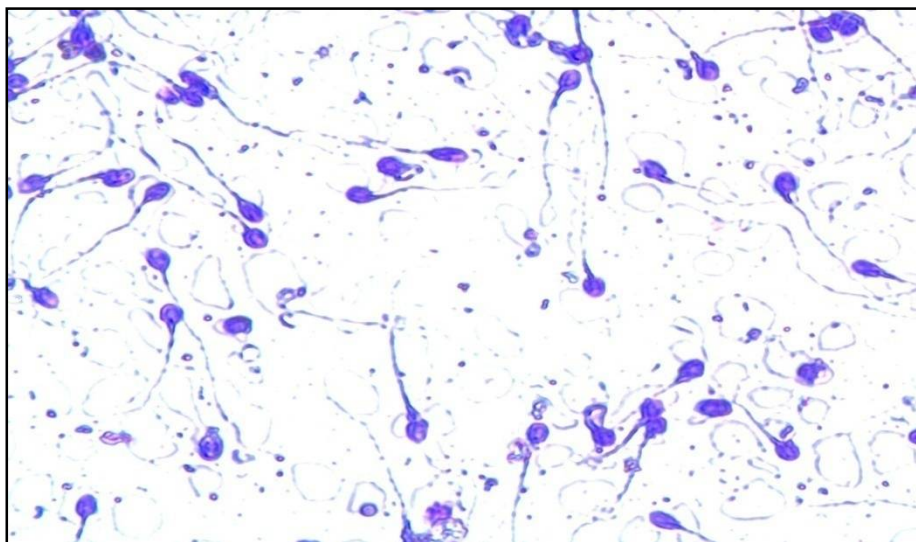

Fig. 3 Image of sperm sample with size 2048x1536 pixels

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(An ISO 3297: 2007 Certified Organization)
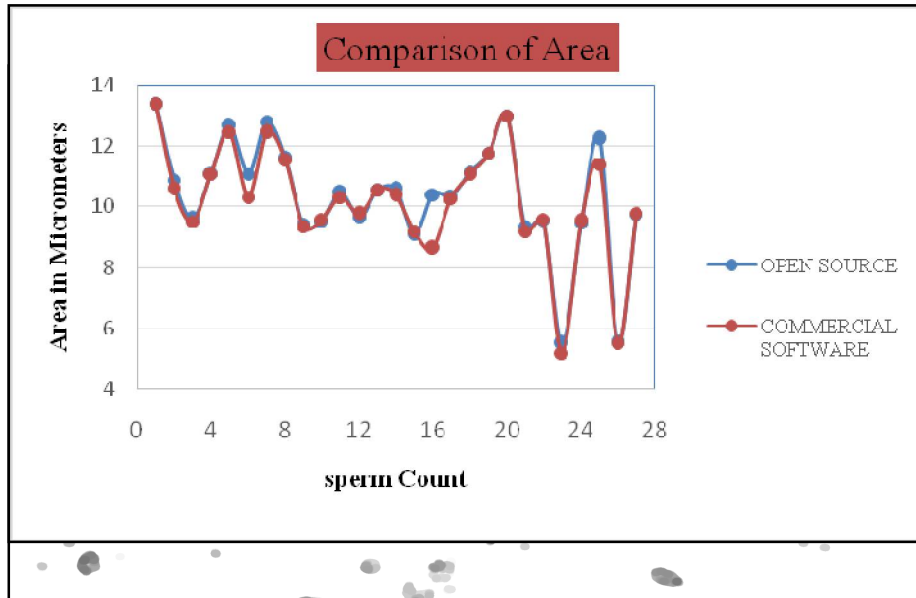
Vol. 5, Issue 6, June 2016
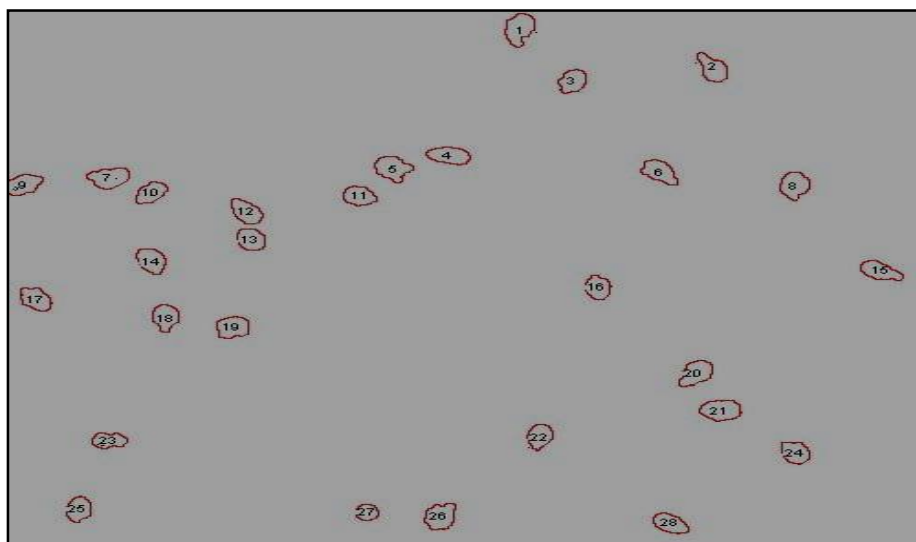

Fig. 4 Output of head extraction filter

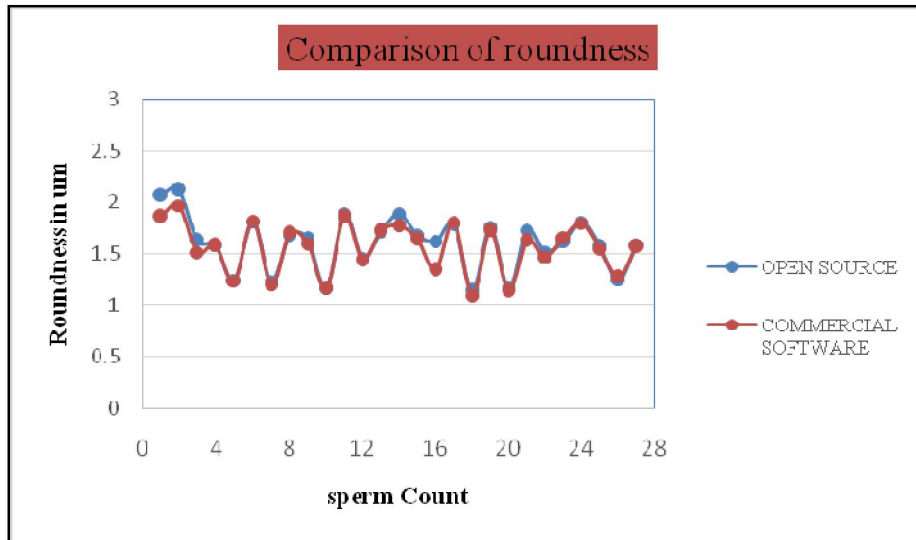Fig .5 Sperm head with mask and label

Fig.6 Comparission of sperm head area obtained from open source and commercial
software
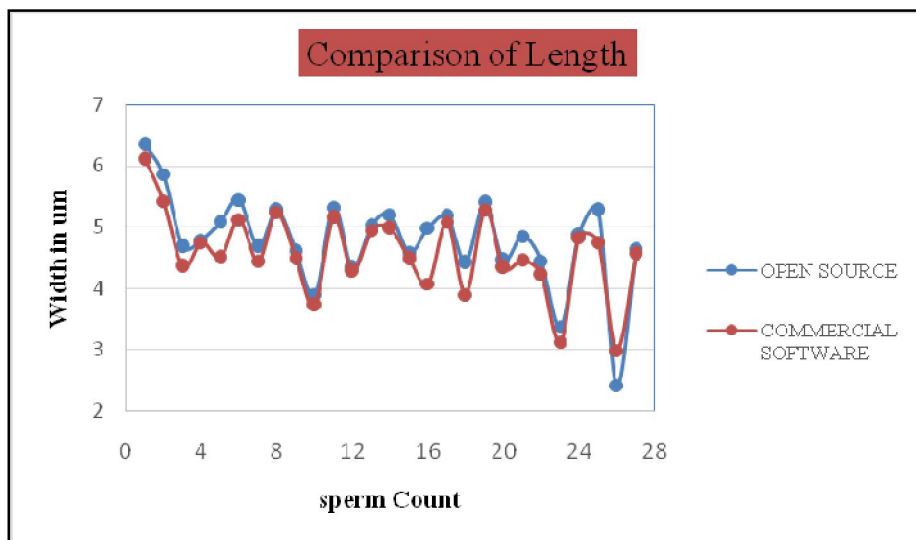
Fig.7 Comparission of sperm head length obtained from open source and commercial
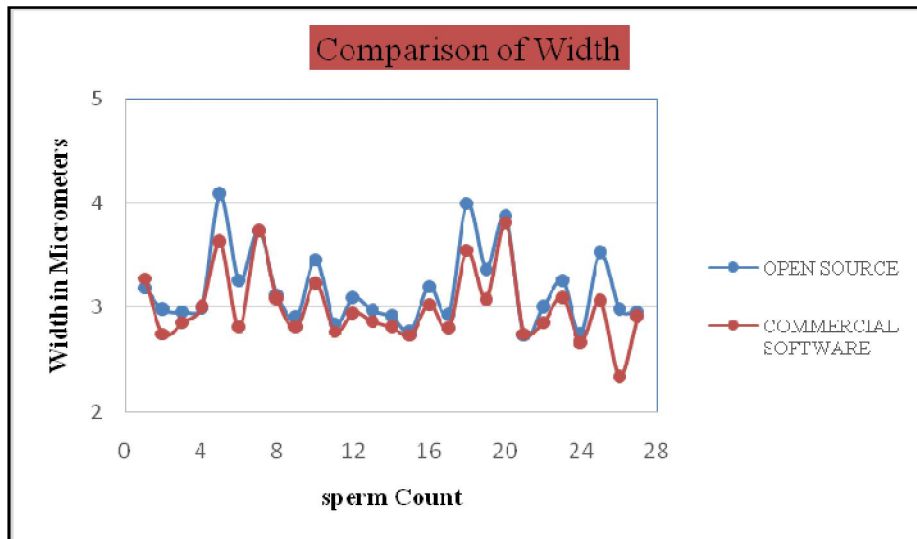software

Fig.8 Comparission of sperm head roundness obtained from open source and commercial software

Fig.9 Comparission of sperm head width obtained from open source and commercial software

The above figures [6- 9] shows measurement on four morphometric parameters for the 27 sperm cells from the sample which are suitable for the analysis. And results using open source software checked for accuracy. Difference in Mean values of length (+ 0.33µm), width (- 0.75µm), area (+ 0.44µm) and roundness (+ 0.06µm) obtained when compared with commercial software MedeaLAB CASA [8].

IV.CONCLUSIONS AND FUTURE WORK

In the present study, we have developed a freely available sperm head morphology analyzer plug-in for open source software. Described the systems functionality and confirmed its validity with respect to the commercial softwares such as Sperm-Class Analyzer [9], Sperm Morphometry Module of ISAS [10, 13], and the Metrix Oval Head Morphology software component of the Hamilton-Thorne CEROS system [11]. Out of nine morphological indices four are automatically measured in the present study (Length, Width, Area and Roundness) [12]. Remaining five morphological indices to be measured automatically in the future work

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