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Real time Analysis of unstained Human Sperm HeadMorphology Using Texture Based Image Processing

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Abstract— About 15-20% couple seek medical advice for difficulty in conceiving, of which nearly 40% require Intra Cytoplasmic Sperm Injection (ICSI) to aid in getting pregnant by assisted reproductive technologies. Semen examination especially morphometric assessment is done to assess and assist the couple but, its done on stained smear of semen from male partner in a diagnostic set up, on the day of procedure morphologically normal sperm is chosen by the embryologist under lower powered optics in an unstained preparation of motile sperms leading to subjectivity. In this paper we developed a realtime computer assisted sperm head morphology analyzer to objectively select morphometrically normal sperm in unstained live specimen using IMAGEJ (Image Processing using JAVA) open source tool.

Keywords—WHO; CASMA; CASA; ROI; IMAGEJ; ART;

I. INTRODUCTION

About 15-20% couple face problems in conceiving in India [1] male factor infertility is contributing about 50% of failure to conceive and about 40% of couple will need advanced intervention of Intra Cytoplasmic Sperm Injection (ICSI) to achieve fruitful fertilization and embryo development [4]. From many years constant research is being done to improve semen parameters in cases of male factor infertility [2]. But, these conventional methods have not fruitful in achieving the desired outcome and

are being revised constantly [5].

Computerised Semen Analysis was proposed to evaluate semen parameters during malefactor assessment to rule out subjectivity and to bring-in standardization in semen analysis [3]. But, they are adopted in assessing male factor during diagnostic phase of couple evaluation, thus, limiting its applicability in a well-planned treatment cycle over to a well trained laboratory expert performing the task of fertilising the oocytes [6]. Hence, there is a need to optimize a realtime computer aided selection of morphometrically normal sperm objectively using unstained sperm samples during treatment cycle

I. MATERIALS AND METHODS

Embryologist selects the sperm under inverted Microscope during process of ICSI first based on its motility in a droplet of 7-10% Polyvinyl Pyrrolidone (PVP) in Hydroxy Ethyl Piperazin Ethane Sulfonic acid (HEPES) buffered medium, immobilizes it by crushing its tail using a pair of micro manipulators. Its then moved to a clean droplet of PVP and The morphological analysis of unstained sperm is done

under 320-400x under Hoffman's Modulation Contrast (HMC)/ Relief Contrast (R.C) Objective. Then an image is taken under 40x Bright Feild (BF) objective using CCD/ CMOSCamera, Image so acquired is analyzed using the program. Figure 1. Depicts experimental Setup to take the images from the unstained semen samplesFigure.2indicates RC images of the sperms for morphology evaluation. Figure.3 shows flow chart of work carried out to examine morphometric parameters of human sperm head. Major steps explained below.

Figure 1. Experimental Setup

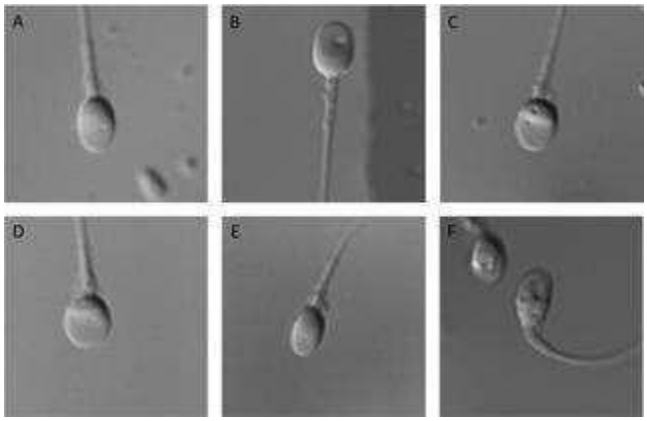

Figure 2.Relief Contrast (RC) images of the sperms

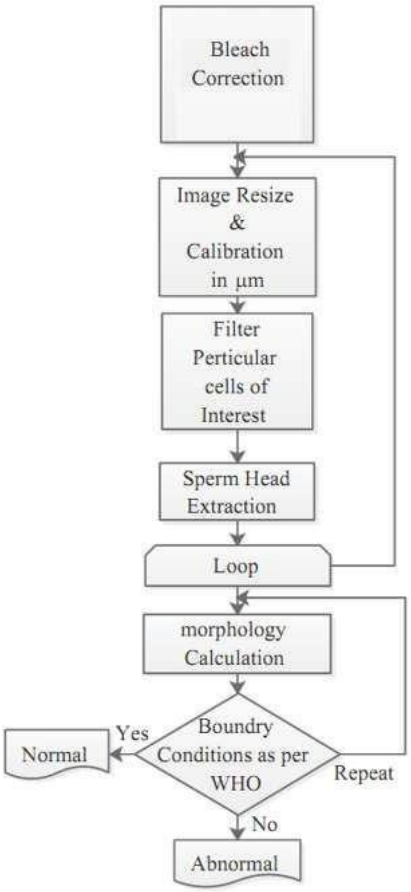

Figure 3.Flowchart of work carried out in the study

1. **Bleach Correction**

The most straightforward method for separating cells in images where the cells have sufficiently and consistently different intensities from their surroundings is thresholding, which labels pixels above the intensity threshold as "object" and the remaining pixels as "background." Disconnected regions can then be automatically labelled as various cells.

Thresholding will fail in the presence of significant noise, autofluorescence, light bleaching, poor contrast, gradients, or halo aberrations (in phase-contrast or differential interference contrast microscopy), necessitating the use of more advanced segmentation techniques.

2. **Image Resize**

All of the photos' lengths and widths must match in order to calibrate the images. Calibration comes before scaling. The sperm cells' trajectory in the image is arbitrary. We must adjust the image such that its height and length are identical if we want to calibrate it in micrometers.

3. **Filter Particular Cells Of Interest:**

I. Convert to Mask

Make a picture in black and white. There will be an inverting LUT on the mask (white is 0 and black is 255). For 8 bit binary pictures, the value (v) of each item in the table is changed to 255-V by an invert LUT, which inverts the current lookup table. With inverted LUTs, pixels with values of 0 and 255 are white and black, respectively.

II. Filling Holes

Filling hole algorithm is used to determine the precise region of the sperm head. When the shape of the sperm is unclear or improperly linked, this algorithm is helpful.

III. First and Second level filter

Thresholding is applied on input image to reduce noise up to some extent. Sperm particles are selected in different stage using particle analyzer by specifying different range of size for an input image.

IV. Third level filter

Applying boundary conditions to area of the sperm and aspect ratio will get only sperm particles in the input image.

v. Sperm Head Extraction

We have used gray morphology algorithm to extract sperm heads in a given image. Gray morphology algorithm works on following principle.

Mathematical Morphology

A theory and method for analyzing and processing geometrical structures, mathematical morphology (MM) is based on set theory, lattice theory, topology, and random functions. Although MM is most frequently used with digital photos, it may also be used with graphs, surface meshes, solids, and a variety of other spatial structures.vi. Morphology Calculation

We have measured all possible (nine) morphometric parameters of sperm head. Along with the status of normal and abnormal of each sperm cell is also displayed.

Result and discussion:

The morphological analysis is part of the image analysis approach that is carried out by the computer system. IMAGEJ was used to program that would analyse the images. Sperm heads were taken into consideration for the analysis in order to quantify the morphology of spermatozoa. Since the head is the feature that most easily distinguishes spermatozoa to the human eye, it was reasonable to employ this feature for automated spermatozoon detection. The head's highly distinctive morphology enables separation of similar-sized items (germ cells, leukocytes) [7]. Figure 4 and Table 1 displayed the various steps carried out to get accurate morphology analysis of sperm head.

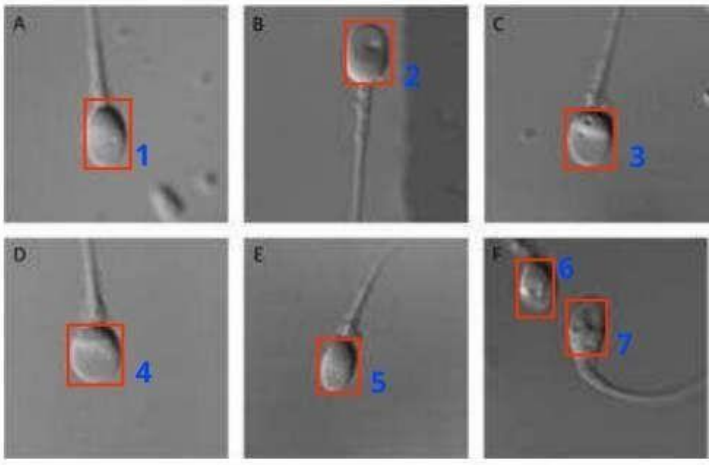

Figure 4. Output images with sperm head extraction

Table 1. Sperm head Morphology parameters with classification.

Morphometric Values in um										
Sperm No.	**Area**	**Perimeter**	**Length**	**width**	**Roundness**	**Ellipticity**	**Rougosity**	**Elongation**	**Regularity**	**Status**
1	9.21	11.31	4.80	2.91	0.51	1.65	0.90	0.24	1.50	Normal
2	9.57	11.7	4.55	3.33	0.6	1.52	0.82	0.25	1.45	Normal
3	8.73	11.45	4.54	3.53	0.54	1.29	0.84	0.13	1.44	Abnormal
4	6.98	11.91	5.18	2.64	0.33	1.97	0.62	0.33	1.54	Abnormal
5	9.52	11.64	4.75	3.14	0.53	1.53	0.88	0.21	1.24	Normal
6	8.41	10.88	4.31	3.43	0.58	1.26	0.89	0.11	1.38	Abnormal
7	8.66	10.77	4.29	3.41	0.52	1.29	0.85	0.15	1.29	Abnormal

IV. CONCLUSIONS AND FUTURE WORK

It is observed that results obtained from the open source tool and from well trained embryologist are comparable. Attempt is made to reduce the cost of computer aided semen analysis system by developing morphology software for realtime evaluation of unstained sperm by open source platform.

This aims to provide objective assessment of realtime unstained sperm head morphometric estimation of sperm and enhances the learning curve of junior laboratory personnel to improve proficiency in their microscopic observational and selection skills. The proposed work can also be extended to assess sperm morphology any other species where there are standards established.

Ethical Committee Consents:

The Proposed Study will be carried out in NIMS Hospital, Jaipur. Study was started after getting approval from the institutional ethical committee.

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