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Image processing for sperm morphology analysis

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Abstract Semen analysis is the first step in the evaluation of an infertile couple. One of the important factors that affect fertilizing capacity of the sperm is its morphology. Manual methods of morphology assessment have resulted not very effective, which has motivated experts to develop automatic procedures.
In this paper we present two techniques proposed for detection and segmentation of sperm heads. The first one is the work of Park et al., published on Annals of Biomedical Engineering in 1997. They select the region of interest for the segmentation of the sperm head using the density difference between the sperm head and background. The boundary of the sperm head was approximated by an ellipse and represented by five parameters investigated by applying the Hough transform strategically [1].
The second one is the recent work of Chang et al., published in Computer Methods and Programs in Medicine in 2014. Their main contribution is the application of a clustering algorithm for detecting sperm heads. Another contribution is the proposal of a novel algorithm to determine which direction the sperm head points. This is a very important issue for posterior stages in the quest for an accurate morphological analysis [2].

1. Introduction

Infertility is a problem that affects up to 15% of couples worldwide. A semen analysis according to standard criteria is the first step in the evaluation of the male factor and sets the basis for all posterior steps for medical treatment of the couple. A typical spermiogram considers concentration, motility, vitality, and/or the fragmentation of the spermatic DNA. In addition, the morphology of the sperm cells is an excellent biomarker of sperm fertilizing capacity [2]. Infertile men show an increased proportion of morphologically abnormal sperm, compared with fertile men, and strict morphology is an excellent biomarker of sperm fertilizing capacity [1].

In 1966, a comparative study in 47 laboratories dedicated to human sperm morphological analysis showed that the manual method of performing the analysis was personality oriented, as well as subjective, qualitative, non repeatable and difficult to teach to students and technicians [2]. These deficiencies in the manual assessment of sperm morphology have provided a major stimulus to develop an objective and automated approaches over the years. These approaches have shown good accuracy, precision, and reproducibility. They are being used for the quantitative and automated analyses of semen with the motional analysis of sperm [1].

2. Sperm anatomy and morphology

Each sperm is composed of head, midpiece and tail. The sperm head contains the nucleus with the genetic material of the father, surrounded anteriorly by acrosome, which contains enzymes used for penetrating the female egg. The mid-piece of the sperm contains mitochondria, which provides the energy for sperm motion. The sperm has a long tail in order to propel the head of the sperm, which carries all the DNA information, towards the egg [3].

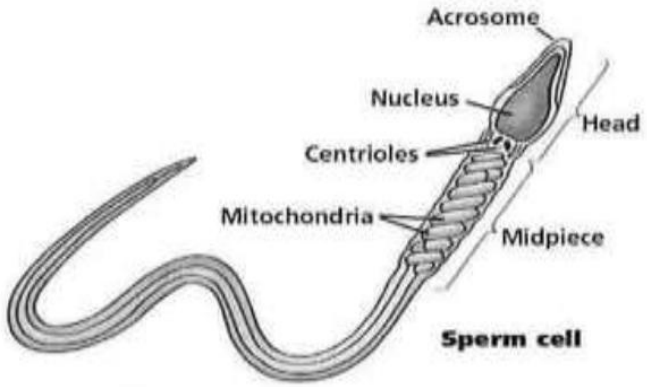

Figure 1. *Anatomy of a sperm.*

For a spermatozoon to be considered normal, the sperm head, neck, midpiece, and tail must be normal.

A normal head should be oval in shape. Allowing for the slight shrinkage that fixation and staining induce, the length of the head should be 4.0-5.0 μm and the width 2.5-3.5 μm. Length-to-width ratio should be 1.50 to 1.75. There should be a well-defined acrosomal region comprising 40-70% of the head area [4].

The following categories of head defects should be noted: namely large, small, tapered, pyriform, round, and amorphous heads, vacuolated heads (>20% of the head area occupied by unstained vacuolar areas), heads with small acrosomal area (<40% of head area) and double heads, or any combination of these [4].

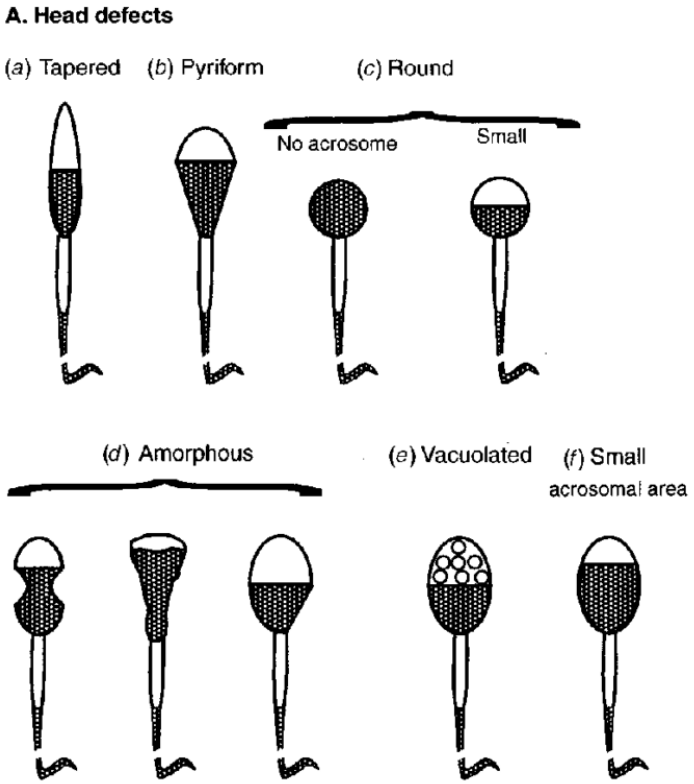

Figure 2. *Schematic drawings of some abnormal forms of human spermatozoa.*

3. Automatic techniques for sperm head morphology analysis

Both of the methods presented on this paper include two main stages. The first stage is detection of the sperms head. This stage's purpose is to get the region of interest of the sperm head, a rectangular area containing individual sperm heads from the image. The second stage is segmentation of the sperms head, defining a boundary between the sperm head and the background.

3.1 The work of Park et al.

3.1.1 Detection

Image pixel values are converted to binary values using a threshold estimated statistically. Pixel values over the threshold are set to white and the others set to black (Figure 3b). Detected white pixels are summed horizontally and vertically for the calculation of horizontal and vertical projection profiles (Figure 3c). Profile intensities are analyzed. If the intensity of the profile over the threshold continued > 25 pixels, this part of the profile is selected for the possible horizontal or vertical band of a sperm head region (Figure 3d). By combining possible horizontal al vertical bands, possible areas for the head sperm region are estimated. Because these regions have been estimated by combining selected bands from the image having more than one sperm, selected regions are usually greater than the minimal rectangular sperm area (Figure 3e). To remove selected regions incorrectly, profile calculation and threshold checking procedure are iterated for each selected region (Figure 3f). [1]

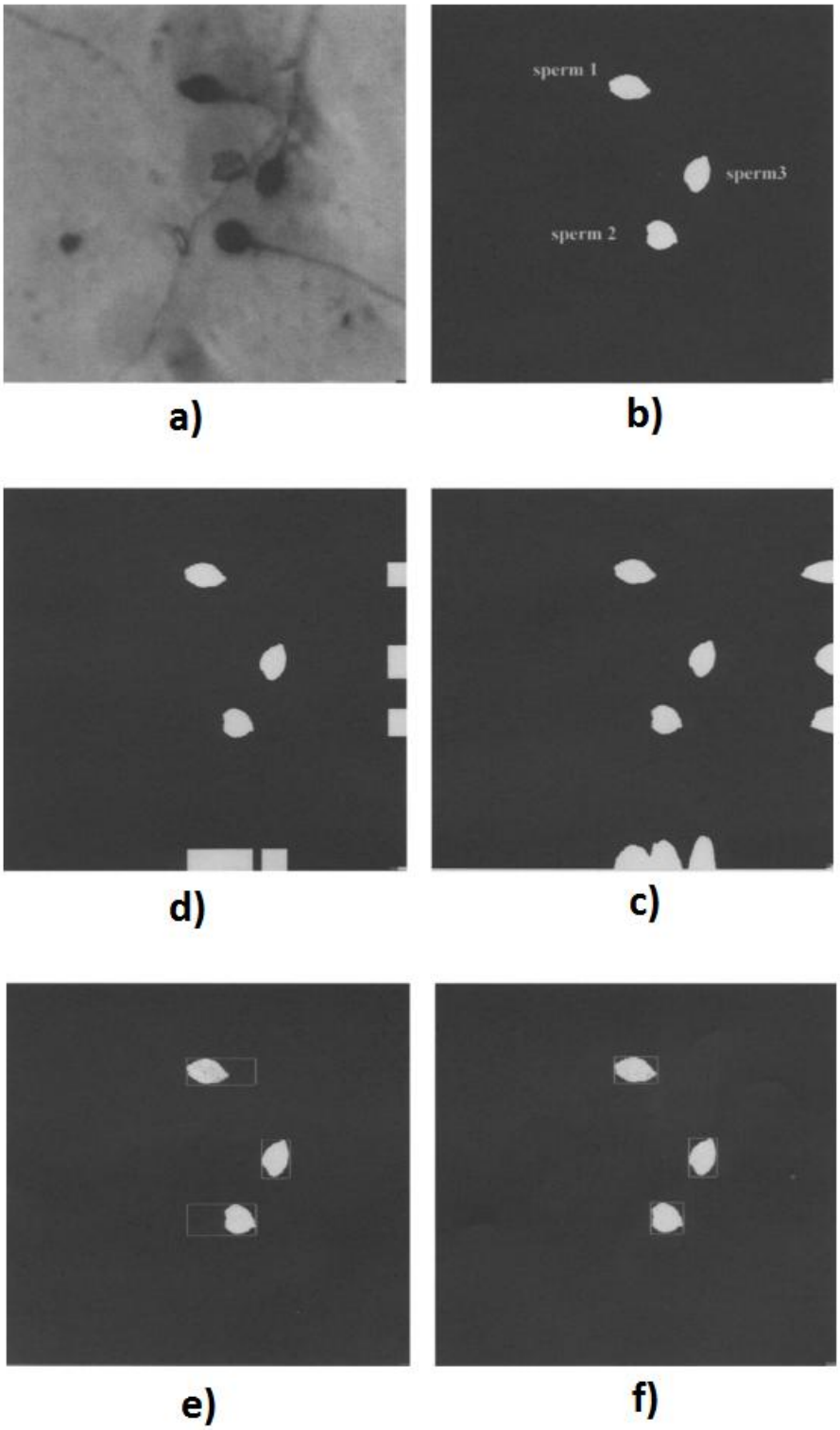

Figure 3. *Detection of sperm heads in the work of Park et al. (a) Original image. (b) Binary image using the preset threshold. (c) Projection profiles from the binary image. (d) Selected bands for the sperm head region. (e) Initially estimated sperm head region. (f) Finally selected sperm head region.*

3.1.2 Segmentation

The Hough transform is one of the powerful methods for detecting determined geometrical shapes like the line, circle, and ellipse. This algorithm uses a matrix, called accumulator, whose dimensions equals the number of unknown parameters. The biggest problem for the efficient use of the Hough transform is the enormous volume of transformed space when the number of parameters is increased.

Lines are represented by two parameters. To detect lines on a plane, calculating 100 values for each parameter, the number of accumulation cells is: 100^2= 10.000. Circles are represented by three parameters. For detection of circles, the number of accumulation cells is 100^3=1.000.000. An ellipse is represented by five parameters. The number of accumulation cells is 100^5= 10.000.000.000. This is why direct application of the Hough transform for the detection of ellipses is practically impossible.

To reduce the size of the transformed volume and increase the calculation speed to a practical level, the initial shape of the ellipse is estimated as a circle, and the center of the circle is located at the center of the selected rectangular sperm head region (Figure 4b). Then, we allocate three accumulation cells for each parameter. For example, being A the initial estimated value: A1= A-STEP, A2=A, and A3=A+STEP. STEP represents the parameter resolution, and is set to 3px, 2px and 1px in for the first, second and third stage of the Hough transform respectively (Figures 4c, 4d). Three values for each of the five parameters make 3^5= 243 accumulation cells, and this number is practical for the calculations. [1]

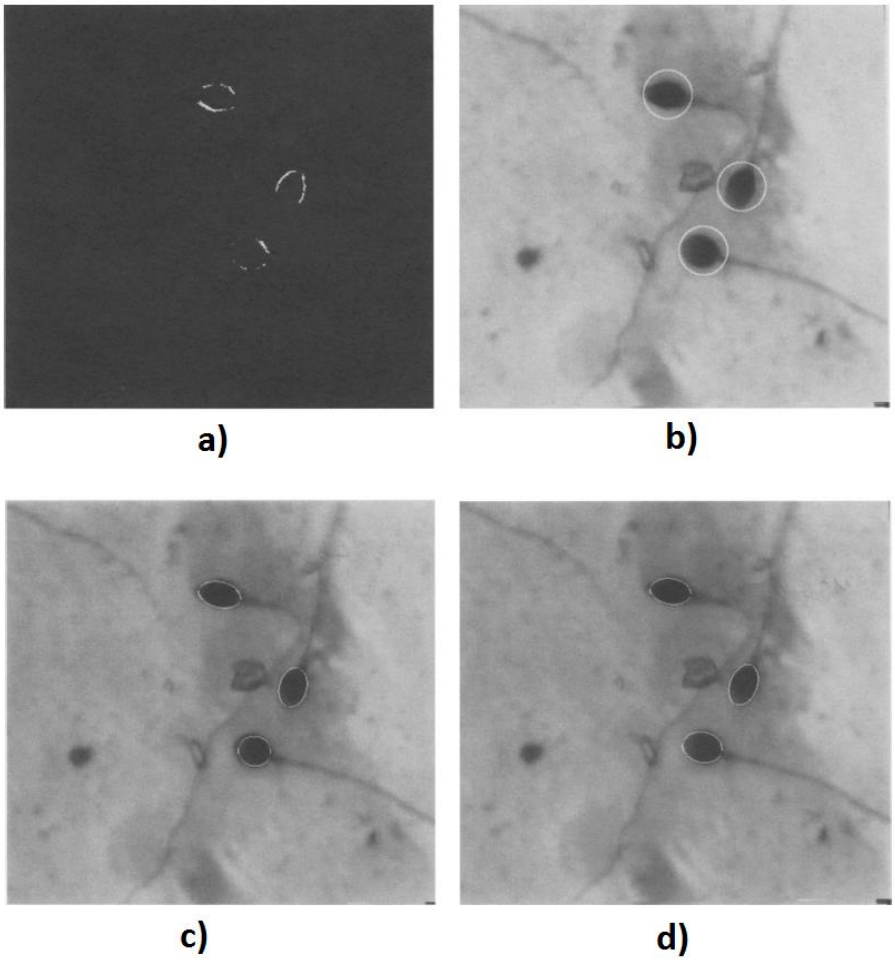

Figure 4. *Segmentation of sperm heads in the work of Park et al. (a) Edge pixels used for the estimation of boundaries. (b) Suggested boundaries from selected sperm head regions, (c) Initially estimated sperm head boundaries, (d) Finally estimated sperm head boundaries.*

3.2 The work of Chang et al.

3.2.1 Detection

K-means clustering algorithm is applied for separation of the sperm cells from the background. Pixels belonging to sperm cells are separated in one cluster, and the pixels belonging other structures and background in a second cluster (Figure 5b). These regions need to be refined for morphological analysis. Other sperm cells which touch the border of the image are eliminated. Then, a procedure referred as “erase tails” is performed. It consists on using a convolution process with a disk-shape kernel of size r and unitary weight. After convolution, all pixels with a resulting value below the threshold are removed.(Figrue 5c). Finally, objects whose size are out of range are discarded (Figure 5d) [2].

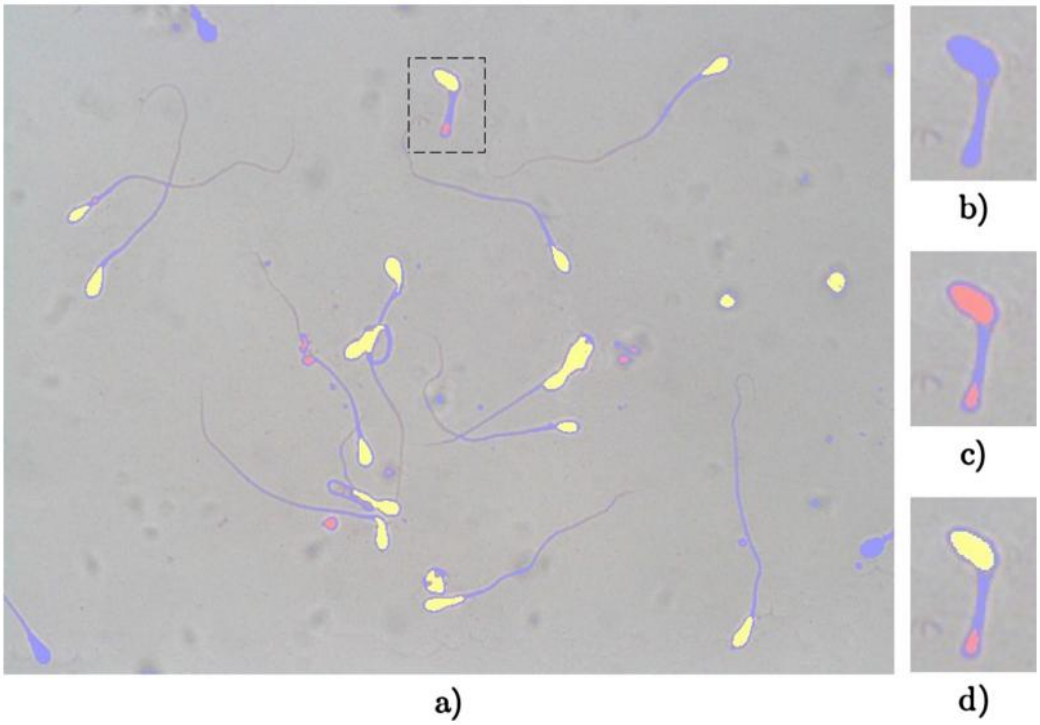

Figure 5. *Detection of sperm heads in the work of Chang et al. (a) Original image in RGB color space with resulting ROIs marked on it. (b) Blue color represents ROIs after applying k-means in RGB and L*a*b* color spaces. (c) Red color represents ROIs after erasing tails and sperm cells at border. (d) Yellow color represents ROIs after erasing by size. Yellow pixels constitute the final ROIs of this stage. Image size: 780 × 580 pixels ≈ 164 × 122 μm.*

3.2.2 Segmentation

K-means is applied only in the particular region of interest to separate the darkest part of the head from the rest. (Figure 6c) As this portion of the head is smaller than the real head, it is needed to enlarge it up to the region of interest. Thus, it is important to determine the front direction of the head.

To determine the direction in which the head points, a two-step method is proposed. First, the orientation of the sperm head is determined as the angle between the X axis and the major axis of the

region of interest. Using this angle, the head is rotated to a horizontal position in which the major axis of the fitted ellipse is parallel to the X axis. Then, the major axis is divided into three similar portions. A fitness value of the two extreme portions with the fitted ellipse is calculated. The portion corresponding to the lowest fitness value indicates the direction to where the head points (Figure 6d).

The pointing direction allows us to build a growing mask for segmenting the whole head, and not only the darkest part of the head (Figure 6e) [2].

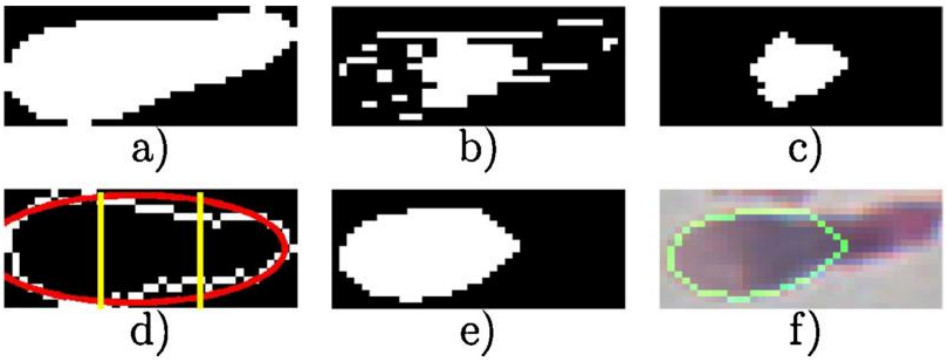

Figure 6. *Segmentation of sperm heads in the work of Chang et al. (a) Detection of sperm head (as returned from Algorithm 1). (b) ROI after applying k-means in L*a*b* and YCbCr color spaces. (c) smallHead detected as the smallest cluster (this constitutes the darkest part of the head). (d) Fitness of (a) with an ellipse (red) for getting which direction the head points. (e) smallHead detected in (c) grows according to growing mask up to (a). (f) Contour superposition of sperm head segmentation*

4. Conclusions

We can appreciate from both methods that techniques are getting more precise through the years, since the first method, proposed in 1997, obtains the segmented area of the sperm head as an ellipse, and the second one, proposed in 2014, results in a not geometrical and more exact segmentation. Despite that, the concept of using information of the geometrical characteristics of the sperm head remains in the newest work, since the fitted ellipse is used in the determination of the sperm head direction.

There is still a lot of work to be done. Even though 17 years separate one work from another, they both present problems in the particular situation of overlapped sperms. This brings up the questioning: will the process of sperm analysis be completely automatized some day, or will it always be place for the human eye on it?

5. References

[1] K. S. Park, W. J. Yi, and J. S. Paick, “Segmentation of sperms using the strategic Hough transform.,” *Ann. Biomed. Eng.*, vol. 25, no. 2, pp. 294–302, 1997.

[2] V. Chang, J. M. Saavedra, V. Castañeda, L. Sarabia, N. Hitschfeld, and S. Härtel, “Gold-standard and improved framework for sperm head segmentation,” *Comput. Methods Programs Biomed.*, vol. 7, pp. 225–237, 2013.

[3] V. S. Abbiramy and D. V. Shanthi, “Spermatozoa Segmentation and Morphological Parameter Analysis Based Detection of Teratozoospermia,” *Int. J. Comput. Appl.*, vol. 3, no. 7, pp. 19–23, 2010.

[4] “WHO laboratory manual for the examination of human semen and sperm-cervical mucus interaction”, 4th ed, WHO Special Programme of Research, Development and Research Training in Human Reproduction, Cambridge University Press, 1999.