Sperm's tail identification and discrimination in microscopic images of stained human semen smear

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***Abstract*— Correct estimating of a man's fertility potential has long been of great interest to researchers. Sperm morphology is assessed routinely as part of standard laboratory analysis in the diagnosis of human male infertility. The evaluation of sperm size, shape and morphological smear characteristics should be assesed by carefully observing a stained sperm sample under a microscope. In order to avoid subjectivity, numerous studies that incorporate image analysis techniques in the assessment of sperm morphology have been proposed appeared. In this paper, we have proposed a new method to identify sperm's tail through some points which are placed on the sperm's tail, accurately. These estimated points could be used to verify the morphological characteristics of sperm's tail such as length, shape and etc. At first, sperm's head and mid-piece are segmented. Then, a pixel which is placed at the end of sperm's mid-piece, is selected as an initial point. At the next step, the proposed method finds the other pixels which are placed on the sperm's tail using structural similarity index in an iterative scheme. In order to stop the algorithm automatically at the end of sperm's tail, local entropy is estimated and used as a feature to determine if a point is located on the sperm's tail or not ,and also to determine the end of sperm's tail.**

***Index Terms*— Structural Similarity Index (SSIM), Entropy, Sperm morphology, Sperm's tail.**

I. Introduction

Infertility is a common clinical problem which causes considerable morbidity, including stress, depression and sexual dysfunction, in those couples affected [1]. The main cause of infertility is an anomaly of the sexual reproductive system. High percentage of these problems are from the male and finding ways to resolve this will be helpful to the physicians for a better and faster cure for couples. To determine infertility, some physical characteristics of the seminal plasma (such as smell, viscosity, pH and aspect) and spermatozoons parameters such as concentration mobility and morphology are analyzed [2]. The subjective assessment of sperm morphology based on visual observation has led to widely varying results due to numerous factors such as the use of different staining procedures or the experience of technicians, among others. So, Methods based on digital image processing have been developed. To estimate semen fertility by means of spermatozoid morphology, sperm head, mid-piece, and tail are considered and digital image processing methods are used to evaluate the morphological characteristics.

In this paper, we have proposed a new method to detect sperm's tail. Sperm's head and mid-piece are segmented using methods reported in previous works [3]. The pixel which is placed at the distal point of sperm's mid-piece is considered as an initial point. The proposed method uses a structural similarity index and entropy in an iterative scheme to estimate sperm's tail with some points which are placed on the sperm's tail, accurately. These estimated points can be used to analyze characteristics of sperm's tail such as length, shape, and etc.

II. Materials and Methods

A. Image Acquisition Technique

Sample Images were acquired from modified Papanicolaou stained sperm smears. Fresh Sperm samples were incubated for 30 to 60 minute in 37° Celsius . The Smear was then prepared after complete liquefaction and the slides were dried in the air before staining with modified Papanicolaou method. The images were captured by means of a 560 TV-line CCD camera mounted on the third eyepiece of a trinocular direct microscope (Proway BK5000) with a total magnification of 1000X using Plan Achromatic Infinity objective lenses. 10 to 25 Images of different fields were captured from each slide. And totally 100 slides were analyzed.

B. Preprocessing

The preprocessing step consists of segmentation of sperm's head and mid-piece. The segmentation of head and mid-piece was performed using a method which is proposed by Carrillo and et al [3]. This method consists of two steps.
At the first step, the original RGB color image is converted to a gray scale image followed by image thresholding using Otsu method [4]. The obtained objects by thresholding are classified in order to eliminate some artifacts. Again, the ramaining objects are classified through histogram analysis and objects with a number of dark pixels are segmented. Then, individual sperms (head and mid-piece) are enclosed using bounding boxes. Finally, individual sperms are extracted in the original RGB color image.
At the next step, $nth\text{-}fusion$ method is applied to the enhanced image to segment the head and mid-piece parts. The $nth\text{-}fusion$ method is based on nth-level thresholding of an image followed by intersection with n special masks. In order to obtain the desired segmentation results, a prior objects morphological model, which is based on the information fusion technique in a feature level is used [5].

After segmentation of sperm's head and mid-piece, the distal point of mid-piece is considered as an initial point. The initial point will be used to sample the sperm's tail. Also, pixels belong to head and mid-piece are set to a different value (say zero), for all three components of the RGB color image.

III. Structural Similarity Index

We have used a structural similarity (SSIM) [6] quality measure from the perspective of image formation which is a function of luminance, contrast and structure. The algorithm's greatest appeal is that it matches human subjectivity. In particular the SSIM Index, like the HVS (human visual system), is highly sensitive to degradations in the spatial structure of image luminances. The luminance of the surface of an object being observed is the product of the illumination and the reflectance, but the structures of the objects in the scene are independent of the illumination. The structural information is defined in an image as those attributes that represent the structure of objects in the scene, independent of the average luminance and contrast. Since luminance and contrast can vary across a scene, the local luminance and contrast are used.

Suppose X $= \{x_i | i = 1, 2, ..., N\}$ and Y $= \{y_i | i = 1, 2, ..., N\}$ are two nonnegative image signals. Let μ_x, σ_x^2 and σ_{xy} be the mean of X, the variance of X, and the covariance of X and Y, respectively. Approximately, μ_x and σ_x can be viewed as estimates of the luminance and contrast of X, and σ_{xy} measures the the tendency of X and Y to vary together, thus an indication of structural similarity. The general form of the Structural SIMilarity (SSIM) index between signal x and y was defined as:

$$\text{SSIM(x,y)} = [l(x,y)]^{\alpha}.[c(x,y)]^{\beta}.[s(x,y)]^{\gamma} \tag{1}$$

where

- $l(x,y)$ is Luminance comparison measure. The luminosity is a comparison of the mean values of each image.

$$l(x,y) = \frac{2\mu_x\mu_y + C_1}{\mu_x^2 + \mu_y^2 + C_1} \tag{2}$$

The constant C_1 is included to avoid instability when $\mu_x^2 + \mu_y^2$ is very close to zero, and

$$C_1 = (K_1 L)^2 \tag{3}$$

where $K_1 \ll 1$ and the dynamic range of the elements of x and y is denoted by the variable L.

- $c(x,y)$ is Constract comparison and is estimated as the standard deviation σ. Structure comparison is done after the local mean subtraction and local variance normalization.

$$c(x,y) = \frac{2\sigma_x\sigma_y + C_2}{\sigma_x^2 + \sigma_y^2 + C_2} \tag{4}$$

The constant C_2 is included to avoid instability when $\sigma_x^2 + \sigma_y^2$ is very close to zero, and

$$C_2 = (K_2 L)^2 \tag{5}$$

where $K_2 \ll 1$ and the dynamic range of the elements of x and y is denoted by the variable L.

- $s(x,y)$ is Structure comparison measure and is estimated from the image vector by removing the mean and normalizing by the standard deviation.

$$s(x,y) = \frac{2\sigma_{xy} + C_3}{\sigma_x\sigma_y + C_3} \tag{6}$$

where $C_3 = C_2/2$.

- α, β and γ are used to adjust the relative importance of the three components..

This structural similarity function (SSIM), $l(x,y)$, $c(x,y)$ and $s(x,y)$ satisfy the following conditions:

1) Symmetry: $S(\text{x},\text{y}) = S(\text{y},\text{x})$;
2) Boundedness: $S(\text{x},\text{y}) \leq 1$;
3) Unique maximum: $S(\text{x},\text{y}) = 1$ if and only if x = y (in discrete representations, $x_i = y_i$ for all i=1,2,...,N);

As mentioned before, we have used SSIM index [6] as a structural similarity function to evaluate quality of similarity in the image, locally.
Let X be a digital image of size $(M \times N)$ which consists of a sperm, and $A(r_a, c_a)$ be a pixel on the sperm's tail which is considered as an initial point (point A). Fig.1 (a) shows a typical sperm image, a section of sperm's tail is zoomed in and shown in Fig.1 (b). Suppose the bright green pixel is point A, to find the next pixel which is on the sperm's tail and has the highest structural similarity index (SSIM) with respect to point A, we have used two windows $(w_1 : p \times p$ and $w_2 : k \times k,\ k < p)$.

w_1 is centered at point A and is applied to limit A's neighborhood. Fig.1 (b) shows w_1 in pink. w_2 is a sliding window which moves over the boundaries of w_1 and computes the local structural similarity (SSIM) index for each pixel with respect to point A. Fig.1 (c) shows pixels which are placed on the boundaries of w_1 in deep pink, also different positions of the sliding window(w_2) which moves over the boundaries of w_1 is shown in blue. So, the SSIM index is calculated within the sliding window (w_2) for all pixels which are placed on the boundaries of w_1 to find the pixel with the highest SSIM index. SSIM index for all pixels are calculated and scaled ([0,255]), these values are shown in Fig.1 (d). The pixel which has the highest SSIM and pixel A are shown in Fig.1 (e). If we do the same algorithm for new selected pixel, it is possible to select previous pixel as a new pixel. So, before running the new iteration for the new selected pixel, a neighborhood of previous pixel is changed to a different value (say zero). This process is done through another window($w_3 : l \times l, k < l < p$). Fig.1 (f) shows this process.
This algorithm is implemented in an iterative scheme, and for every new selected pixel, local entropy is computed to detect if we have reached the end of the tail or not.

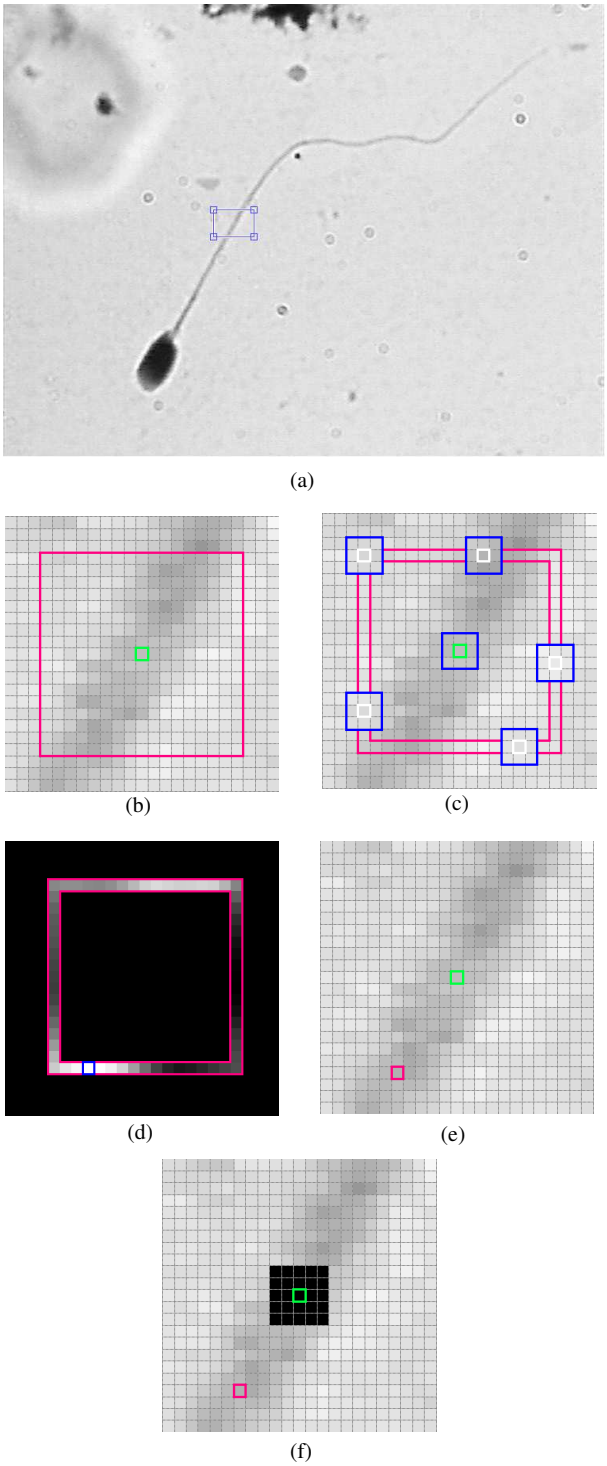


Fig. 1. Use of SSIM index to find pixels with the most similarity: (a) A typical sperm image (Red component of RGB color image), (b) the initial pixel (bright green) and w_1 (deep pink), (c) w_2, sliding window (blue), which computes SSIM for each pixels over the boundaries of w_1 and the initial pixel, (d) SSIM values over the boundaries of w_1, (e) The new selected pixel with the highest SSIM index (deep pink) and the initial pixel(bright green), (f) A neighborhood of the initial pixel is set to zero.

IV. Entropy and Checking for the End of Tail

In the information theory, information entropy is a measurement of the system uncertainty degree. The term of entropy in the theory of information was first introduced by Shannon, in 1948, in [7].

A. Shannon Entropy

Let $X = \{x_1, ..., x_n\}$ be a discrete random variable with values in S and probability mass function $p(x)$, the information (or uncertainty) of each possible event is

$$I(x_i) = -\log p(x_i) \tag{7}$$

the mean of $I(X)$ is introduced as Shannon entropy and is denoted by $H(X)$

$$H(X) = E(I(X)) = -\sum_{x \in \mathcal{S}} p(x)\ \log p(x) \tag{8}$$

$H(X)$ varies from zero to $\log(|S|)$, zero meaning that there is no uncertainty, whereas $\log(|S|)$ is reached when all elements of X have equal probabilities, in this case, the uncertainty is at its maximum [8].

B. Rényi Entropy

Rényi further expanded the concept of Sannon entropy, and defined the $q(q \geq 0, q \neq 1)$ order Rényi [9] entropy of probability density function $p(x)$ as

$$H_q(X) = \frac{1}{1-q} \log \sum_{x \in \mathcal{S}} p(x)^q \quad , \ q \neq 1 \tag{9}$$

Rényi entropy is a non-increasing function of q and It can be verified, by applying the l'$H\hat{o}pital$ rule that Shannons entropy is the Rényi entropy of order 1.

V. Entropy Estimation

In this paper, Rényi entropy [9] is selected as entropy measure and estimation of this type of entropy which will be quite fast, is based on Leonenko and et al [10].
Let $X \in \mathbb{R}^n$ be a random vector with probability measure μ having the density p. The proposed method by Leonenko estimates H_q from a sample of N independent and identically distributed (i.i.d.) random variables $X_1, ..., X_N, N \geq 2$ based on nearest-neighbor distances in the sample. This work is resumption of the method which estimates H_1 and proposed by Kozachenko and Leonenko [11].
Leonenko's method estimates I_q, Eq.10, for $q \neq 1$ through the computation of conditional moments of nearest-neighbor distances.

$$I_q = I_q(p) = \mathbb{E}\{p^{q-1}(x)\} = \sum_{x \in \mathcal{S}} p(x)^q \quad , \ q \neq 1 \tag{10}$$

A. Estimators

Suppose that $X_1, ..., X_N, N \geq 2$, are i.i.d. with a probability measure μ having a density p with respect to the Lebesgue measure. Let $\rho(x, y)$ denote the Euclidean distance between two points x, y of $\mathbb{R}^m$. For a given sample $X_1, ..., X_N$, and a given X_i in the sample, from the $N-1$ distances $\rho(X_i, X_j), j = 1, ..., N, j \neq i$, the order statistics $\rho^{(i)}_{1,N-1} \leq \rho^{(i)}_{2,N-1} \leq ... \leq \rho^{(i)}_{N-1,N-1}$ are formed, so that $\rho^{(i)}_{k,N-1}$ is the

kth nearest-neighbor distance from X_i to some other X_j in the sample, $j \neq j$. I_q in eq.10 is estimated for $q \neq 1$, by

$$\hat{I}_{N,k,q} = \frac{1}{N}(\zeta_{N,i,k})^{1-q}; \quad (11)$$

where

$\zeta_{N,i,k} = (N-1)C_k V_m(\rho^{(i)}_{k,N-1})^m$
$V_m = \pi^{m/2}/\Gamma(m/2+1)$
(V_m is the volume of the unit ball $\beta(0,1)$ in $\mathbb{R}^m$)
$C_k = [\Gamma(k)/\Gamma(k+1-q)]^{1/(1-q)}$

So, H_q in Eq.9 is estimated as below

$$\hat{H}_{N,k,q} = \log(\hat{I}_{N,k,q})/(1-q), \quad (12)$$

For every new selected pixel, the local entropy is estimated and compared with a $threshold$. If the estimated value is greater than the $threshold$, it means that the selected pixel is placed on the sperm's tail. In other words, the local entropy is considered as a criterion which shows that if we are on the sperm's tail or not.
To estimate the local entropy for the new selected pixel, a window with odd size ($w_4, h \times h$) is centered at that pixel, then for every pixel which is placed in the window, only Red and Green components are extracted as a two dimensional feature. The local entropy is estimated by estimation of the entropy of these samples.

We have experimentally observed that a two dimensional feature which contains Red and Green components, gives more strict $threshold$ in comparison with other features. Fig.2 shows the variations of the local entropy at different points placed on the sperm's tail.

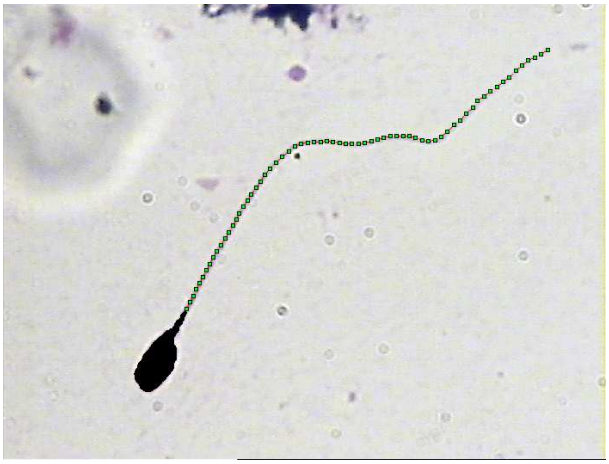
VI. Algorithm

The algorithm includes two major modules:

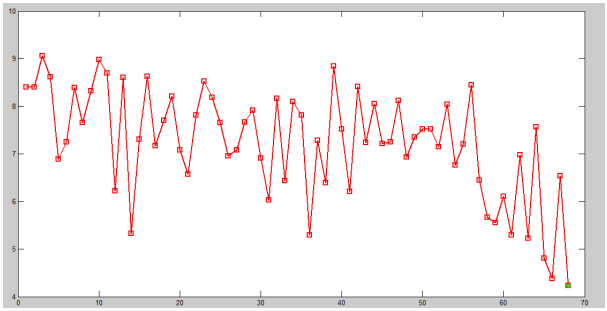
- Selecting an initial pixel (A) which is placed at the end of mid-piece, and finding the pixel (B) with the highest SSIM index with respect to that.
  To compute and compare the structural similarity (SSIM) index, only the Red component of the RGB color image is considered as a separate image and is used to perform this module.
- Verifying if the selected pixel (B) is on the sperm's tail or not, this procedure is based on local entropy estimation.
  To evaluate local entropy, only the Red and Green components of RGB color image are used as a two dimensional feature.

The steps of proposed method are summarized as below

1) Setting pixels on the sperm's head and mid-piece to zero.
2) Smoothing the input image using an average filter.
3) Selecting the end of sperm's mid-piece as the initial point pixel (A).
4) Centering the window w_1 at pixel A.



(a)



(b)

Fig. 2. Result of applying the proposed algorithm to the sperm's image: (a) Estimated points, as the result of proposed method, (b) Use of local entropy changes as a measure for identification of end of tail.

5) Moving a sliding window (w_2) over w_1's boundaries and compute SSIM index, locally, through Eq.(1), for each pixel over the boundaries of w_1 and pixel A.
6) Finding the pixel with the largest amount of SSIM Index (B).
7) Estimation of the local Entropy at pixel B to understand if it is placed on the sperm's tail or not. If the estimated value is less than a $threshold$, stop the algorithm, otherwise go to the next step.
8) Setting pixels around the pixel (A) to a different value (say zero), through the window w_3.
9) Replacing the initial pixel (A) with the new selected pixel (B) and go to step 4.

VII. Simulation Results

The results of proposed method, for different sperms are shown in Fig.3. Fig.3 (a) shows the original sperms images. The proposed method is applied to sperms images, and the estimated points are shown in Fig.3 (b). It shows that the estimated points are exactly located on the sperm's tail. Fig.3 (c) shows how the estimated local entropy changes with the position of estimated point on the sperm's tail. The last points in each tail have the lowest value of local entropy in comparison with the other points, these values are less than the $threshold$, and the algorithm was stopped because of these points.

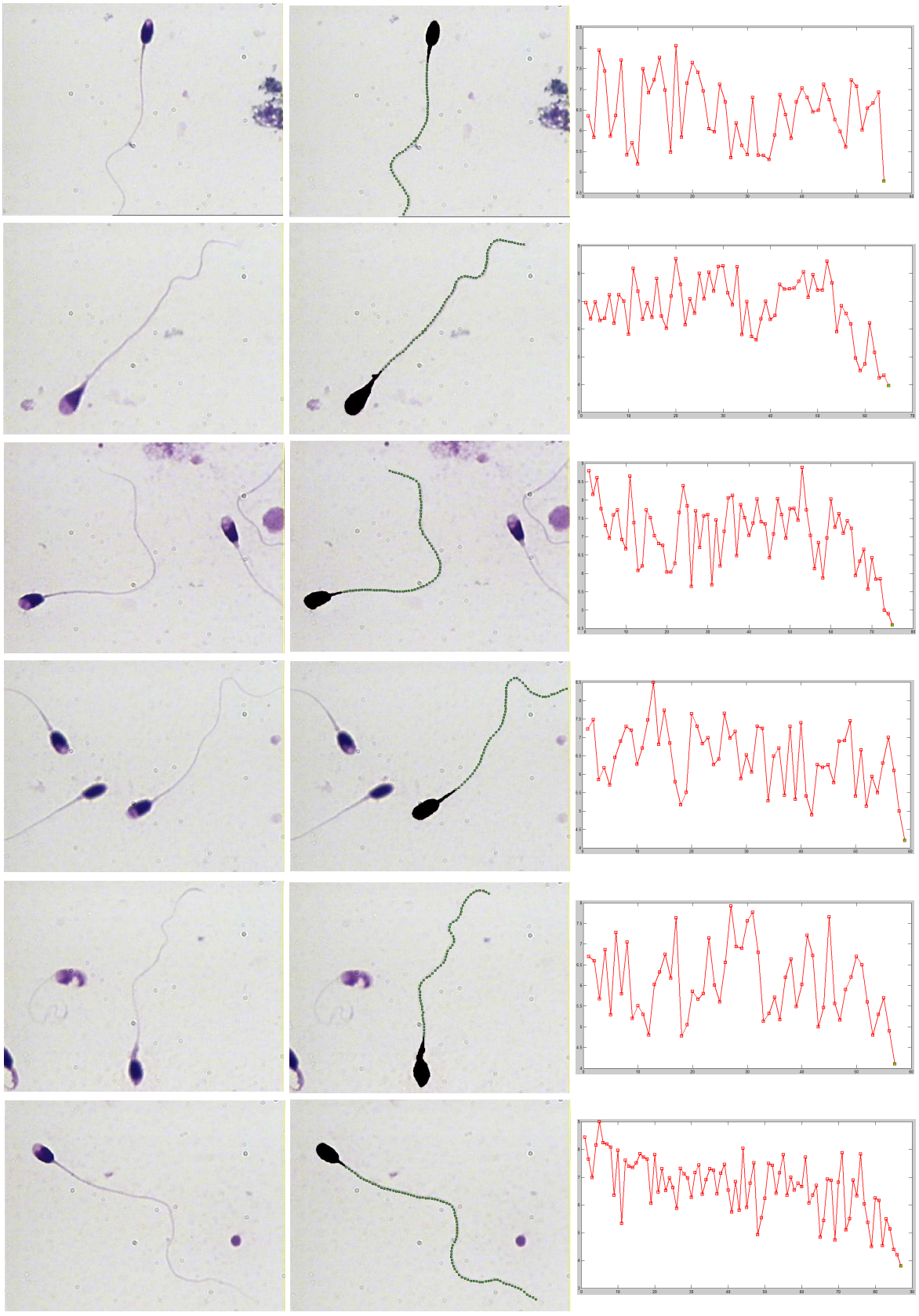


Fig. 3. Result of applying the proposed algorithm to different sperms images: (a) Original sperms images, (b) Estimated points, as the result of proposed method, (c) Use of local entropy changes as a measure for identification of end of tails.

VIII. CONCLUSION

In this paper, we have proposed a new method for identification and discrimination of sperm's tail in microscopic images of stained human semen smear. The algorithm uses the structural similarity (SSIM) index and local entropy estimation in an iterative scheme. At first, sperm's head and mid-piece are segmented, this process is done by a method which is proposed by Carrillo and et al [3]. At the next step, the distal point of mid-piece is considered as an initial point. Knowing that a pixel is located on the sperm's tail (such as the distal point of mid-piece), to find a new pixel on the sperm's tail, SSIM index is computed and checked, locally. To automatically stop the algorithm at the end of sperm's tail, local entropy is selected and estimated as a feature. Experimental results show that the proposed method is able to detect sperm's tail, accurately. Also, the estimated points can be used to obtain morphological assessment of sperm's tail, such as length and shape and etc. For all sperms in database, sperm's tail is computed manually and compared with the estimated value through the proposed method. Success rate is defined below

$$\text{Success rate} = \frac{\text{Estimated length}}{\text{Computed Length}} \tag{13}$$

and computed for all sperms. The overall success rate of this approach is 97.6 %. One of the important advantages of the proposed method is the ability to detect sperm's tail in the low-contrast sections. Also, the execution time of implemented algorithm is too low. In future investigations we intend to use various geometric features to design a powerful method which works under uncontrolled conditions, automatically.

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