POSTER: Parametric 3D model of sperm cell surface applied in confocal microscopy

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ABSTRACT

Confocal microscopy combined with 3D graphics enable to study spatial characteristics of biological objects on microscopic level. Human sperm cells have a typical shape with marked asymmetry in its frontal-back part. 2D analysis with optical microscope gives mainly information about horizontal plane of the cell. Presented parametrical model provides important geometrical parameters describing 3D shape of the cell surface using 3D reconstruction of horizontal cross-sections acquired by a confocal microscope.

Keywords

3-D Modeling, Surface Reconstruction, Parametric Model, Confocal Microscopy, Sperm Cell.

1. INTRODUCTION

The relationship between the shape and the function of a cell is the subject of intensive research. Moreover, cell morphology represented by texture and shape indexes is applicable in medicine ([TDH+08]). Sperm cell (especially its head) has a typical shape with marked asymmetry of its frontal-back part. Although there were attempts to describe the 2D contour of the cell's head [BCV05], 3D models are still rare. The reason is that traditional optical microscopy cannot provide high-resolution 3D images. Atomic force microscopy (AFM) [KCS+05] and confocal laser scanning microscopy (CLSM) [DBF+97] opened new possibilities to obtain detailed information about 3D topology of sperm cell. Most of images show a marked flatness of the frontal part of the cell. This deformation (hydrodynamic profile) is not included in 2D models and can be an important factor of the cells movement in the surrounding environment.

The output of confocal microscope is a series of digital images representing individual cross-sections acquired from the different depth of the sample. 3D surface can be reconstructed from these optical sections by using image processing techniques. 2D contours of sperm cell at all levels of depth consist of pixels that can represent surface voxels in 3D. Fitting of all these voxels by a proper parametric model allows both efficient visualization and shape analysis. A novel parametric model of sperm cell surface is proposed in this contribution. The shape analysis allows elimination of artifacts in fluorescent microscopy where their irregular shape and random position lead to the variable intensity of emitted light. We expect that individual parameters can serve as a feature vector for classification of sperm cells.

2. MODEL

A lot of surface models in computer graphics are based on superquadrics however, they are not proper to fit wrinkles in the front part of sperm cell. The basis of our model (Figure 1) is an ellipsoid with main axes a_0, b_0, c_0 (in y, z, x directions) however, its surface is modified in cylindrical coordinate system through a parametrical function $\rho(v,\theta)$ with variables θ (lateral angle) and $v = t/c_0$ (relative distance) :

$$\rho(v,\theta) = \sqrt{\frac{1-v^2}{(cos\theta/a(v))^2 + (sin\theta/b(v))^2}} \quad (1)$$

where for the main axes holds

$$a(v) = a_0\,(1 + \delta_a v) \quad (2)$$

$$b(v) = b_0 \cdot flat \quad (3)$$

and flatness is given by the equation

$$flat = 1 - d_f/\left(1 + exp\left[-k_f\left(v - v_f\right)\right]\right) \quad (4)$$

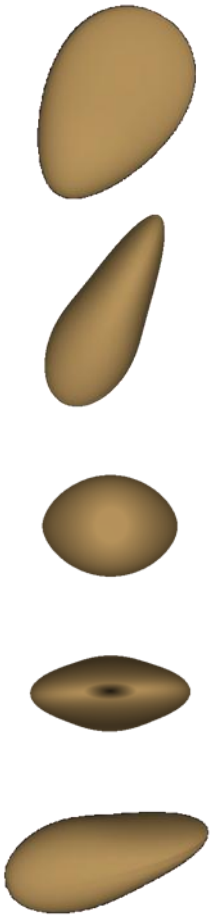

Figure 1. Illustration of a 3D-model surface of the cell (top, side, back, frontal and a rotated view), depicted using K3DSurf [K3DS].

The above defined model has 7 parameters specified in Table 1, Figure 3 and incorporates basic shape features of the sperm cell (Figure 2):

After backward transformation of Eq.(1)-(4) to the Cartesian coordinate system:

$$x(v, \theta) = v \quad (5)$$

$$y(v, \theta) = \rho(v, \theta)\, cos(\theta) \quad (6)$$

$$z(v, \theta) = \rho(v, \theta)\, sin(\theta) \quad (7)$$

Figure 2. Selection of horizontal slices of a real cell acquired by confocal microscope (inverted).

3. CONFOCAL MICROSCOPY

Purified sperm cells were obtained from the patients of Centre for Assisted Reproduction with the institutional ethical approval. After fixation, human sperm cells were stained with a fluorescent dye Ethidium Bromide. Stained cells were then placed into polyacrylamide gel environment to guarantee

their stable position during scanning. Subsequently, the microscopic preparation was created by the standard way.

a_0, b_0, c_0	the main axes of the ellipsoid
δ_a	asymmetry of the cell's contour in its horizontal plane
v_f	location of the vertical flatness of the cell relative to its center
k_f	steepness of the vertical flatness
d_f	depth of the vertical flatness

Table 1. Parameters used in shape model

Samples were scanned by fluorescence confocal microscope which offers a series of digital images captured in different depth of sample (0.4 micrometers apart).

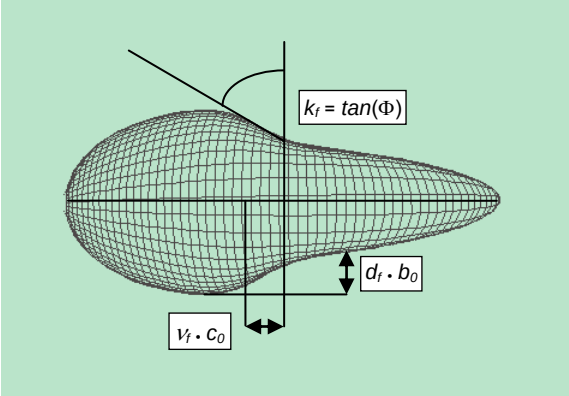

Figure 3. Model parameters interpretation, depicted using K3DSurf [K3DS].

Two types of confocal microscopes have been used - Leica DM 2500 CSQ V-VIS and Leica TCS SP5 (objective 100, resolution xy-139nm, z-236nm) both supplied with Leica Application Suite Advanced Fluorescence (LAS AF). Surface points coordinates *x*,*y*,*z* were calculated from contours acquired by segmentation of calibrated microscopy scans of horizontal cross-section (Figure 2). A simple thresholding segmentation algorithm followed by edge tracking contour detection was used (Figure 4). Approximation of the cell surface with the defined model resulted in noise reduction.

4. DATA PROCESSING

As the cell's centre and orientation differs from the basic position of the model, it is necessary to use 6 additional parameters (3 coordinates of the center and 3 angles of the orientation) for model analysis. In order to increase the reliability of calculations and accelerate computing, parameters of the cell's placements and orientation are calculated at first by sequential fitting of the surface with the main planes of the ellipsoid. Their intersection defines the main axis and its center the cells' center (Figure 5).

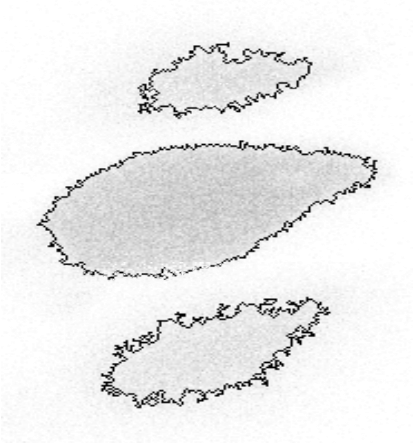

Figure 4. Segmentation of individual optical sections.

Consecutively, the cell surface points are transformed into cylindrical coordinate system with origin in the cell's centre oriented toward its main axis and these data are used in the subsequent analysis. Downhill simplex algorithm was used for least square optimization of proposed parametric model.

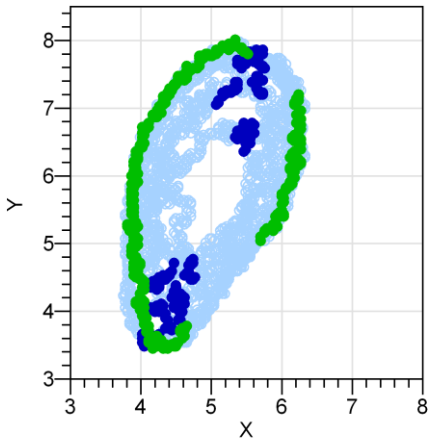

Figure 5. Schematic drawing of surface points projection (light blue dots) and projection of the nearest points to the cell main planes fits (blue – horizontal plane, green– vertical plane) from side view.

5. RESULTS

Nonlinear regression methods (Figure 6) were used to gain geometrical parameters of a real cell: a_0 = 1.5 µm, b_0 = 1.1 µm, c_0 = 2.4 µm, δ_a = 0.06, $t_f = v_f \,.\, c_0$ = 0.7 µm, k_f = 11.5, d_f = 0.35 with standard deviation of the surface 0.1 µm and with correlation coefficient 0.94. Figure 7 shows the visualized model of sperm cell surface fitting the real image data acquired by the confocal microscope.

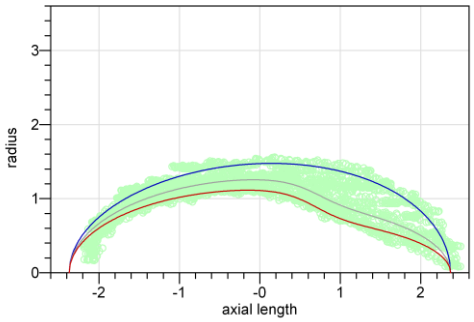

Figure 6. Longitudinal half-contours of the model surface of the cell with the real cell contours in the background. Three longitudinal contours corresponding to angles 0°-blue, 45°-gray, 90°-red are depicted.

6. CONCLUSION

We have presented 3D-reconstruction of real human sperm cell surface from confocal microscopy optical sections using novel parametric surface model. The extracted parameters occur in region of real cell dimensions and as we believe could be useful to 3D-shape cell classification.

7. ACKNOWLEDGMENTS

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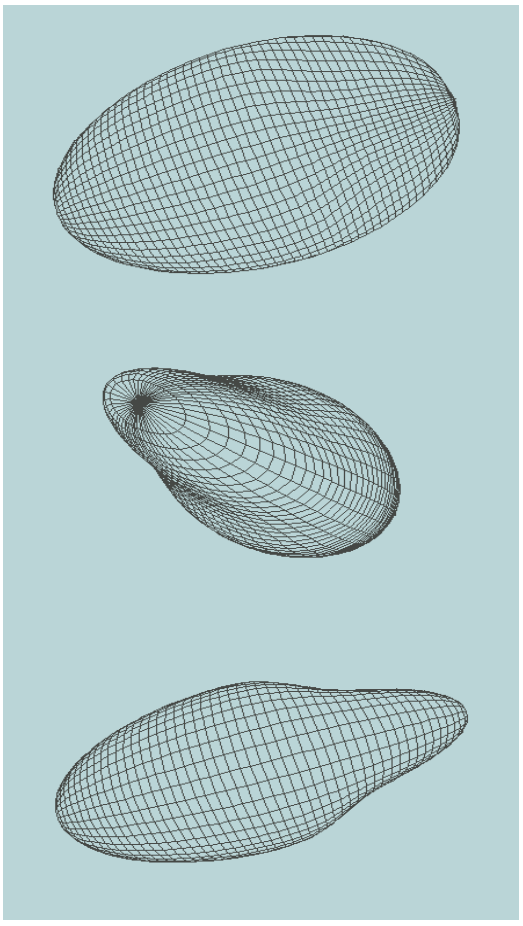

Figure 7. Visualization of the surface model of sperm cell head (top, rotated and side views), depicted using K3DSurf [K3DS].