Geometrical Modelling of Muscle Cells Based on Functional Representation of Polygons

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ABSTRACT

A geometrical modelling tool allowing construction of models of living cells and their organelles would facilitate understanding of biological processes at the cellular level. Here we describe a technique of skeletal muscle cells modelling, and a construction of the model based on the theory of implicit surfaces and their binary operations. The geometry of the cell is defined by means of parallel modelling planes perpendicular to its longitudinal axis. In each plane, the number, shape and topology of cell organelles is defined by means of functionally represented polygons. The model is obtained by interpolation between the planes. This approach allows to generate approximations of surfaces and to estimate the volume and surface densities of organelles in the model.

Keywords

Muscle cells, Geometrical 3D modelling, Implicit surfaces.

1. INTRODUCTION

Structure is a fundamental category intimately associated with function of biological objects. Functional features of living cells are often related to specific intracellular organelles or to coordinated action of several organelles. An important determinant in their function is the size, which ranges from few nanometers to many microns, the shape, the surface area, and the volume. These data can be obtained by methods of modern stereology based on geometrical statistics [Wei73]. Three-dimensional geometrical characteristics of the organelles are estimated from electron microscopic images of very thin sections of the specimen. There are several practical problems, such as appropriate number of images for analysis, or selection of a proper test grid with respect to the size, density and orientation of intracellular structures. In other words, and in analogy to classical metrological problems, stereology of the microworld needs a practical calibration tool that could be used in everyday practice.

A typical feature of the striated muscle cells, or muscle fibres, is their prolonged shape. Along the longitudinal axis an elementary motif, the sarcomere, is repeated giving rise to a striated pattern seen under the microscope. The sarcomere consists of myofibrils, which span the length of the cell. In the cross section, the myofibrils show a curved polygonal shape of about few hundred nanometers in diameter. The mitochondria of irregular rounded shape are localized around the myofibrils. Transversal (T) tubules are distributed perpendicularly to the long axis of the muscle fiber. Sarcoplasmic reticulum forms a fine network of tiny tubules and cisterns surrounding myofibrils along their length. An important feature of individual structures (organelles) is their disjunctive character. Therefore, each structure should be bound in exact delimited space. The free space between two neighbouring organelles can reach many nanometers. With respect to these conditions, building of the model should allow definition of the shape, topology and location

WSCG POSTERS Proceedings
WSCG'2004, February 2–6, 2004, Plzeň, Czech Republic. Copyright UNION Agency – Science Press

of individual organelles.

Recently, substantial progress has been made in medical applications working with discrete images representing sections of an object obtained by sophisticated instrumentation, which allows reconstruction of specific objects in 3D. There is a need, however, to generalize the 3D features of a given class of objects, or in other words, to work with an average, as opposed to a typical 3D object, which characterizes an entire class, e.g., an average heart. Such objects can be created by means of modelling in 3D. Turk and O'Brien [Tur99] presented an approach for creating an n-dimensional implicit function from parallel sets of (n-1)-dimensional scattered data. There are methods for computation of two-dimensional signed distance fields from inside/outside characteristics of a structure in the image of a section. Pasko *et al* [Pas96] developed a theory of functional representation of polygons, which may serve as the basis for creation of two-dimensional distance-based real functions approximating closed polygons and their interconnection with the three-dimensional implicit solids. This implicit surface representation may then enable to accomplish modelling of 3D objects from stereological data.

The aim of this work was to develop basic tools for building models to be used for verification of stereologic measurements of muscle cells. The software is based on functional representation of objects with consideration of specific geometrical properties of cellular organelles and of their topology within the cell.

2. FUNCTIONAL REPRESENTATION OF POLYGONS

A two-dimensional non-self-intersecting (simple) polygon is defined by a finite set of segments. These segments are edges closed by vertices. In a functional representation, the function $F(x, y)$ describing a simple polygon takes zero values at polygon edges [Pas96]. A convex polygon can be represented as an intersection of half-planes, defined by edges of the polygon, using the *R-function* defining the intersection. Representation of concave polygons is in general more complex. Dobkin and colleagues [Dob93] presented an efficient algorithm for finding a monotone boolean formula. This formula represents a concave polygon by set-theoretic operations where each half-plane appears exactly once and no additional half-planes are used (Figure 1). In contrast to other techniques, this formula does not generate artifacts caused by "internal zeroes" [Pas96].

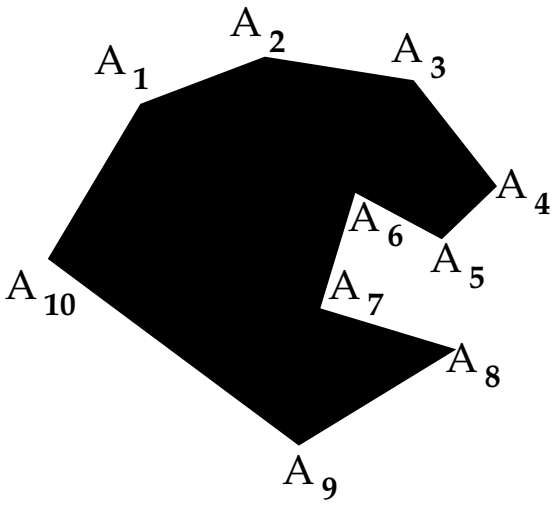

Figure 1: An example of a concave polygon. The corresponding formula: $F = f_1 \wedge f_2 \wedge f_3 \wedge ((f_4 \wedge f_5) \vee f_6 \vee f_7) \wedge f_8 \wedge f_9 \wedge f_{10}$, where f_i is a halfspace defined by a line passing through points A_i and A_{i+1}, and $\vee, \wedge$ are *R-functions* of an union and an intersection.

3. MODELLING STRATEGY

In modelling the striated muscle cells we limit ourselves only to one or at most several sarcomeres, which represent the basic repetitive pattern of the cell. The geometry of the muscle fiber is defined by means of parallel modelling planes perpendicular to the longitudinal muscle cell (z) axis. In each plane (Figure 2) the number, shape and topology of cell organelles is defined by means of functionally represented polygons.

The global cell structure within each modelling plane is defined by a planar continuous graph which divides the plane into areas limited by closed polygons. The functional representation of polygons, as it was introduced in Section 2., exactly describes a given polygon, with straight edges and sharp corners. However, our goal is to model smooth objects with irregular shapes. Therefore instead of the basic *R-functions* we use blending functions [Pas94] based on *R-functions* that join objects in a single complex with smooth edge transition.

In the case when a single organelle is represented by several disjunctive contours within the modelling plane, its resulting functional representation is given by their union.

Organelles of a given muscle cell are defined by means of an ordered sequence of modelling planes along the z axis of the cell (Figure 3). A functional representation of the organelle in an arbitrary point is interpolated from in-plane values.

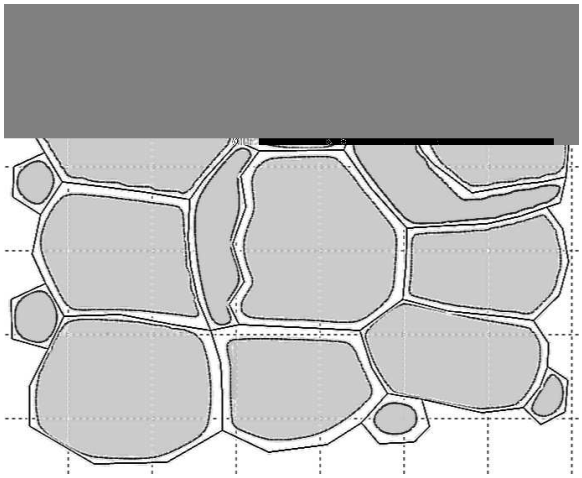

Figure 2: A modelling plane divided into closed polygons and their corresponding contours.

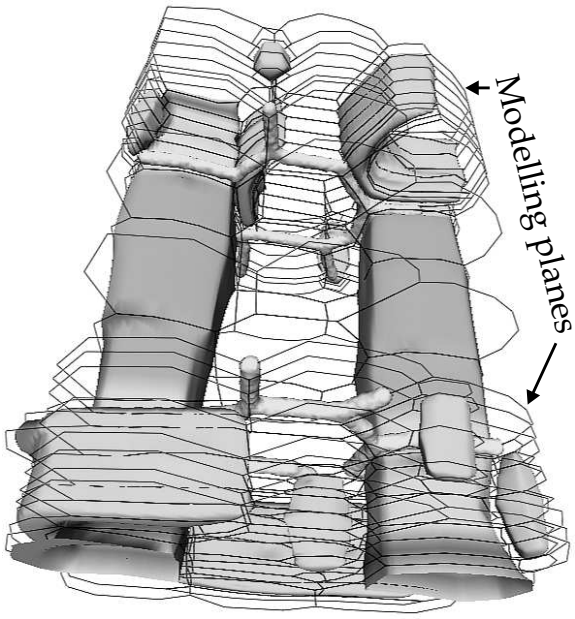

Figure 3: Creation of the muscle cell model by linear interpolation between 16 modelling planes.

4. THE MUSCLE FIBER MODEL

We model four basic organelles of a muscle fiber (Figure 4): myofibrils, mitochondria, T-tubules and sarcolemma.

Myofibrils are cylindrical objects, oriented in parallel and organized in bundles. They span the whole length of the cell (z-axis) and usually have rounded shapes. Since we model only a part of the cell (the sarcomere), myofibrils cross all modelling planes and are not closed from top and bottom.

Mitochondria are closed, elliptically shaped, objects of irregular form. They spread between and around myofibrils. Since they are closed, each is defined in a finite number of modelling planes. In order to create smooth "rounded cap" to close a mitochondrion, we modified the interpolation function by a negative weight between the two uppermost and the two lowermost modelling planes, where the mitochondrion is defined.

Sarcolemma is a membrane envelope that surrounds the muscle cell. The value of the volume limited by sarcolemma is needed to compute the volume and surface densities of intracellular organelles. The functional representation of the sarcolemma in a given modelling plane is defined by the outer edges of the planar graph that defines the myofibrils and mitochondria.

T-tubules are continuations of the sarcolemma that form a planar network around the myofibrils, perpendicular to the z axis. Each T-tubule is generated as *R-function* of a union of segments—skeletal contributions [Fer97]. These segments are defined by a "randomly" chosen contiguous subset of the set of polygonal edges defining myofibrils and mitochondria.

For all organelles in the cell, the condition of disjunction must hold. Therefore the T-tubule system has to lie in between the remaining structures. The required minimal distances between the T-tubule and the polygon contours representing the myofibrils and mitochondria is achieved either by shrinking the neighbouring organelles,i.e., by changing their corresponding iso-contour values, or by changing the width of the T-tubule,i.e., by choosing an appropriate distance value from the leading edge shared by the neighbouring polygons.

5. CONCLUSION AND FUTURE WORK

We have developed a modelling tool, based on parallel modelling planes and blended functional representation, which allows construction of 3D models of striated muscle cells. The generated models satisfactorily represent ultrastructural features of the cells from the point of organelle composition, i.e., their shape, size, surface and relative positioning, as well as from the point of stereology, i.e., the related volume and surface densities of cellular components. The principles used to define the myofibrils, mitochondria, t-tubules, and sarcolemma, were found very well-behaved and useful. Nevertheless, they were not appropriate for generation of the sarcoplasmic reticulum. Due to its very

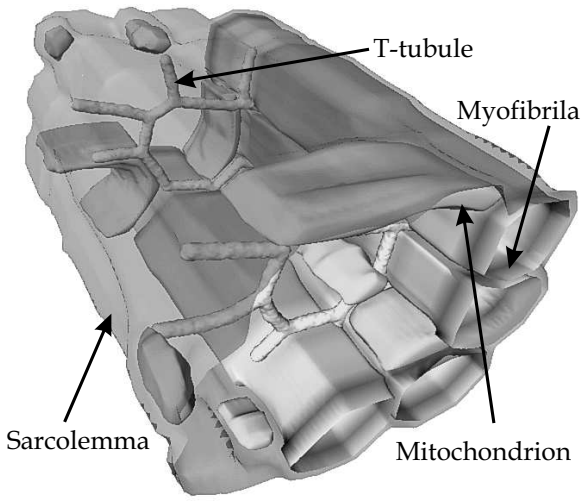

Figure 4: An example of the muscle fiber model. For clarity, some organelles under sarcolemma are hidden.

complex and fine structure, modelling of the 3D features of the sarcoplasmic reticulum asks for a different method of its generation.

Testing of the software with models reproducing real cells revealed some possible improvements. The Marching Cubes algorithm [Lor87], used to tesselate the organelle surfaces for visualization, was found to generate an unnecessarily high number of triangles. An alternative approach would be to use algorithms such as boundary tracking for finding an isosurface [Art80] or the application of mesh simplification techniques [Kob98]. Altogether, the generated models can be used not only for presentation purposes but also for testing of alternative stereological methods for their applicability in specific scientific problems.

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