APPLICATION TO TWO CASES

Relax-to-Rigor Transition

In the rigor state, S-1 heads of HMM are locked into an angle on the f-actin. Since the method used here is sensitive to anisotropic elements and their changes when they go into rigor, the measured quantities monitor the orientational changes of intrinsically anisotropic elements and packing changes of isotropic elements. Plotted in Fig. 2 are the relaxed (1 mM ATP) and rigor state data at several ionic concentrations, μ, of the bathing medium. It is apparent that at any μ condition, the rigor state has both a lower depolarization ratio, r, as well as a lower total birefringence, Δn (2), when compared with its corresponding relaxed state values. A decrease in the depolarization ratio upon rigor is consistent with the idea that as S-1 binds, the connecting S-2 element subtends a larger angle with respect to the fiber axis. These results together are consistent with the idea that when a fiber goes into rigor, intrinsic anisotropy changes dominate over form birefringence changes.

Low Ionic Strength Solutions

Brenner et al. (3) noted that a decrease of the total ionic strength of the relaxing solution causes an increase in the dynamic stiffness of the fiber. X-ray diffraction studies have indicated that an increase in the mass concentration at the actin occurred after the decrease of solution ionic strength (4). In our experiment, ionic strength was varied at both the relaxed (1.0 mM ATP) and the rigor (0.0 mM ATP) states. The trend of Δn and that of r with decreasing ionic strength differ. As the ionic strength is lowered from the normal 100 mM to 20 mM, there is a strong increase in Δn while the change in r is almost negligible. When the same experiment is conducted at rigor state, the trends are similar but the overall values of Δn and r are both smaller. The difference in our results between the rigor state and the low ionic strength state is consistent with the idea that the nature of movement and redistribution of matter are different for these two states.

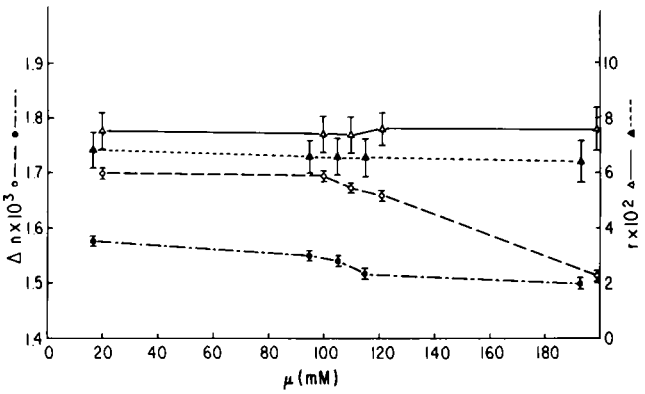

FIGURE 2 Ellipsometry parameters, Δn and r, plotted against the solution ionic strengths, μ. The relaxed fiber data, obtained at 1 mM ATP, is given by ○ for Δn, and △ for r; the rigor state fiber data is represented by ● for Δn, and ▲ for r. Straight line connections from point to point have been used to illustrate the trends.

We acknowledge the skillful assistance of Mrs. Quan You Li in the preparation of the single fibers.

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THREE-DIMENSIONAL RECONSTRUCTIONS OF OPTICALLY IMAGED SINGLE HEART CELL STRIATION PATTERNS

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INTRODUCTION

The quantification of sarcomere length in cardiac muscle is of critical importance for the unambiguous interpretation of contractile performance. Myocardial mechanics are routinely characterized by the interrelated parameters of length, force, velocity, and time. Any unknown variation in

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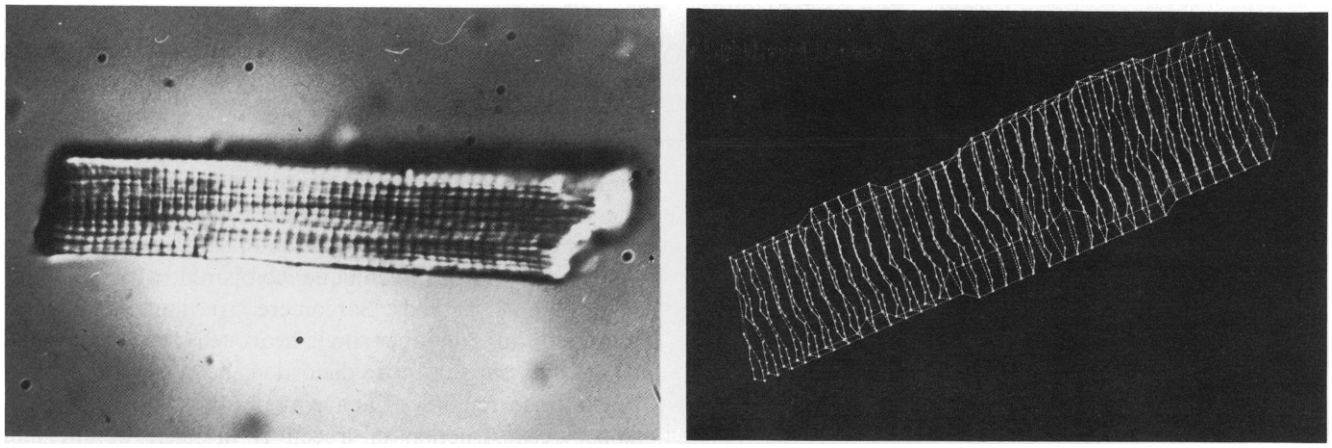

FIGURE 1 Photomicrograph and three-dimensional reconstruction of a cell. The photomicrograph (*a*) illustrates the striation pattern from a single focal plane of a cell. A simplified two-plane three-dimensional reconstruction of the same cell (*b*) is viewed from the 10-0-20 rotation (looking from the top down, but rotated back by 10° and around the optical axis by 20°). These two planes of data contain 916 discrete striation positions each marked with an *x*. Adjacent *x*'s are joined by solid lines to simulate striations. The dash-dot lines continue each simulated striation between nonadjacent *x*'s in the direction indicated by the photomicrograph. Each plane's periphery is defined by dotted lines. The reconstructed striations are joined from plane-to-plane at the cell periphery by dashed lines. The length, width, and depth scaling is not in exact proportion due to the perspectives involved in cell rotation. Plane separation is 3.0 μm; average sarcomere periodicity is 1.852 ± 0.105 μm.

one of these parameters, such as length, can lead to the erroneous evaluation of any of the other related parameters and therefore to overall myocardial performance (3). It is the long-range goal of these studies to quantify precisely the individual sarcomere striation positions throughout the volume of single cardiac muscle cells to evaluate individual and average sarcomere lengths in terms of other measurable parameters (3, 5).[1] A computer-interfaced high resolution optical microscope apparatus has been developed (4, 5)[1] and three-dimensional optical sectioning image reconstruction techniques used (1, 2, 6)[1] to characterize the sarcomere striation patterns at the myofibrillar level at rest or during dynamic contraction.

METHODS

Striation Pattern Image Acquisition

Ca^{++}-tolerant isolated heart muscle cells or myocytes (Fig. 1 *a*) are prepared by coronary perfusion of excised adult rat hearts with a 0.1% collagenase solution (5).[1] The cells are maintained in a physiological Ca^{++}-Tyrode's solution and exhibit clearly visible A-, I-band striation patterns. A dilute slurry of these cells is placed on the glass-bottomed chamber of the microscope. A cell meeting specific morphological and functional criteria (5)[1] is selected and imaged through the 63×, 1.2 numerical aperture (NA) Zeiss Plan Neofluar objective and Nomarski differential interference contrast (DIC) microscope system (Carl Zeiss Inc., Federal Republic of Germany). The cell may be directly viewed for selection and orientation, or photographed with a 35 mm camera, or imaged with the 1 × 1728 pixel charge-coupled device (CCD) array (Reticon Inc., Sunnyvale, CA). The selected cell is oriented on the *X–Y* rotating stage so that its striations are perpendicular to the long axis of the 1728 pixel solid-state CCD imaging array and the maximum contrast enhancement of the DIC. Cell image data from the CCD is sequentially digitized and stored in the memory of a PDP 11/34*a* minicomputer (Digital Equipment Corp., Maynard MA) as fast as 2.5 ms/complete 1728 pixel frame of data using a custom designed direct memory access (DMA) interface (4). Multiple sequential frames of data can be obtained from quiescent cells (at any frame rate) for later signal-averaging noise reduction or from contracting cells (at 10 ms/frame or less) for temporal quantification of sarcomere dynamics. These data are stored in real time for later analysis.

The combination of the high NA-DIC equipped optical microscope and the CCD sensor provide the means to section optically the cell's image

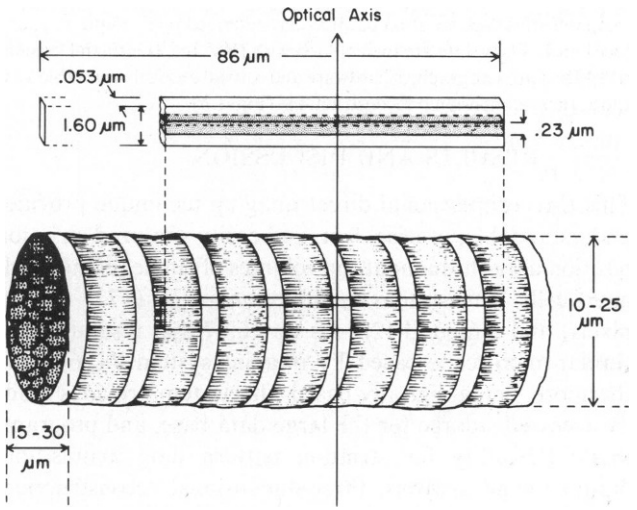

FIGURE 2 Optical subvolume. Volumetric subsections of the heart cell image are obtained from the combination of the focal slicing capability of the high resolution DIC optical microscope and the discrete nature of the CCD detector. The optical subvolume is illustrated both within and above the sample cell. The entire length of the cell and subvolume rectangle are not shown in order to clarify the presentation. The dimensions are not to scale.

[1]Roos, K. P. Sarcomere length uniformity determined from three-dimensional reconstructions of resting isolated heart cell striation patterns. Manuscript submitted for publication. Fig. 1 will illustrate the direct three-dimensional imaging apparatus.

laterally and focally into subvolumes as shown in Fig. 2. At the usual system magnification of 300 ×, the imaged subvolume is 86.4 μm in length, 0.053 μm in width, and 1.60 μm in depth of image influence. The light-sensitive area of the 1 × 1728 pixel array determines the subvolume length and width dimensions. Each CCD pixel is 16 μm wide and separated by 15 μm centers. (86.4 μm L = 1728 × 15 μm/300; 0.053 μm W = 16 μm/300). Using the equations of Allen et al. (2), the depth of sharp focus has been calculated to be 0.23 μm and the depth of image influence to be 1.60 μm for this DIC objective/condenser combination.

A complete three-dimensional volumetric characterization (using x, y, and z coordinates) of a cell's sarcomere striation positions is obtained by sequential lateral scanning in multiple focal image planes. First, a striation pattern planar map at a given focal plane is obtained by moving the CCD sensor physically across the width of the cell's image in a series of 0.34 to 1.01 μm controlled steps. The CCD sensor is mounted on a precision sliding track oriented perpendicular to the long axis of the cell's image and is moved laterally with a motorized micrometer (Ardel Kinematic Inc, Stratford, CT) under external control. The movement of the sensor across the image is more precise and repeatable than moving the cell under the microscope's objective because it takes advantage of the optical system magnification. Finally, to achieve three-dimensionality, the microscope's focus is changed by 2.0–3.0 μm and the planar map procedure repeated. Thus, three-dimensional, x, y, and z-coordinate multiple plane representations of striation positions throughout the volumes of resting heart cells are obtained at nearly the myofibrilar level. For dynamic studies on electrically paced cells, 50–100 sequential frames of data are acquired during a single contraction/relaxation cycle at each x–y position to provide a fourth dimension, time.

Three-dimensional Image Reconstruction

Discrete striation positions are determined from each cellular subvolume frame of image data by interactive digital processing techniques (1, 4 ,5).[1] There are typically 20–50 striations monitored along the length of a given cell in a single 1728 pixel scan depending on the sarcomere periodicity and the amount of nonstriated material (nuclei and mitochondria) within that specific imaged subvolume. Each striation position is determined with a precision of at least 0.05 μm and given a z-coordinate value (5). Each scan of z coordinate striation positions is assigned x (width) and y (focal plane) coordinates (in μm) determined from the externally controlled sensor and focal position steps. Therefore, the data for a given cell are stored in the computer with each striation position characterized by a unique x, y, or z coordinate. These data are then plotted with IBM Inc. (German Division) IDAMS computer graphics hardware and software as fully rotatable x, y, and z, three-dimensional reconstructions (Fig. 1 *b*).

RESULTS AND DISCUSSION

This three-dimensional direct imaging technique provides understandable, precise, fast, and easily analyzed striation position data throughout the volumes of single, living adult heart cells. This system has superior spatial (1728 vs. 512 pixels) and temporal (2.5 ms vs. 16.67 ms) resolutions to similar microscope-based TV imaging systems (1, 6). Furthermore, being digitally based, the system provides readily accessed storage for the large data base, and programmatic flexibility for striation pattern data acquisition, digital image analysis, three-dimensional reconstruction, and statistical evaluation.

This imaging system has been used with various protocols and analyses to evaluate the three-dimensional striation pattern uniformity of resting unloaded cells,[1] to examine unloaded cellular sarcomere dynamics (5), and to compare and evaluate real light diffraction patterns to those calculated from three-dimensional sarcomere arrays.[2] Fig. 1 is a direct comparison of a photomicrograph (a single focal plane) to a simple two plane, three-dimensional reconstruction of the central 39 striations of the same resting cell. This simple reconstruction demonstrates the improved visualization of sarcomeric structure achieved with this technique compared with standard micrographic methods. Sarcomere striations within most regions of this and other single heart cells are uniform and in register, but subject to distortions near the noncontractile nuclear regions.[1] Each planar map or three-dimensional reconstruction of a cell is in itself unique and corresponds directly to (but more precisely than) their photomicrographs. These reconstructions and their three-dimensional database provide the means for additional evaluation of sarcomere periodicity through rigorous statistical analyses.[1] Three-dimensional striation pattern evaluation and reconstruction provide the basis for unambiguous quantification of sarcomere lengths and improved interpretation of myocardial contractile performance.

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