THREE DIMENSIONAL DEFORMATION OF THE EPICARDIUM OF THE ISOLATED RABBIT HEART

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INTRODUCTION

Over 60 million Americans suffer from various forms of cardiovascular disease [1]. Of these diseases, coronary artery disease is responsible for more deaths than all other forms combined. Coronary artery disease is generally a regional phenomenon, leading to regional ischemia, hence regional changes in the heart. As these changes can result in altered deformation patterns within specific regions of the heart, it is necessary to determine deformation with high spatial resolution in the heart.

We have previously reported the ability of computer aided speckle interferometry (CASI) to determine two dimensional regional deformation with high spatial in the isolated rabbit heart [2] [3]. However, regional ischemia can lead to systolic bulging, or significant out-of-plane displacement. It is therefore necessary to measure deformation in three dimensions.

Herein, we report the use of two techniques to measure three dimensional deformation. CASI is able to determine two dimensional in-plane displacement, whereas digital fringe projection (DFP) is able to determine out-of-plane. By combining the data obtained with these techniques we are able to reconstruct the three dimensional displacement of the surface of the isolated rabbit heart.

MATERIALS AND METHODS <u>Protocol</u>

All animals received humane care in compliance with the "Principles of Laboratory Animal Care" formulated by the National Society for Medical Research and the "Guide for the Care and Use of Laboratory Animals" prepared by the National Academy of Sciences and published by the National Institutes of Health (NIH Publication No. 85-23, revised 1985). Furthermore, the Institutional Animal Care and Use Committee at Stony Brook reviewed and approved the protocol followed in this study (IACUC # 0834). In this study rabbits were chosen because their hearts could easily be isolated and they are commonly used in a Langendorff apparatus, which is a well-designed model for myocardial function.

For all studies involving rabbits, New Zealand rabbits, weighing 2.5-3.5 kg, were anesthetized with sodium pentobarbital (30 mg/kg) and anticoagulated (1,000 units sodium heparin) by an ear vein. The chest was opened via bilateral thoracotomy, the heart rapidly excised and placed in iced Krebs-Henseleit solution.

A latex balloon, attached to a vertically adjustable water filled reservoir, was placed in the left ventricle of the hearts via an incision made to the left atrial appendage. Adjusting the height of the reservoir controlled the intracavitary pressure within the ventricle. Small silicon carbide particles (approximately 40 µm diameter) mixed with retroreflective beads (60 µm diameter) were dispersed randomly on the anterior wall of the left ventricle. These particles provide a dark/light contrast, referred to as a speckle pattern, for digital imaging. A 1024 x 1024 pixel CCD camera was used to record the speckle image at 0 mmHg intracavitary pressure. After the image was acquired for CASI analysis, a digital projector was used to project vertical fringes upon the surface of the heart. Images were then captured with the same CCD camera used to acquire the speckle images for CASI analysis. The intracavitary pressure was then increased to 10 and 20 mmHg intracavitary pressure and data were again acquired.

Data Analysis

CASI Analysis: CASI is a modern version of conventional speckle interferometry techniques. The technique has previously been described in detail [4] [5] [6]. In summary, two digital images are divided into "subimages". The deformation of the subimage between the two images is then determined. To accomplish this, a two dimensional Fourier transform is applied to both subimages. The images are then combined through an interference function and a second two dimensional Fourier transform is performed on the resultant data. This results in an impulse function with a peak corresponding to the u (displacement along the x axis) and v (displacement along the y axis) displacement between the two subimages. This process is repeated for each subimage in the region of interest, providing two dimensional displacement at every subimage within the region of interest.

Digital Fringe Projection: Three separate digital fringe patterns, each with 120° phase shift, are sequentially projected onto the surface of the heart with the following light intensity distributions:

$$I_{i}(x,y) = I_{0}(x,y) \{1 + \gamma(x,y) \cos[\phi(x,y) + \theta_{i}]\}$$
(1)

where i = 1, 2, 3 and θ_1 , θ_2 , $\theta_3 = 0^\circ$, 120°, 240° respectively. I₀ is the average brightness at a "point" (e.g. pixel), $\gamma(x,y)$ the fringe visibility, $\phi(x,y)$ is the phase angle to be determined. The three equations can be solved to yield:

$$\phi = \arctan\left(\frac{I_3 - I_2}{2I_1 - I_2 - I_3}\right)$$
(6)

from which the height variation can be deduced. A more detailed description of the DFP technique has been previously reported [7].

RESULTS

A region of interest on the anterior surface of the heart was selected for analysis. This region was 3.6 x 3.6 mm. The displacement along the x axis (u) and y axis (v) are shown in Figure 1. The resolution of the image is 18 µm/pixel. The displacement over the region of interest is approximately 92 µm along the x and 110 µm along the v direction.

The out-of-plane displacement at 0 and 20 mmHg intracavitary pressure for the same region of interest in which CASI analysis was performed is shown in Figure 2. The change in curvature between the two loads was relatively small. The three dimensional deformation is then computed as the sum of the u, v, and w (out-of-plane displacement) displacements.

DISCUSSION

Herein, we present a novel method to determine the three dimensional deformation of the epicardium. This method can determine deformation with high spatial resolution, which is needed to assess myocardial function in the regionally ischemic heart. We have previously reported that CASI produces equivalent results to that obtained by sonomcirometry[3]. However, CASI is able to determine deformation with over 100 times higher spatial resolution than sonomcirometry. An inherent limitation of CASI is that a three dimensional surface is projected into two dimensions. This can lead to error due to curvature and displacement along the camera axis. With DFP we are able to determine both the curvature and the displacement along the camera axis. These data can be used to correct the two dimensional displacement determined by CASI.

Recent advances in bioengineering are allowing novel ways to restore myocardial function. For example, angiogenesis factors are being used to stimulate new blood vessel growth in ischemic myocardium. However, it is currently unknown if an increase in blood vessel formation leads to an increase in regional function, as some groups have reported no change in regional function with increases in vessel formation[8]. Most studies that determine the effectiveness of such therapies are based on global (whole heart) parameters. The combination of CASI and DFP will provide information about changes in the regional function of the heart. This will allow for improved assessment of various therapies and deliveries of such therapies.

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Figure 1. Displacement gradients (u on the left, v on the right) over the region of interest determined by CASI. Each contour represents approximately 10µm.



Figure 2. The region of interest where CASI analysis is performed outline over the 3-D reconstruction of the rabbit heart at 0 (left) and 20 (right) mmHg intracavitary pressure.