Measurement of Displacement in Isolated Heart Muscle Cells using Markerless Subpixel Image Registration

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***Abstract*— Cellular mechanics is an area that has been readily explored, yet there is still much that is unknown about the behaviour of heart muscle cells (myocytes) during contraction in both healthy and diseased states. Currently available systems for contractility measurements expect myocytes to be aligned within the imaging system and expect them to contract axially. However, myocyte contraction is not always uniform, and hence localised changes in the myocyte may not be captured in 1D measurements such as average sarcomere and cell length. With the availability of new and robust image correlation techniques, there is new potential to track displacements of cells without the need for external markers. Here, we present the use of a subpixel image registration (accurate to the millipixel) to track the internal movement of an isolated myocyte during unloaded contraction. Additionally, methods were developed to estimate the orientation of a contracting myocyte frame by frame, removing the need for myocytes to be perfectly aligned in the imaging system. This will allow measurement of sarcomere lengths and displacement regardless of what angle the myocyte is at. Furthermore, it offers the potential to find localised myocyte angle during contraction and therefore localised sarcomere lengths.**

Keywords—image registration, myocyte, displacement measurement, tracking, sarcomere length

I. Introduction

The heart, arguably the most important organ in the body, is made up of millions of muscle cells (myocytes), which contract in synchrony to pump blood to the rest of the body. Heart disease often stems from the dysfunction of cardiac myocytes, yet much remains unknown about the function and mechanics of these cells in both healthy and diseased states. Once isolated from the heart, muscle cells are relatively transparent to visible light. Thus, transmission imaging allows visualisation of their sarcomeres – the basis contractile units with the cell [1] – while avoiding the heterogeneous electrical and stress/strain distributions and non-uniform alignment of myocytes that occurs in many multicellular preparations [2]. A deeper scientific understanding of cellular force-producing and shortening mechanisms and how these change with pharmacological, physiological, and optogenetic interventions will aid the development of treatments for cardiac disease.

While some systems for examining myocyte contractility are commercially available [3], [4] the measurements they offer are limited. Such systems can estimate sarcomere length (using the fast Fourier transform (FFT) analysis) and myocyte length (using edge detection) in real-time, yet they do not give information on the spatial variation of motion within the myocyte. Myocytes do not always contract uniformly, and available systems require the myocyte to be oriented such that contraction occurs axially in order to provide an accurate estimate of contractility and sarcomere length.

Other methods for measurement of cellular mechanics, such as traction force microscopy, allow the tracking of multiple points on the myocyte and can be used to determine localised force production of cells [5], [6]. However, these methods typically require cells to be bound to a substrate of known stiffness in which particles are embedded and tracked. The tracking is therefore dependent on these external markers; localised displacement calculations are limited by the density of markers, and consequently other measurements, such as calcium transients can be obscured. Here, we propose a method that uses subpixel registration of transmission images for markerless tracking of an unloaded myocyte, as well as methods for sarcomere length estimation of unoriented myocytes – capabilities not offered by previous systems.

While an image correlation method has previously been used for tracking internal displacements of myocytes during contraction [7], the technique uses optical flow methods, which are computationally expensive, and can result in inaccurate measurements with large displacements. Moreover, the method used in [7] does not allow sub-pixel displacements to be resolved. In this study, we used a novel, robust and accurate image registration technique developed by HajiRassouliha *et al.* [8] to perform internal displacement field calculations of an isolated myocyte during unloaded contraction using only the intrinsic features of the cell. The resolution of the image registration algorithm is in the order of millipixels, offering the ability to measure extremely small deformations. Additionally, FFT analysis techniques were used to estimate sarcomere length and orientation of a myocyte during contraction. By tracking the orientation of the myocyte, displacements of the myocyte can be calculated along its long axis, and therefore a better measure of myocyte length can be obtained, independent of orientation, and used for in-depth analysis of cell contractility.

II. Methods

A. Sample Preparation

All experiments were conducted in accordance with protocols approved by the Animal Ethics Committee of The University of Auckland (AEC 002049). A male Wistar rat (~300 g) was deeply anaesthetised with isofluorane (5% in O_2), injected with heparin (1000 IU/kg). Following cervical dislocation, the heart was rapidly excised.

The heart was Langendorff-perfused with a Tyrode's solution until the heart was clear of blood (containing 143 mmol/L NaCl, 5.47 mmol/L KCl, 0.5 mmol/L $MgCl_2$, 0.33 mmol/L NaH_2PO_4, 1.8 mmol/L $CaCl_2$, 5 mmol/L HEPES, and 5.5 mmol/L glucose; pH adjusted to 7.4 using TRIS ($C_4H_{11}NO_3$) and continuously bubbled with 100 % oxygen). The heart was then perfused with calcium-free Tyrode's solution (as above, without $CaCl_2$) continuously bubbled with 100 % oxygen until the heart ceased beating. Oxygenated calcium-free Tyrode with 0.01 % collagenase (Yakult, EC3.4.24.3) was perfused until the heart was pale and flaccid to digest the extracellular matrix. Following this, the heart was perfused with Kraft-Brühe (containing 70 mmol/L KOH, 0.5 mmol/L EGTA, 10 mmol/L HEPES, 50 mmol/L L-glutamate, 3 mmol/L $MgCl_2$, 20 mmol/L taurine, 10 mmol/L glucose, and 20 mmol/L KH_2PO_4; pH adjusted to 7.3 using KOH) for five minutes. The heart from removed from the apparatus and the ventricles cut open. The tissue was gently prised apart using forceps. The supernatant containing dissociated myocytes was collected and cells were left to gravity settle in a centrifuge tube for 5 minutes.

The myocytes were placed unloaded in carbogenated Krebs-Henseleit buffer with a $CaCl_2$ concentration of 1.5 mmol/L (containing 118 mmol/L NaCl, 4.75 mmol/L KCl, 1.18 mmol/L $MgSO_4$, 1.18 mmol/L KH_2PO_4, 24.8 mmol/L $NaHCO_3$, 1.5 mmol/L $CaCl_2$ and 10 mmol/L glucose; pH adjusted to 7.4 by bubbling with carbogen - 5% CO_2 and 95% oxygen).

B. *Experimental Set-Up*

The experiment was performed at room temperature, where a myocyte was stimulated at 1 Hz (1300 Vm^{-1}, 10 ms duration stimulus pulse) using a GRASS S9 stimulator. The myocyte was imaged (Fig. 1) using brightfield microscopy using a Nikon Eclipse TI microscope, equipped with a Nikon LWD 20X /0.40 1.2 Ph1 ADL WD 3.0 microscope objective. A USB3 camera (FLIR Flea3, FL3-U3-13Y3M-C, 1280 pixel × 1024 pixel) was used to capture the videos of the myocyte at 110 fps. The microscope system was estimated to provide a spatial resolution of 236 nm/pixel.

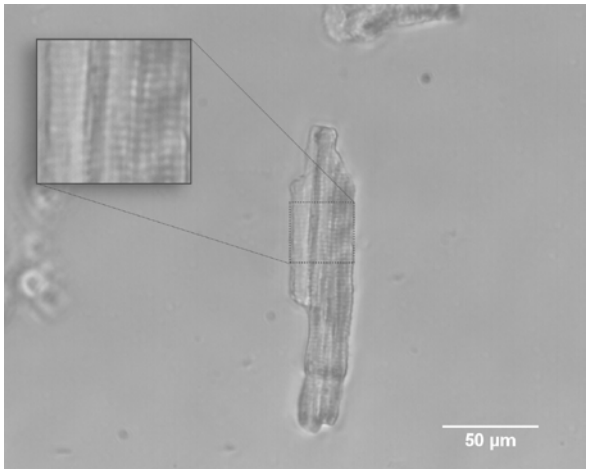

Fig. 1. An image of a myocyte taken with the system described above, showing the myocyte in a relaxed state. Inset shows sarcomere striations

C. *Displacement Calculations and Material Tracking*

A form of Digital Image Correlation (DIC) was employed to calculate the displacements of points within the myocyte. Frame to frame displacements were calculated using the subpixel image registration algorithm developed by HajiRassouliha *et al.* [8], implemented in MATLAB R2017a. In this study each frame was divided into subimages of 64 pixel × 64 pixel (approximately 15.1 µm × 15.1 µm) with an overlap of 54 pixel (12.7 µm). Control points were defined as the centre of each subimage. A region of interest (ROI) was chosen manually by outlining the myocyte to calculate the displacements for control points within the ROI.

A regular grid of points 15 pixel apart was defined for the reference image (first frame) within the ROI. Using previously calculated displacements, a scattered interpolant was used to determine the displacements of the grid of points between the first and second frame and therefore determine the new position of each point in the second frame. This was repeated for each frame to track the material points within the myocyte, with reference to the first frame.

D. *Sarcomere Length Estimation*

Sarcomere length was estimated using FFT methods implemented in LabVIEW 2016. A 64 pixel × 64 pixel Hamming window was applied to a cropped image of the myocyte and a 2D FFT was performed. The spectrum was shifted to centre the DC term and the magnitude of the FFT signal was computed (Fig. 2(a, b)). A section of the FFT containing spatial frequencies data associated with the sarcomeres was extracted (Fig. 2(a, b)). The width of this section was twice the width defined using a 1.5 µm lower and 3 µm upper bound of sarcomere length translated into frequency of 0.40 μm^{-1} and 0.33 μm^{-1} respectively. The height of the section was half of the section width.

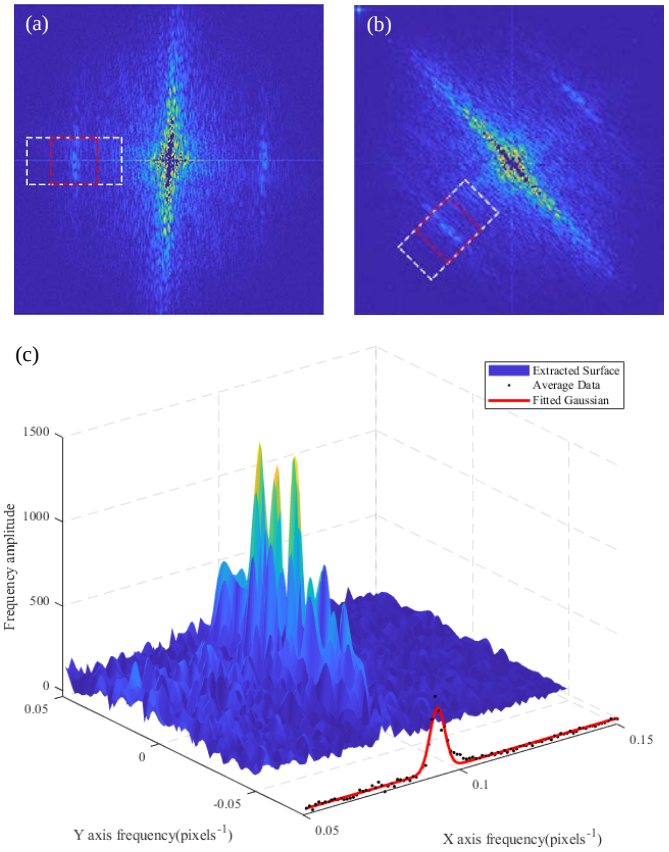

Fig. 2. 2D FFTs of myocyte image (a) When myocyte is aligned horizontally (b) When myocyte is at an angle of approximately 45°. White boxes indicate the initial area extracted to calculate exponential fit. Red box indicates area from lower to upper sarcomere length bounds. (c) Example FFT extracted from a red bounding box, showing averaged vertical bins and fitted Gaussian.

The vertical bins (Y axis) within the sarcomere length bounds were averaged (Fig. 2c). An exponential function was fit to the data corresponding to frequencies outside the range of expected sarcomere lengths. The fitted exponential was then subtracted from the line profile. A Gaussian was fit to the resulting line trace, and the location of peak of this curve was used to estimate sarcomere frequency and therefore average sarcomere length across the myocyte.

E. Angle Estimation

A rotated myocyte (with respect to the x-y axes), will result in the FFT performed on the image also being rotated. Therefore, to determine the region to extract (Fig. 2b) for estimation of sarcomere length, the angle with which the data is rotated is required.

The FFT of a myocyte image displays a strong central DC term, which is perpendicular to the long axis of the myocyte and the angle of this term can therefore be used to determine the orientation of the myocyte. A half-circle arc segment on the FFT was taken (see Fig. 3a) using the centre of the 2D FFT as the centre of the arc. The outer arc boundary was set to the upper sarcomere length bound (3 µm) and the width of the arc was 10 frequency bins. The magnitudes of the FFT were interpolated along the width of the arc and remapped into a rectangular coordinate system. The magnitudes for each angle were averaged to produce a line profile across the arc (Fig 3b). A Gaussian curve was fit to the line profile, and the location of the peak was used to estimate the angle of the myocyte.

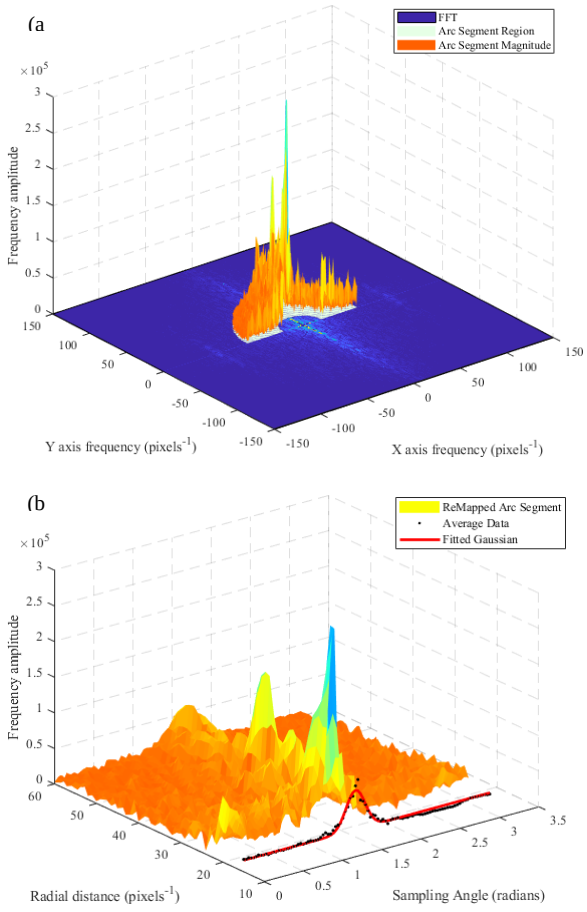

Fig. 3. (a) 2D FFT of myocyte image, with half-circle arc segment region highlighted, and arc segment FFT magnitudes plotted. (b) Arc segment region magnitudes after interpolation and re-mapping onto a rectangular coordinate system. For each angle the data is averaged and a Gaussian is fit to the data.

To validate the angle estimation, a myocyte image was synthetically rotated by known angles (1° increments for 180°, bicubic interpolation). The estimated angle was corrected by subtracting the estimated angle of the reference image (no rotation) as the myocyte in this image is not perfectly aligned with the image axes. The error in estimation was defined as the difference between the estimated angle (after correction) and the known angle rotation.

Following validation of angle estimation, contracting myocyte videos were rotated and sarcomere lengths were calculated throughout contraction for each video. Each frame of a video was rotated by the same angle, to produce videos of contracting myocytes at 0° up to 90° in increments of 5°. For each frame the angle of the myocyte was estimated. A 2D FFT was performed on a square region of the image (chosen manually to capture the entire myocyte at all angles). The section of the FFT required (Fig. 2) to calculate sarcomere length was extracted by transforming the coordinates of the rectangular section using the appropriate angle. The magnitude of the FFT at the new coordinates were interpolated and the sarcomere length was estimated as in Section II (D). This process was repeated for each frame of the contraction video, to produce sarcomere length traces. The traces were compared across different angles of rotation.

III. Results

A. Displacement Calculations and Material Tracking

Internal displacement fields of an isolated myocyte were calculated using subpixel image registration techniques. Fig. 4 shows a non-uniformly contracting myocyte with overlaid displacement vectors at several time steps during systole. In this recording, the left-most end of the myocyte does not appear to move significantly during the contraction. The average displacements in the *x* and *y* directions and sarcomere lengths were plotted over time (Fig. 5). There is a net movement of the myocyte downwards during contraction, and the rate of relaxation appears to be slower that contraction.

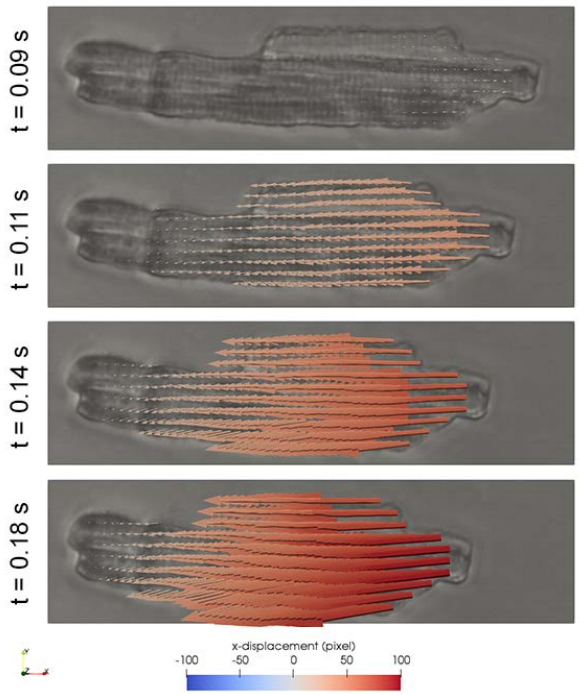

Fig. 4. Visualisation of the displacements at four time points, *x*-component is represented by the colour map.

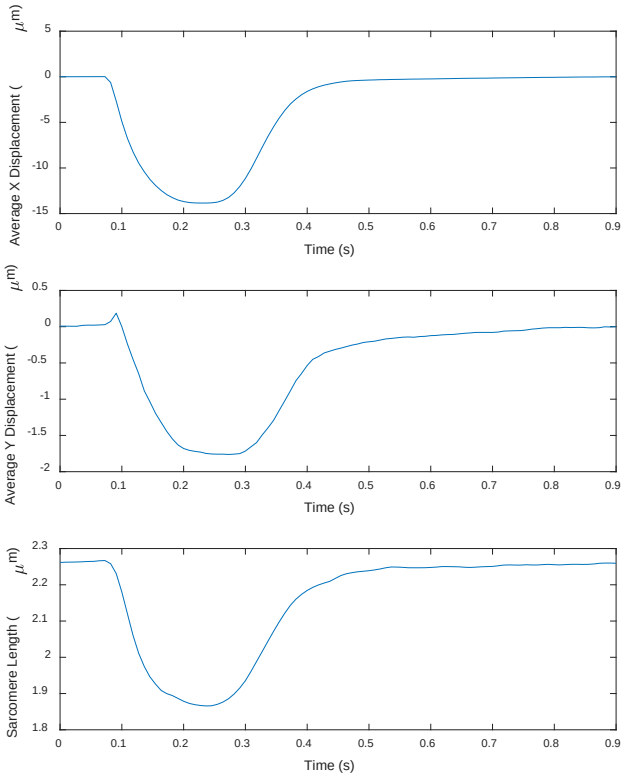

Fig. 5. Average displacements in *x* and *y* directions, and sarcomere length during contraction.

B. *Angle Estimation Validation*

The error between the known rotation angle, and the calculated angle for angle estimation validation is plotted in Fig. 6. The mean error (± standard deviation (std dev)) is -0.27° ± 0.22°, suggesting that there may be a negative bias in the error. Although there may be error associated with the measurement of the angle of the myocyte, the more important measure is the error in length estimates that would arise from a wrong estimation of angle. The percentage error in length estimates were calculated and are plotted in Fig. 7. With the maximum error in angle (-0.92°) the percentage in error that would arise in length estimations is 0.013 %. The mean error (± std dev) for length estimates is 0.002 % ± 0.002 %.

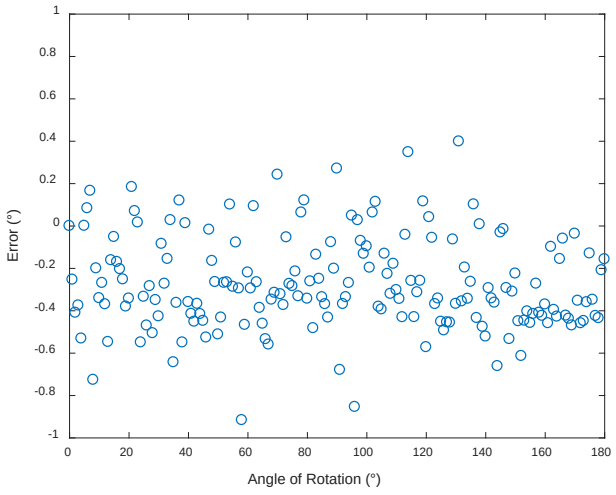

Fig. 6. Angle estimation error using method of finding the location of the peak DC term.

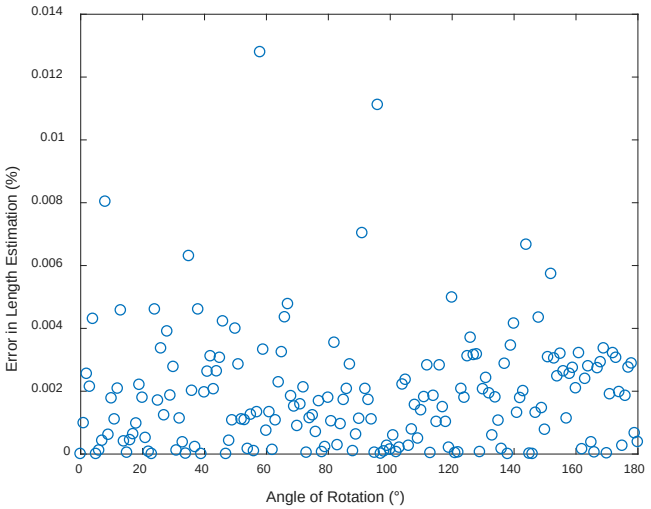

Fig. 7. Error in length estimates that would arise at each angle of rotation given the error.

C. *Sarcomere Length Estimation*

The sarcomere length throughout the myocyte contraction was calculated for each rotated video. The traces were plotted below in Fig. 8 and appear to be in close agreement with each other. The average of all traces at each time point was calculated and the difference between all traces and this average was calculated. The mean difference for each trace is displayed in Tab. 1. The mean difference between the averaged trace and traces for each angle was less than 0.004 µm.

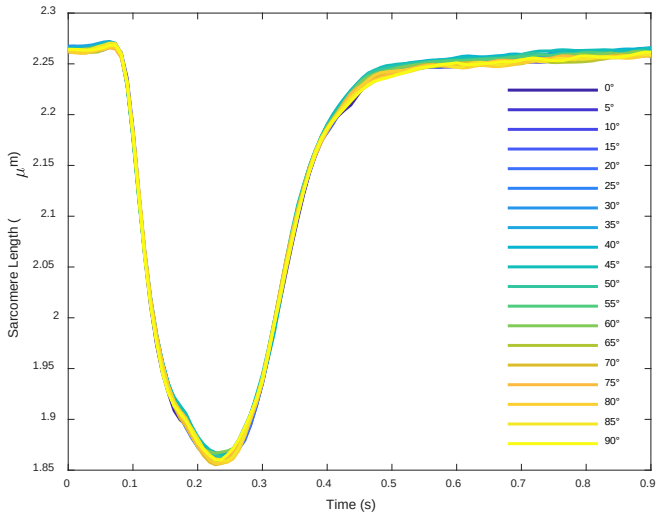

Fig. 8. Overlaid sarcomere length traces estimated for each rotated video (from rotations of 0° to 90°)

Tab. 1. Error in length estimates that would arise at each angle of rotation given the error.

Angle (°)	Mean (µm)	Std Dev (µm)
0	-0.0025	0.0040
5	-0.0020	0.0033
10	-0.0008	0.0029
15	0.0000	0.0031
20	-0.0006	0.0037
25	0.0003	0.0022
30	0.0018	0.0026

35	0.0027	0.0030
40	0.0027	0.0040
45	0.0039	0.0031
50	0.0018	0.0027
55	0.0011	0.0023
60	0.0009	0.0029
65	-0.0019	0.0031
70	-0.0011	0.0029
75	-0.0011	0.0029
80	-0.0020	0.0030
85	-0.0016	0.0035
90	-0.0015	0.0035

IV. Discussion

Displacements were successfully calculated for an unloaded isolated myocyte during contraction. The robustness, accuracy and speed of the subpixel image registration used for calculation of localised displacements within the myocyte has been covered elsewhere [8]. Unlike other methods, the technique developed by HajiRassouliha *et al*. is able to account for large displacements, while also picking up registering small localised displacements to subpixel precision, without the requirement for the addition of external markers. It therefore offers a simple way to explore the dynamics of the cell at the individual sarcomere level. There is also the possibility of implementing the subpixel image registration algorithm on a field-programmable gate array (FPGA) or graphics processing unit (GPU) to offer real time displacement field calculations.

The net movement of this myocyte in the *y* direction, although small relative to the movement in the *x* direction, indicates that contraction is not purely axial. As a result, 1D measurements such as myocyte and sarcomere length, may not be adequate to fully capture the complexity of the mechanics of cellular contraction. 2D displacement fields would allow a much better understanding of how loads on the cell would affect its behaviour as a whole, but also they may affect spatio-temporal movements of different parts of the cell. Additionally interventions that may have local effects can be measured using such methods.

In this study, a method for estimating the orientation has been developed to provide the ability to analyse myocytes that may not be aligned with the imaging system axes, something that is commonly required in commercial systems. The proposed method is able to determine the angle of the myocyte, and is accurate to within a degree on images of myocytes. The angle estimation is done automatically, and as a result, could be used in any system without requiring the myocyte to be aligned with the imaging axis. Knowledge of the orientation of the myocyte can also be used to correct displacement calculations to find shortening of the cell in its long axis to get a more accurate measure of axial contractility. The negative bias may stem from errors in calculating the angle of the first image, as this is subtracted from all other estimates to compare to the synthetic rotations.

The mean difference between the sarcomere length traces is around 0.2% (0.004 µm for a minimum sarcomere length of ~1.85 µm) when compared to the averaged trace. This indicates that the angle estimation error (which is estimated to produce at maximum an error of 0.002% in length for the same set of images) is not the only contributing factor to the differences in sarcomere length. This may have occurred due to sarcomeres not all being in the same exact orientation, or some sarcomeres being out of focus. Other possible sources of error are not having the same region of interest between the rotated videos. For each rotated video, while the entire myocyte is present within the square region (that the FFT is performed on), different areas of the background are selected due to the rotation, which would result in the FFT having slight differences. Nevertheless, the mean differences between the traces and averaged trace are very small and may be considered negligible. (Tab. 1). Although the results are promising, this method has not been validated using low resolution, low contrast or noisy images, which may cause issues with both angle estimation and measurement of sarcomere length.

As with displacement field calculations, angle estimation could also be implemented in real-time to determine myocyte orientation during experiments, and therefore actively correct sarcomere length. Being able to estimate the angle on a frame to frame basis offers the capability to make length (cell and/or sarcomere) measurements of a myocyte that may be moving in solution throughout the experiment, or is contracting in a non-uniform manner (e.g. twisting). Methods may even be extended to looking at not only displacements but sarcomere length in specific regions of the myocyte by calculating localised myocyte orientation.

V. Conclusions

Here, we have demonstrated the use of an accurate, robust and fast image registration code for tracking internal displacements of a myocyte during contraction. The displacements calculated and average displacements follow the same trend as sarcomere length measurements. This work has the potential to be implemented in real-time in order to visualise cellular mechanics on the fly during contraction. We have also successfully performed automatic estimation of myocyte angle, allowing sarcomere measurement regardless of the orientation of the myocyte. The error in length estimates caused by angle estimation error is less than 0.002 %, and the error in angle estimation is less than 1°, indicating that this method is a reliable tool for sarcomere lengths. We envision that real-time angle estimation will be used in conjunction with displacement tracking to obtain real-time and robust measures of contractility and a better understanding of cellular mechanics.

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