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Measurement of tissue movement with POC-based analysis

Analyzing mechanical traits of engineered heart tissue

Abstract

Recently, research aiming to elucidate the causes and therapies of diseases using artificial tissues derived from disease-specific induced pluripotent stem cells has been active. An important part of this approach is to quantitatively measure the functional health or performance of the artificial tissue of interest. Specifically, in the engineered heart tissue (EHT), it is very important to measure its contraction and relaxation forces. To this end, we develop a method based on the Phase-only Correlation for measuring contractile and diastolic forces by culturing cardiomyocytes on a pair of pillars with known mechanical properties and capturing video of tissue movement in response to a cyclic stimulus using a fluorescence microscope. The method tracks the tissue movement over the frames and then determines its maximum deflection and frequency by fitting the detected deflection to a sine function. We show that our method enables the measurement of the mechanical performance of the EHT.

Kinetics approach to cardiomyopathy

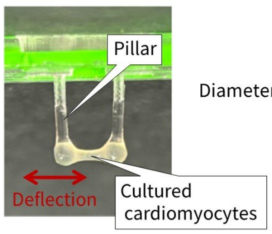
- Pumping blood is the heart's basic function.
- Mechanical properties of cardiomyocytes are important indicators of their health.

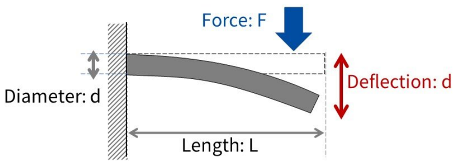
Research on cardiomyopathy models using engineered heart tissue (EHT) created from disease-specific iPSCs*

*iPSC: induced Pluripotent Stem Cell

Contraction and relaxation forces of EHT can not be measured directly.

Conventional approach: State estimation via calcium image analysis
Our method: Force estimation via cantilever beam mechanical model





Culturing cardiomyocytes on a pair of pillars and inducing tissue movement in response to a cyclic electric stimulus

Capturing video of tissue movement ⇒ Converting its deflection: d to force: F according to the following equation.

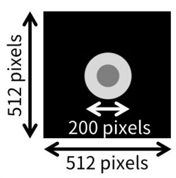
$$F = 3EId/L^3 = \{3pEd^4/64L^3\}\,d$$

L: length, d: diameter, E: Young's modulus

How to estimate tissue's deflection?

Live cardiomyocytes have few feature points for tracking.
⇒ We employed template matching for tracking issue.

Related works: HOG *1 based method (using brightness information)
Our method: POC *2 based method (using phase information)



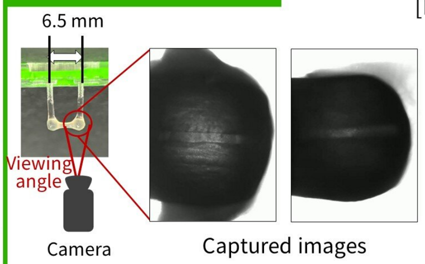
Performance assessment with artificial images

Evaluation using the averaged RSE in each frame

HOG + GF *3: 0.25 pixel
Our method : 0.01 pixel

⋆1 Histogram of Oriented Gradient
⋆2 Phase-Only Correlation
⋆3 Gaussian Fitting

Experiments



[Image capturing condition]

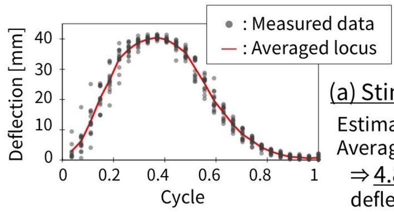
Objective lens: x4
Image size: 700 x 960 pixel
Resolution: 3.79 mm/pixel
Bit depth: 8 bit
Frame rate: 40 fps
File format: mp4
Frequency of electric stimulus: 1 Hz, 1.5 Hz
Recording time: 10 sec.

Experimental procedure and results

Step 1: An initial boundary box is specified by user and tracked among captured images to obtaining tissue displacements.

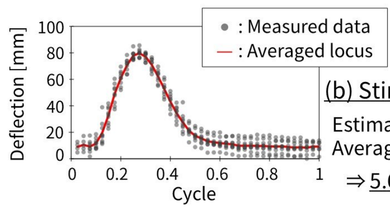
Step 2: The locus of tissue displacements is fitted to a sine curve and its frequency is estimated.

Step 3: The maximum deflection: d is determined from the average locus of data of whole cycles and converted to force: F.



(a) Stimulus frequency: 1.5 Hz

Estimated frequency: 1.48 Hz
Average standard deviation : 1.86 mm
⇒ 4.8% of the maximum deflection.



(b) Stimulus frequency: 1.0 Hz

Estimated frequency: 0.99 Hz
Average standard deviation : 4.53 mm
⇒ 5.6% of the maximum deflection.

Our study demonstrated that the mechanical performance of EHT can be measured with improved accuracy using POC-based video analysis.

This achievement has been used for functional evaluation of cardiomyocytes created from iPSCs, and advanced insights related to cardiomyopathy are beginning to be obtained[1].

References

[1] M. Hasegawa, K. Miki, T. Kawamura, I. Takei-Sasozaki, Y. Higashiyama, M.Tsuchida, K. Kashino, M. Taira, E. Ito, M. Takeda, H. Ishida, S. Higo, Y. Sakata, S. Miyagawa, "Gene correction and overexpression of TNNI3 improve impaired relaxation in engineered heart tissue model of pediatric restrictive cardiomyopathy," *Development Growth and Differentiation*, pp.1-14, 2024.

[2] K. Takita, T. Aoki, Y. Sasaki, T. Higuchi, K. Kobayashi, "High-accuracy subpixel image registration based on phase-only correlation." *IEICE Trans. of Fundamentals*, Vol. E86-A, no.8, 1925-1934, 2003.

[3] M. Tsuchida, M. Hasegawa, K. Miki, S. Miyagawa, K. Kashino, "Mechanical performance of engineered heart tissue can be measured with POC-based video analysis," in Proc. *The 46th Annual International Conference of the IEEE Engineering in Medicine and Biology Society (EMBC)*, under review.

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