Automated detection and observation of beating cardiomyocytes derived from human iPS cells

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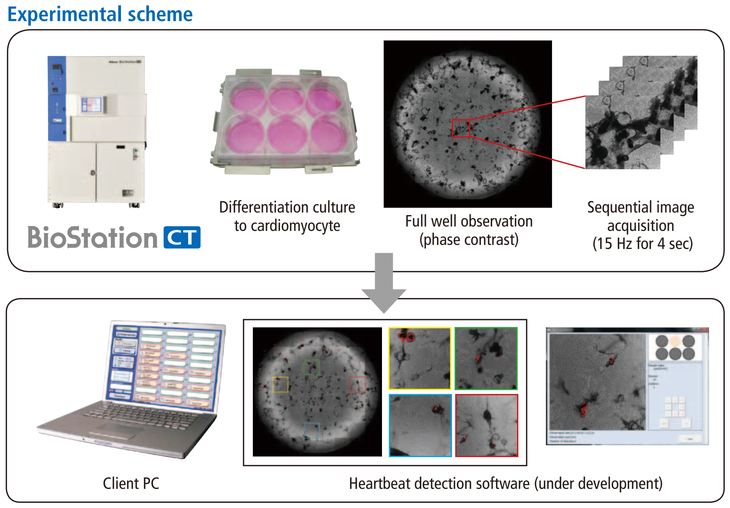
Introduction

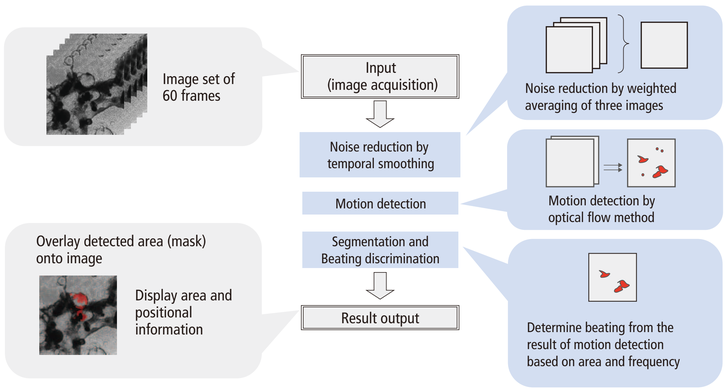
Cardiomyocytes (CMs) differentiated from hES/iPS cells are promising experimental materials for basic research of heart diseases and for drug discovery screening. For functional cardiomyocytes, one indication of differentiation is the beating of a cell or cell clump. However, conventional beat detection methods require the operator to manually scan the culture vessel under a microscope while visually inspecting for beating cardiomyocytes, which is both laborious and inconsistent. In many cases, the cells must also be taken out of the culturing environment for observation/inspection, potentially increasing the risks of contamination and temporal weakening of beating. Such issues cannot be overlooked in the mass production of cardiomyocytes and in applications that require consistent quality and quantitative evaluations. For this reason, we developed an algorithm for automatically detecting beating cardiomyocytes under a culturing environment.
Human iPS cells were induced to differentiate into cardiomyocytes while cultured in flat-bottom plates, and observed inside Nikon's BioStation CT. For beat detection, phase-contrast images of the entire culture vessel were captured at 15 fps for a period of 4 seconds. We developed the algorithm to detect the beating of the differentiated cardiomyocytes based on the optical flow method.
The developed algorithm detected more than 80 % of beating colonies. On the other hand, the degree of detection by skilled operators greatly varied among individuals, ranging from 55 ~ 91%, indicating the difficulty of quality control by manual detection. The threshold of the algorithm for beat detection can be adjusted, which allows the detection criteria to be set according to the purpose.
The algorithm significantly reduced the effort and time to find beating cardiomyocytes used by the operators while eliminating inconsistency due to human operations, thereby contributing to quality management. It is expected that non-invasive automated detection using the algorithm will become an essential tool in drug discovery and regenerative medicine involving human iPS cells.

Methods

Experimental scheme



Detection of beating cardiomyocytes



Cell culture

■ Human iPS cells-derived cardiomyocytes were plated on 6-well plates and cultured in the BioStation CT (NIKON COPORATION).

Time-lapse image acquisition and analysis

■ Phase contrast images (1000x1000 pixels, 4x4 mm) were observed at 2x magnification using BioStation CT (NIKON CORPORATION) under an incubation environment (37ºC, 5% CO_2). At each x-y position, sequential images were captured at 14.7 fps for 4 sec. Images were recorded at 100 x-y positions so that the entire well was covered.

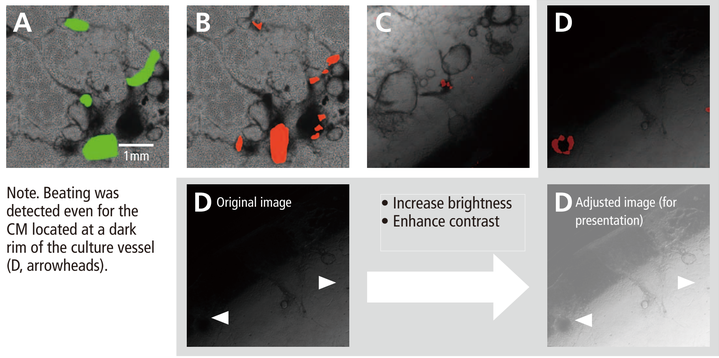
■ Captured images were automatically transferred from the BioStation CT to its client PC. Image analysis was performed by the software (NIKON CORPORATION, under development).

Results

Representative images showing detection outcome

True positive

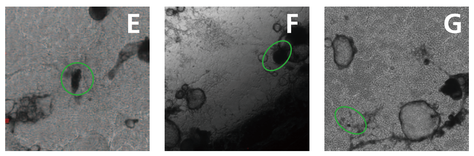
A-D. Red masks indicate the beating area detected by the developed algorithm.



Note. Beating was detected even for the CM located at a dark rim of the culture vessel (D, arrowheads).

False negative

E-G. Green circles indicate examples of beating CMs the algorithm failed to detect.



Note. The algorithm missed out on detecting beating CMs, possibly due to the reasons such as the following.
(E) the motion is imperceptibly small.
(F, G) the colony images are obscure.

False positive

H-J. Blue circles indicate examples of false-positive detection.

Note. Some area was detected (blue circles) although these were not true beating CMs.
H: Image noise.
I, J: Weltering colonies.

31 out of 35 beating CMs were detected (89% True-Positive).

Performance evaluation

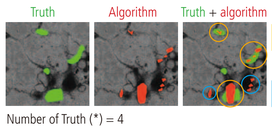
Evaluation indicator

Definitions

$$\text{Detection rate [\%]} = \frac{\text{Number of beating CMs detected by the algorithm}}{\text{Number of Truth (*)}} \times 100$$

$$\text{False detection rate [\%]} = \frac{\text{Number of false-detected pixels}}{\text{Total number of detected pixels}} \times 100$$

Example



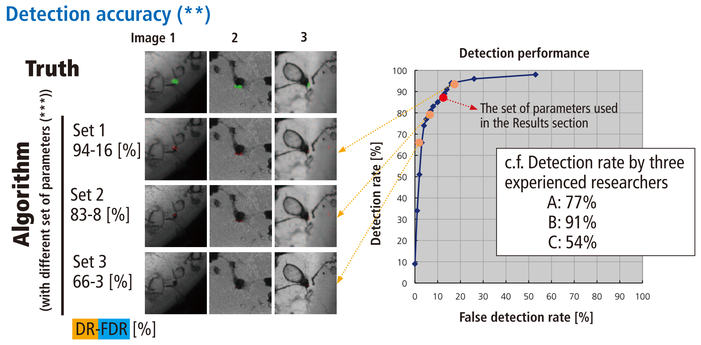
Number of Truth (*) = 4

$$\text{Detection rate} = \frac{3}{4} = 75\ [\%]$$

$$\text{False detection rate} = \frac{2{,}880\ [\text{pixels}]}{16{,}000\ [\text{pixels}]} = 18\ [\%]$$

(*) Number of Truth:
Number of Truth was defined as the total number of beating CMs found in the entire data set used for evaluation experiments. Truth was determined based on the results by three experienced researchers as well as the results obtained from the software detection.

Detection accuracy (**)



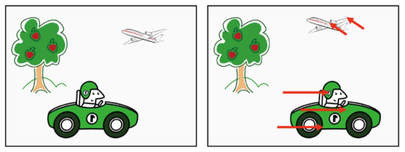
(**) Experimental conditions:
Examined beating CM colonies: 35
Total images: 65 view fields (1000x1000 pixels, 4x4 mm/field)

(***) Parameters varied (number of conditions):
- Window size for spatial smoothing (5)
- Threshold for motion intensity (8)
- Threshold for detection frequency (4)
- Threshold for detected area (8)

Appendices

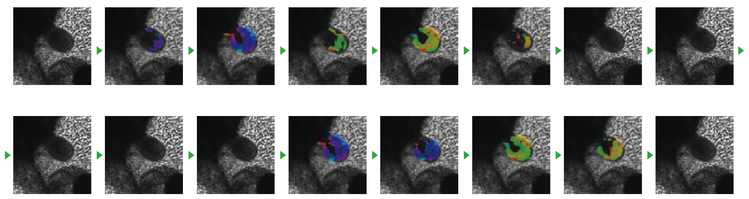
Optical flow

Optical flow is the pattern of motion vectors that are calculated from two consecutive image frames. As typical techniques, matching method and gradient method [1, 2] are well known.



Conceptual diagram showing optical flow. Red arrows indicate motion vectors.

Direction of motion can be expressed in terms of colors, as indicated in the right circle. For example, red represents that the object has moved to the right direction.



Consecutive optical flow.

Farneback method [3]

Farneback method is one of the algorithms for two-frame motion estimation. With the method of Farneback, the first step is to approximate the surface brightness value of the neighborhood using a quadratic polynomial (eq. 1). Then estimate displacement fields by comparing the polynomial expansion coefficients between two-frame (eq. 2). By assuming that the displacement field is only slowly varying, we can integrate information over a neighborhood of each pixel and increase the robustness of the algorithm. d(x) minimizing equation 3 is the estimated motion.

$$f(\mathbf{x}) = \mathbf{x}^T A\,\mathbf{x} + b^T\mathbf{x} + c \qquad \cdots (1)$$

$$\frac{A_1(\mathbf{x}) + A_2(\mathbf{x})}{2}\,\mathbf{d}(\mathbf{x}) = -\frac{1}{2}\left(\mathbf{b}_2(\mathbf{x}) - \mathbf{b}_1(\mathbf{x})\right) \qquad \cdots (2)$$

$$\sum_{\Delta x \in I} w(\Delta\mathbf{x}) \left\| A(\mathbf{x}+\Delta\mathbf{x})\mathbf{d}(\mathbf{x}) - \Delta\mathbf{b}(\mathbf{x}+\Delta\mathbf{x}) \right\|^2 \qquad \cdots (3)$$

Reference

[1] B. D. Lucas and T. Kanade, An iterative image registration technique with an application to stereo vision. Proceedings of Imaging Understanding Workshop, pages 121-130, 1981
[2] B.K.P. Horn and B.G. Schunck, "Determining optical flow." Artificial Intelligence, vol. 17, pp 185-203, 1981.
[3] Farneback G. Two-frame motion estimation based on polynomial expansion. SCIA '03 Proceedings of the 13th Scandinavian conference on image analysis, vol. 2749, Springer 2003, pp. 363-370.

Acknowledgement

We thank Drs. Akina Hagino, and Makoto Honda for CM detection and helpful discussion.

Conclusion and discussion

■ A new algorithm was developed to detect beating of CM colonies derived from human iPS cells under culture.
- Detection rate was 94% at best parameter set.
- Detection by experienced researchers varied greatly (54 ~ 91%).
- Different sets of parameters are applicable depending on purposes

■ The algorithm used phase-contrast images captured in an incubation environment for beating CM detection.
- Non-invasive
- Beating detection at physiological condition

■ The algorithm was integrated into the culture and imaging system, BioStation CT.
- Automation through the entire detection process: incubation, observation, image acquisition, data transfer, beating detection, and result output
- Reducing human effort
- High reproducibility