Supplementary Information (SI) for Lab on a Chip. This journal is © The Royal Society of Chemistry 2024

Supplementary information for

Compact lens-free imager using thin-film transistor for long-term quantitative monitoring of stem cell culture and cardiomyocyte production

Taishi Kakizuka^{1, 2}, Tohru Natsume³ and Takeharu Nagai^{1, 2, *}

¹ SANKEN, The University of Osaka, Mihogaoka 8-1, Ibaraki, Osaka 567-0047, Japan

² Transdimensional Life Imaging Division, Institute for Open and Transdisciplinary Research Initiatives, The University

of Osaka, Yamadaoka 2-1, Suita, Osaka 565-0871, Japan

³ Cellular and Molecular Biotechnology Research Institute, National Institute of Advanced Industrial Science and

Technology, 2-3-26 Aoumi, Koto-ku, Tokyo 135-0064, Japan

* Corresponding author: ng1@sanken.osaka-u.ac.jp

This PDF file includes:

Fig. S1 to Fig. S8 Supplementary movie legends S1 to S3

Other supplementary materials for this manuscript include:

Movies S1 to S3

Supplementary figures:



Fig. S1 Structure of the TFT image sensor pixel



Fig. S2 Cross-sectional view of the sensor module and the base module



Fig. S3 Real-time monitoring from a control PC outside of the incubator



 $65 \ \mu m \ grids$

Fig. S4 Bright field images of hiPSCs forming dense colonies: The same region was imaged every 24 hours on a conventional inverted microscope. 65 μ m grids were marked for comparison to the pixel pitch of INSPCTOR.



Fig. S5 Evaluation of the cell detection accuracy of INSPCTOR at the pixel level (A) A fluorescence image of nucleus-stained hiPSCs observed at a subcellular resolution. (B) Detected colonies in the image were shown as yellow outlines. (C) Enlarged image of the white dot square region in B. (D) An INSPCTOR image of the same region as A. (E) Pixels above a threshold in D were shown in red. Colony detection results based on the fluorescence image from B were overlayed as yellow outlines. (F) Enlarged image of the white dot square region in E, which is the same region as C. Cyan arrowheads in B and E are the same false positive regions caused by background noise. Cyan arrowheads in C and F are the same false positive regions placed outside of the contour

regions of large colonies. White arrowheads in C and F are the same false negative regions due to the small size of the colony. (G) Quantification results of the fluorescence and INSPCTOR intensity from the same regions corresponding to each 9,108 INSPCTOR pixel. The accuracy of INSPCTOR when assuming that judging by fluorescence intensity is true was calculated. (H) The quantitative relationship between the colony size and intensity in INSPCTOR image. (I) Relationship between the colony size and detection efficiency by INSPCTOR. Scale bars: 650 µm in A, B, D, and E, 260 µm in C and F.



Fig. S6 Examples of zoom-in images of the first derivative calculation: (A) A typical example from the control sample. (B) A typical example from the CHIR-treated sample. Scale bars: 500 µm.



Fig. S7 Examples of integer multiple frequencies and their shape in temporal intensity fluctuation: (A, B) Single frequency (A) results in a sine curve (B). (C, D) Adding one double-frequency component (C) results in a thinner peak width (D). (E, F) A case with four integer multiple frequency components (E) results in a further thinner peak width (F).



Fig. S8 Confluencies of cardiomyocyte culture after reseeding on day 11: Typical images from day 12 and day 16 were shown. Scale bars: 200 μm.

Supplementary movie legends:

Movie S1. Repeated contraction of iPSC-derived cardiomyocytes on day 11: A representative movie of iPSC-derived cardiomyocytes differentiated on the control well of the INSPCTOR taken on day 11 of differentiation by a conventional inverted microscope with 10 fps. Scale bar: 500 µm.

Movie S2. Reentry-type contraction conduction of iPSC-derived cardiomyocytes on day 11: A representative movie of iPSC-derived cardiomyocytes showing a reentry-type conduction pattern. The cardiomyocytes were differentiated on the control well of the INSPCTOR. This movie was taken on day 11 of differentiation by a conventional inverted microscope with 10 fps. Scale bar: 500 µm.

Movie S3. Synchronised contraction of matured iPSC-derived cardiomyocytes: A representative movie of iPSC-derived cardiomyocytes reseeded on the control well of the INSPCTOR. This movie was taken on day 30 of differentiation by a conventional inverted microscope with 10 fps. Scale bar: 200 µm.