SUPPLEMENTAL MATERIAL

Versatile open software to quantify cardiomyocyte and cardiac muscle contraction in vitro and in vivo
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Supplementary Methods

Model Cell
To establish and test the algorithm of MUSCLEMOTION we first created an in silico CM-like shape (Fig. 1d,f,g) using Blender v2.77 (Stichting Blender Foundation). This three-dimensional squared sphere had an aspect ratio of 1:1:0.26 (XYZ) when contracted and 2:1:0.26 (XYZ) when relaxed. A diffuse “shader” with a foggy, patterned texture was then applied to the object to simulate extremely repetitive phase contrast image features. Temporal input parameters were then imposed: 100 ms for time-to-peak (t₁) and 350 ms for relaxation time (t₂). A beating frequency of 1 Hz and sampling rate of 100 frames per second were simulated. Modifications of cell shortening (25% and 50% of baseline) and time-to-peak (50% and 200% of baseline) were generated to validate the algorithm linearity.

Patch Clamp Recordings on hPSC-CMs
Electrophysiological recordings of isolated hPSC-CMs were performed using the perforated patch clamp technique as previously described1. Briefly, cells were paced at 1 Hz through the glass capillary and were perfused with warm (37 °C) Tyrode’s solution containing (mM): 154 NaCl, 5.4 KCl, 1.8 CaCl₂, 1 MgCl₂, 5 HEPES-NaOH, 5.5 D-Glucose; pH was adjusted to 7.35 with NaOH. Pipette solution contained (mM): 125 K-Gluconate, 20 KCl, 10 NaCl, 10 HEPES; pH was adjusted to 7.2 with KOH. Amphotericin-B was dissolved in DMSO right before each experiment and added to the pipette solution to reach a final concentration of 0.22 mM.

Movement of embedded beads
Fabrication of gelatin-patterned polyacrylamide gels containing fluorescent beads and contraction force measurements were performed as described previously2, with an increased recording frame rate to 56 frames per second. For nifedipine experiments, cells were paced through an external stimulator to ensure a constant and triggered beating rate. Experiments with isoprenaline were performed in spontaneously beating aligned hPSC-CMs to investigated the pharmacological effect on beat rate. hPSC-CMs on patterned polyacrylamide gels were measured in Pluricyte® medium (Pluriomics b.v.). Drug treatment was performed with additive doses which were compared to baseline.

Monolayers of hPSC-CMs
Pluricytes® (Pluriomics b.v.) were cultured following the manufacturer’s instructions. Cells were plated at a density of 25k-40k cells/coverslip on Matrigel-coated glass Ø10 mm coverslips for 7 days before measurements of contraction. Monolayers were paced by an external field stimulator (bipolar square pulse of ~15V for 5 ms) and superfused with a warm (37 °C) Tyrode’s solution (described above). Drug treatment was performed with additive doses which were compared to baseline.

Cardiac Organoids
Cardiac organoids composed of hPSC-CMs and hPSC-derived endothelial cells were generated as previously described3. Cardiac organoids were paced through an external field stimulator (bipolar square pulse of ~15V for 5 ms) and superfused with a warm (37 °C) Tyrode’s solution (described above). Drug treatment was performed with additive doses which were normalized to baseline.

Adult cardiomyocytes
CMs were isolated from New Zealand White male rabbits as previously described4. Animal experiments complied with the ARRIVE guideline for animal studies. The procedures used complied with current regulations and were approved by the Research Committee of the University of Glasgow. In total, 3 animals were used for these experiments. Sarcomere shortening was estimated using an ImageJ macro that calculates, for each image in a stack, the spatial frequency of the pixel intensity
profile along a fixed, elongated rectangular region of interest placed inside the cell boundary and on a part of the cell showing clear banding. At each time point, mean sarcomere length corresponded to the location of the peak frequency value of the power spectrum obtained by taking the Fourier Transform of the intensity profile. Drug treatment was performed with single dose additions which were normalized to their pre-treatment baseline.

Membrane labelling
hPSC-CMs were plated on Matrigel-coated glass-bottom 24-well plates in either BPEL or Differentiation Medium C (Pluriomics b.v.) at a density between 20k-70k cells per well. Live cells were labelled with Cell Mask Deep Red (Thermo Fisher Scientific Inc.) and imaged with a Nikon Ti-U Eclipse with a CamRecord CR600x2 monochromatic camera (Optronis GmbH) at 333 Hz after amplification of the image with an intensifier tube (Photonis b.v.).

Engineered Heart Tissues
EHTs were generated and analyzed as previously described. In brief, hiPSC were differentiated into CMs with a growth factor based protocol in suspension culture using spinner flasks. Following dissociation with collagenase, fibrin-based EHTs were generated with 1x10^6 cells per 100 μL EHT construct. Contraction analysis based on a video-optical figure recognition algorithm (100 frames per second) was performed under electrical stimulation in serum-free Tyrode’s solution in a humidified gas (40% O2, 7% CO2) and temperature (37 °C) controlled incubation chamber. Drug treatment was performed with additive doses which were compared to baseline.

Zebrafish hearts
All experiments were conducted in accordance to the ARRIVE guidelines and approved by the local ethics committee of the Koninklijke Nederlandse Akademie van Wetenschappen (KNAW). Wild-type, sibling and gnb5a/gnb5b double mutant embryos (5 days post fertilization) were embedded in 0.3% agarose (Ultra Pure agarose, Invitrogen) prepared in E3 medium containing 16 mg/ml tricaine and mounted on glass bottom dishes. Recordings were performed at 28 °C using an inverted light microscope with climate chamber. Recordings were performed at 150 frames per second for 10-30 seconds using a C9300-221 high-speed CCD camera (Hamamatsu Photonics K.K). Basal heart rates were recorded first. Subsequently 400 μM carbachol (CCh; Sigma-Aldrich) was added and incubated for 30 min before heart rates were measured a second time.

Echocardiograms
Anonymized ultrasounds of 5 adult patients who had undergone transthoracic echocardiography between 2012 and 2017 on clinical indication, were selected from the echocardiography database of the Leiden University Medical Center. Transthoracic echocardiography was performed using a GE Vivid7 or E9 (GE-Vingmed, Horten, Norway) ultrasound machine with standard views from the parasternal, subcostal, suprasternal and apical window. For MUSCLEMOTION analysis, 4 chamber views were used. The images were converted to avi-format, preferably containing multiple beats. For this analysis of clinically acquired data, the Institutional Review Board waived the need for patient written informed consent.

Supplementary References


3. Giacomelli E, Bellin M, Sala L, van Meer BJ, Tertoolen LGJ, Orlova VV, Mummery CL. Three-
dimensional cardiac microtissues composed of cardiomyocytes and endothelial cells co-differentiated from human pluripotent stem cells. *Development.* 2017;144:dev.143438–1017.


Online Figures

Online Figure I

Linearity of the contraction tool.

a) Profiles of displacement (top) and velocity (bottom) when the block is moving along the x-axis;
b) Profiles of displacement (top) and velocity (bottom) when the block is moving along the y-axis;
c) Profiles of displacement (top) and velocity (bottom) when the block is moving along both axes. In all figures, the linearity between displacement and velocity is clearly preserved.
Online Figure II
Out-of-bounds limitation of the contraction tool.
Demonstration of the out-of-bounds limitation occurring when the differences of moving areas in $img_i$, an $img_{i-1}$ relative to $img_{ref}$ reached the maximum level. This is clearly visible in the clipping of the contraction plot. However, the velocity plot is still valid, since it calculates the difference between $img_i$ and $img_{i-1}$. 
Online Figure III
Automatic region of interest selection using maximum projection.
First the difference from $\text{img}_i$ to $\text{img}_\text{ref}$ is calculated. Next, the projection of the maximum intensity of the resulting image stack is generated. This projection is subjected to a threshold (standard set to the mean intensity of the projection image plus one standard deviation) and made binary. Next, this mask is used to analyze only the moving (contracting) areas.
Online Figure IV
Automatic reference frame detection.

a) Example of measured velocity profile.
b) Velocity$_n$ values are plotted against velocity$_{n+1}$ values. In order to select the point that has both low velocity and neighbour with similar values (two characteristics of a flat diastolic period, where the derivative is ideally zero) a number of points (in this example 50, blue) are selected that have the smallest distance to the origin.
c) Magnification of a region in b). For each of the selected points, a number of points (in this example 20, red) that are closest to the unity line (dotted red) is selected. Finally, of those points, one point (green) is selected as the smallest combination of values and distance from unity line.
Online Figure V
Examples of linearity checks for various cardiac models.
The measured speed by frame-to-frame analysis (black) and calculated speed from the frame to reference frame analysis (red) of a) isolated adult rabbit cardiomyocyte contraction, b) fluorescent bead displacement by hPSC-CM on gelatin patterned polyacrylamide, c) engineered heart tissue contraction, d) contraction of fluorescently labelled monolayer, e) hPSC-CM monolayer contraction, f) hPSC-CM organoid contraction, g) single hPSC-CM contraction during patch clamp, h) zebrafish heart in vivo.
Online Figure VI
Results from EHT and Adult gold standards
a) Representative average contraction profile obtained by pole deflection analysis of a single EHT during the recording period. b) Average dose-response curves (black traces) and single measurements for several parameters obtained in EHTs treated with isoprenaline (top, red) and nifedipine (bottom, green) by pole deflection analysis. c) Representative contraction profile obtained by sarcomere fractional shortening analysis of an adult rabbit CM. d) Average dose-response curves (black traces) and single measurements for sarcomere fractional shortening obtained in adult CMs treated with isoprenaline (top, red) and nifedipine (bottom, green).
P-values baseline versus dose. Panel b i) 1 nM: 1; 3 nM: 1; 10 nM: 1; 30 nM: 1; 100 nM: 1. Panel b ii) 1 nM: 0.01517; 3 nM: 0.00016; 10 nM: 2.8·10⁻⁵; 30 nM: 8.8·10⁻⁶; 100 nM: 2.9·10⁻⁶. (N=6; 6; 6; 6; 6). Panel b iii) 3 nM: 1; 10 nM: 1; 30 nM: 1; 100 nM: 3.8·10⁻⁷. Panel b iv) 3 nM: 0.2095; 10 nM: 0.00002; 30 nM: 5.2·10⁻¹⁰; 100 nM: 1.2·10⁻¹⁴. (N=6; 6; 6; 6; 6). Panel d i) 1 nM: 1; 3 nM: 0.018. Panel d ii) 10 nM: 1; 30 nM: 1; 100 nM: 0.00013; 300 nM: 0.00676.
Online Figure VII

MUSCLEMOTION behaviour with different camera exposures.

The output parameters of MUSCLEMOTION are shown for the same monolayer sample recordings with different exposure times. The contraction amplitude output increases linearly with increased exposure time while all temporal output parameters are independent of the lighting conditions within a reasonable range (i.e. the image should not be dark and it should not be saturated completely).
Online Figure VIII
Negative controls of MUSCLEMOTION.

The response of MUSCLEMOTION to a) a video of a single cell hPSC-CM that does not contract; b) a video of a contracting single cell hPSC-CM with baseline (i.e. diastolic period without contraction) indicated; c) a movie with extremely low exposure (i.e. black frames) and d) extremely high exposure (i.e. white frames).
Online Movie Legends

Online Movie I: test bench of block displacements.
Online Movie II: single human stem cell derived cardiomyocyte during a patch clamp experiment.
Online Movie III: human stem cell derived cardiomyocyte monolayer culture.
Online Movie IV: human stem cell derived cardiac organoid.
Online Movie V: Engineered heart tissue from human stem cell derived cardiomyocytes.
Online Movie VI: isolated adult rabbit cardiomyocyte.
Online Movie VII: Fluorescent bead displacement by a single hPSC-CM aligned on a gelatin patterned polyacrylamide substrate.
Online Movie VIII: hPSC-CMs monolayer culture plated on a MultiElectrode Array.
Online Movie IX: Fluorescently labelled hPSC-CMs monolayer.
Online Movie X: Optical recording of a zebrafish heart (wildtype) in vivo.
Online Movie XI: Human echocardiogram from healthy individual.
### Online Table I
Overview of hPSC lines used in this study.

<table>
<thead>
<tr>
<th>Cell culture configuration</th>
<th>hPSC cell line used</th>
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</thead>
<tbody>
<tr>
<td>Single cells with patch clamp</td>
<td>LUMC0059iCTRL Clone 01</td>
</tr>
<tr>
<td>Single cells aligned</td>
<td>NIH CRM-5 (NCRM-5)</td>
</tr>
<tr>
<td>Monolayers brightfield</td>
<td>WT2&lt;sup&gt;a&lt;/sup&gt;, Pluricytes&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Monolayers membrane labelling</td>
<td>NIH CRM-1 (NCRM-1)</td>
</tr>
<tr>
<td>Monolayers on MEA</td>
<td>WT2&lt;sup&gt;a&lt;/sup&gt; and LQT1&lt;sup&gt;R594Qa&lt;/sup&gt;</td>
</tr>
<tr>
<td>Organoids</td>
<td>NKX2-5&lt;sup&gt;cGFP/w&lt;/sup&gt;</td>
</tr>
<tr>
<td>EHTs</td>
<td>C25&lt;sup&gt;c&lt;/sup&gt; and MYH7&lt;sup&gt;R453C&lt;/sup&gt; isogenic triplet</td>
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<sup>a</sup> Zhang et al., 2014.  
<sup>b</sup> from Elliott et al., 2011.  
<sup>c</sup> Mannhardt et al., 2016.