A Novel Wireless ECG System for Prolonged Monitoring of Multiple Zebrafish for Heart Disease and Drug Screening Studies

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Equal Contribution

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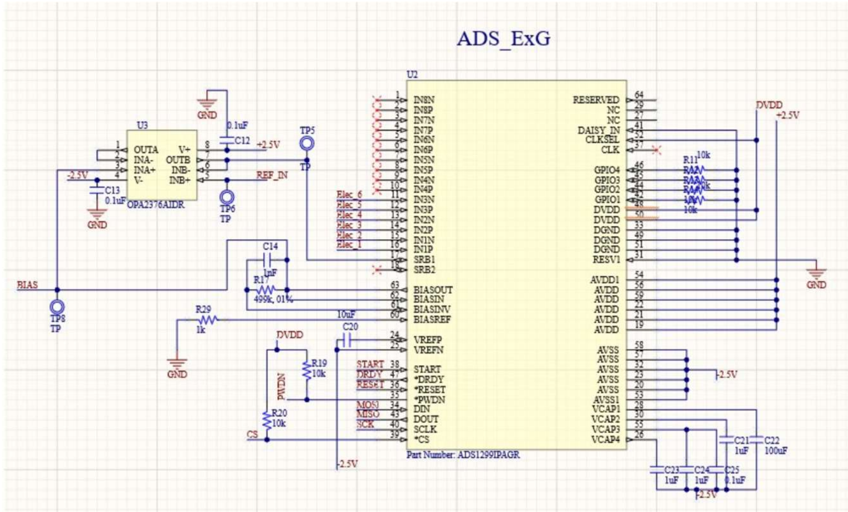
Supplementary document

Supplementary Section 1. Drug Administration

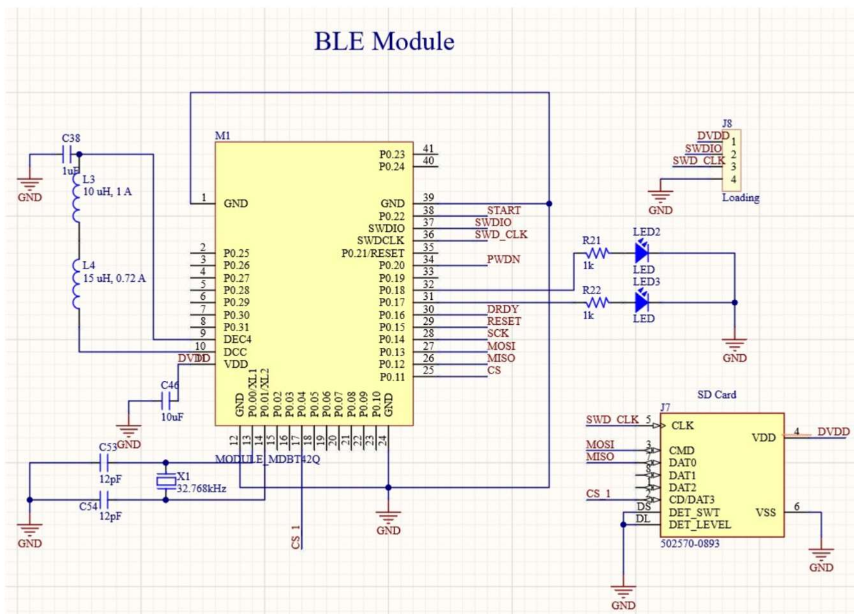
To anesthetize fish, a buffered solution of 200 parts-per-million (ppm) Tricaine (Sigma, USA) was used (Le et al. 2019). Tricaine was dissolved in distilled water to a final concentration of 7,000 parts-per-million (ppm) as a stock, and the pH value was adjusted to 7.2 with sodium hydroxide (Sigma).

Amiodarone (Sigma) was dissolved in water at 65ºC for 2 hours and stocked as 900 μM at 4ºC. Before use, the solution was re-dissolved at 65ºC for 1 hour (Chen et al. 2012). The fish were immersed in a tank with 100 μM amiodarone for 1 hour and then returned to fish water for 15 min before conducting experiment.

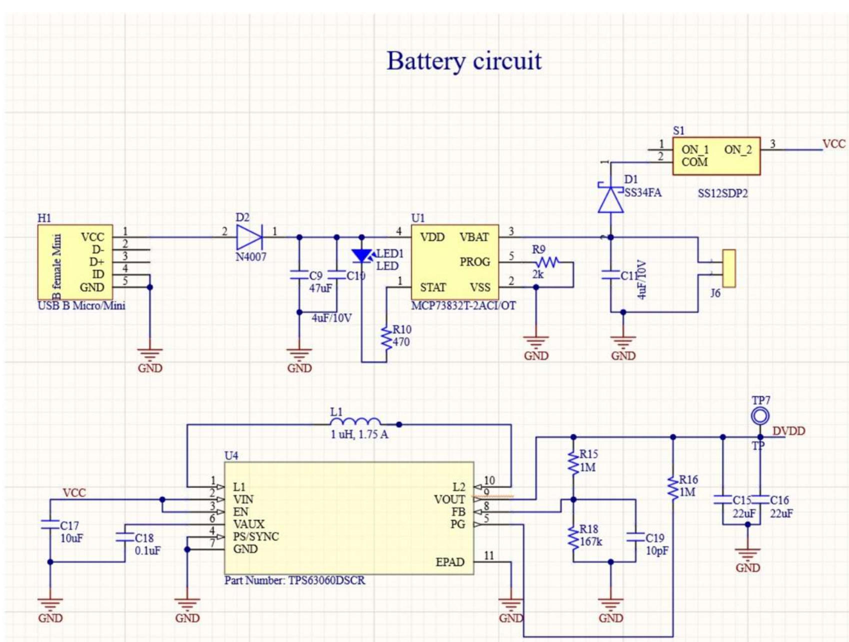
(a)


ADS_ExG
U2
U3
OPA2376AIDR
OUTA
INA-
INA+
V-
V+
OUTB
INB-
INB+
0.1uF
C12
+2.5V
-2.5V
C13
0.1uF
GND
TP5
TP6
TP
REF_IN
BIAS
TP8
C14
1nF
R17
499k, 01%
R29
1k
10uF
C20
DVDD
R19
10k
R20
10k
PWDN
CS
START
DRDY
RESET
MOSI
MISO
SCK
Elec_6
Elec_5
Elec_4
Elec_3
Elec_2
Elec_1
IN8N
IN8P
IN7N
IN7P
IN6N
IN6P
IN5N
IN5P
IN4N
IN4P
IN3N
IN3P
IN2N
IN2P
IN1N
IN1P
SRB1
SRB2
BIASOUT
BIASIN
BIASINV
BIASREF
VREFP
VREFN
*DRDY
*RESET
*PWDN
DIN
DOUT
SCLK
*CS
RESERVED
NC
DAISY_IN
CLKSEL
CLK
GPIO4
GPIO3
GPIO2
GPIO1
DVDD
DGND
RESV1
AVDD1
AVDD
AVSS
AVSS1
VCAP1
VCAP2
VCAP3
VCAP4
R11
10k
C21
1uF
C22
100uF
C23
C24
C25
1uF
+2.5V
Part Number: ADS1299IPAGR

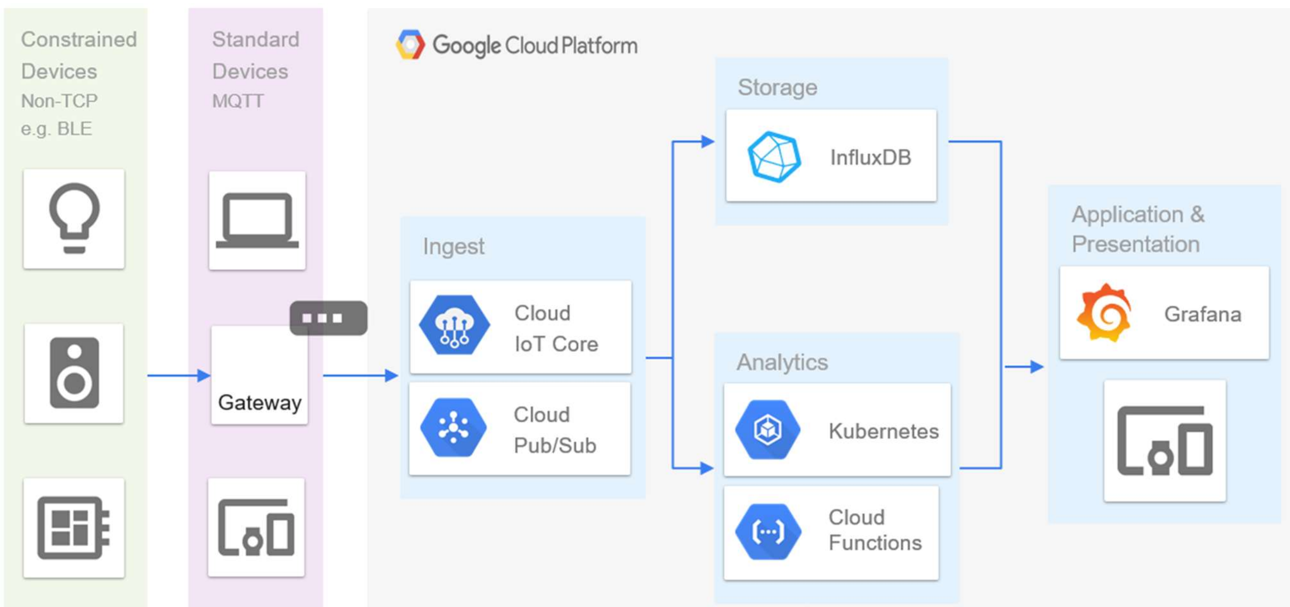
(b)


BLE Module
M1
C38
1uF
GND
L3
10 uH, 1 A
L4
15 uH, 0.72 A
P0.25
P0.26
P0.27
P0.28
P0.29
P0.30
P0.31
DEC4
DCC
VDD
DVDD
C46
10uF
C53
12pF
C54
12pF
X1
32.768kHz
MODULE_MDBT42Q
CS_1
P0.23
P0.24
P0.22
SWDIO
SWDCLK
P0.21/RESET
P0.20
P0.19
P0.18
P0.17
P0.16
P0.15
P0.14
P0.13
P0.12
P0.11
START
SWD_CLK
PWDN
DRDY
RESET
SCK
MOSI
MISO
CS
R21
1k
R22
1k
LED2
LED3
LED
J8
DVDD
SWDIO
SWD_CLK
Loading
SD Card
J7
CLK
CMD
DAT0
DAT1
DAT2
CD/DAT3
DET_SWT
DET_LEVEL
VDD
VSS
502570-0893

(c)

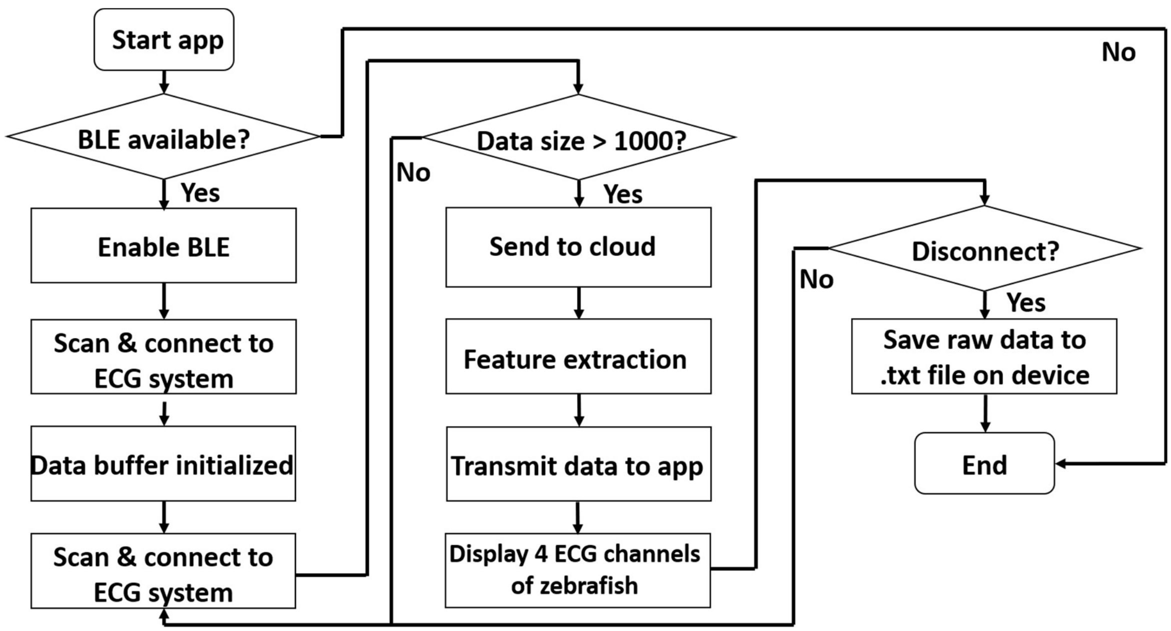

Battery circuit
S1
ON_1
ON_2
COM
VCC
SS12SDP2
D1
SS34FA
H1
VCC
D-
D+
ID
GND
USB B Micro/Mini
D2
N4007
C9
47uF
C10
4uF/10V
LED1
LED
U1
VDD
VBAT
PROG
STAT
VSS
MCP73832T-2ACI/OT
R9
2k
R10
470
C11
4uF/10V
J6
GND
L1
1 uH, 1.75 A
U4
L1
VIN
EN
VAUX
PS/SYNC
GND
L2
VOUT
FB
PG
EPAD
Part Number: TPS63060DSCR
VCC
C17
10uF
C18
0.1uF
R15
1M
R16
1M
R18
167k
C19
10pF
C15
22uF
C16
22uF
TP7
TP
DVDD

Supplementary figure 1. Schematic of the ECG acquisition circuit. (**a**) The analog front-end circuit, including ADS chip with 4 differential channels, an OP-AMP utilized to reduce common mode noise. (**b**) wireless transmission circuit, including a system-on-chip nRF52832. (**c**) The power-supply module included a charge management controller (MCP73832, *Microchip Inc*), a buck boost converter TPS63060 (*Texas Instrumentation*) to maintain a 3.5 V output for the digital system regardless of the fluctuation of battery voltage level and two low-dropout regulators (*i.e.*, TPS72325 and TPS73225) provide stable ±2.5 V, respectively.

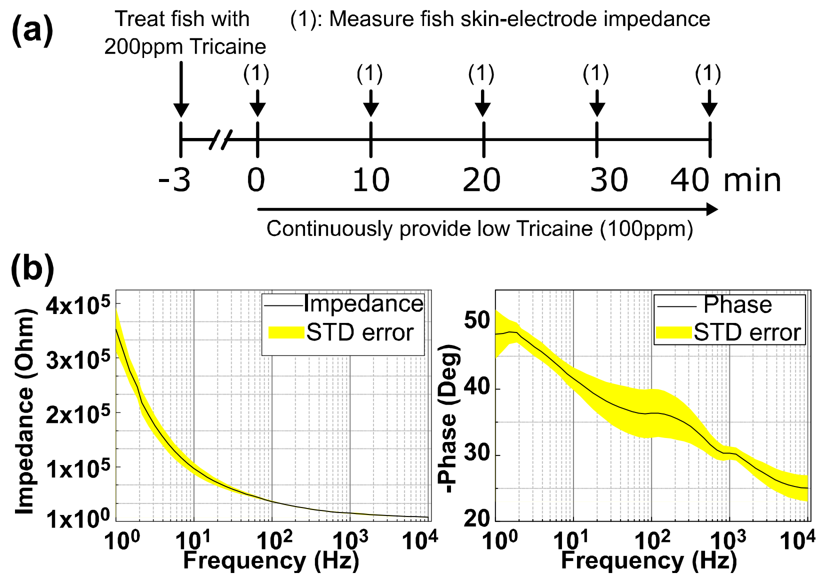


Supplementary figure 2. **The IOT system integrated with the prolonged ECG system.** The IoT Core provides the functionalities to manage and configure connected devices conveniently and securely. Once the ECG data are acquired from the measurement device, it is transmitted in real-time to the cloud platform through the Message Queuing Telemetry Transport (MQTT) protocol. The MQTT protocol is designed to maintain a long-lived connection between the device and the client with minimal communications overheads to save bandwidth for data transmission. Cloud Pub/Sub is an asynchronous communication medium between the device and the servers on the IoT cloud. The communication is based on the notion of topics that cache durable messages. Zebrafish ECG published on a certain topic by the device can be pushed to or pulled from the servers that subscribe to the same topic for storage and analytics.

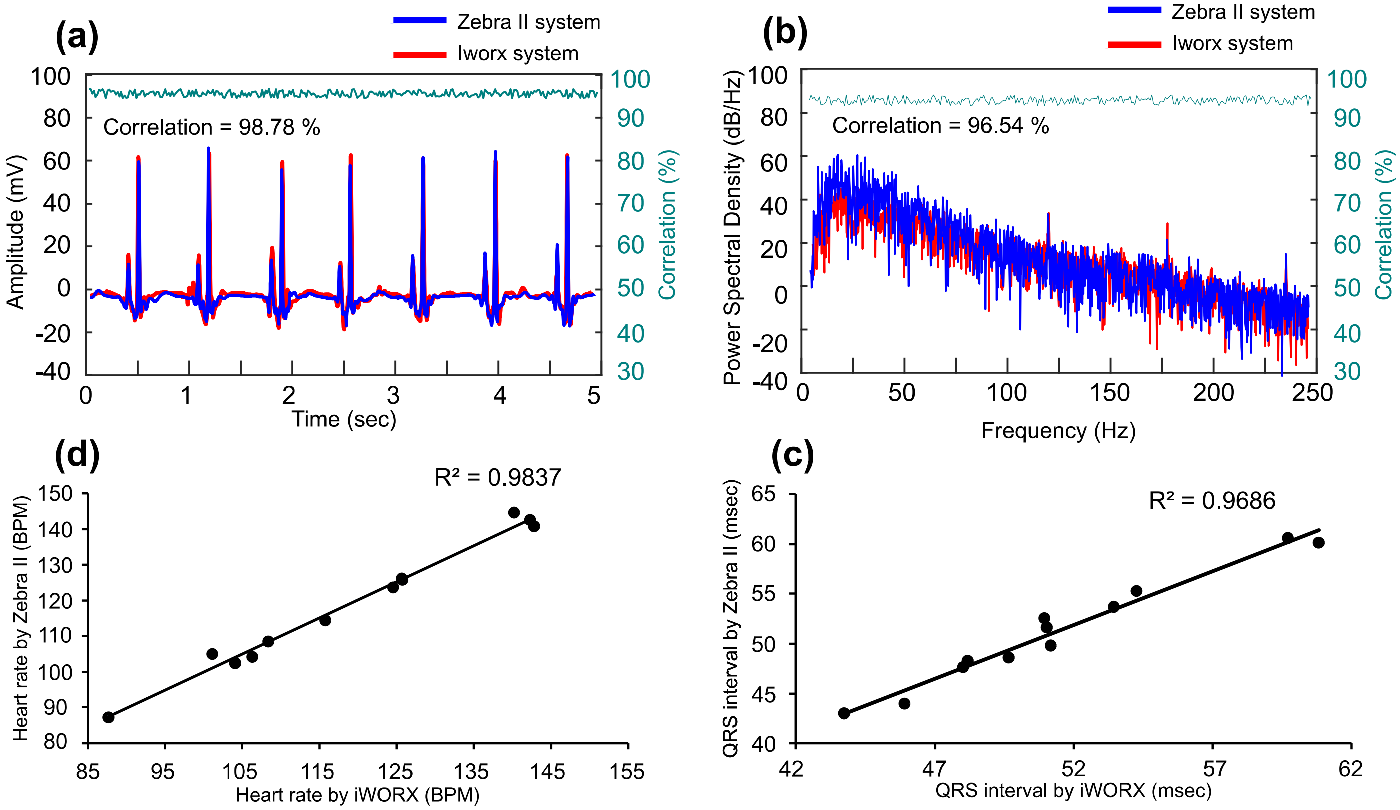
The storage layer is responsible for storing the real-time zebrafish ECG data into the database. As one of the most promising time-series databases, InfluxDB is employed in the proposed system for timely and reliable storage. As an advanced non-relational database, InfluxDB resolves the performance bottleneck of traditionally used databases such as MySQL and provides greater flexibility and read-write speed. In the analytics layer, Cloud Functions, a serverless execution environment, run processing techniques such as denoising, filtering, normalizing, detecting P wave, QRS complex, or T wave, and extracting other useful features. Furthermore, machine learning approaches can be applied to these data with the aid of the Kubernetes Engine to train and deploy the models in containerized applications. Computationally intensive process and analysis tasks are carried out in powerful servers, which greatly eases the burden of local devices. The graphical user interface is responsible for data visualization and management, providing an easy access to the data in the IoT cloud. Grafana is an open platform that is utilized for designing dashboards to represent the ECG data. Users can log onto the cloud to acquire visualized ECG data on either web pages or mobile applications. Based on the results of data analysis, users can observe and understand the real-time conditions of zebrafishes. In the event of any anomalies or suspicious readings, the IoT cloud will notify users in time.



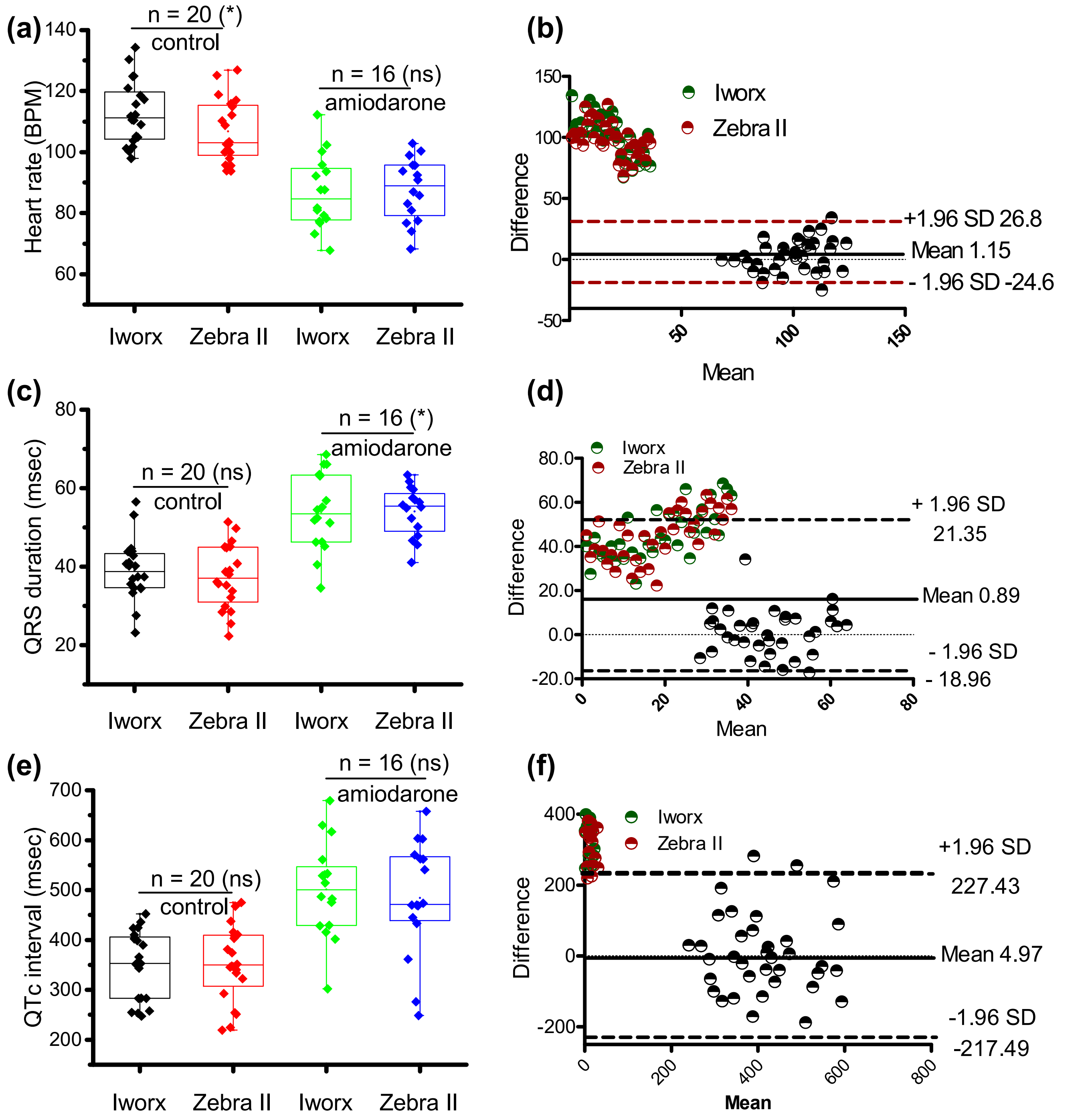
Supplementary figure 3. Wireless data transmission to mobile application. An Android application developed in Java that connects to the ECG system via BLE communication for data collection, displaying, and logging. An Android smartphone application in Java was developed to connect to the Bluetooth Low Energy (BLE) device for data collection, displaying, and saving. Through BLE protocols, the application connects to the Zebra II and reads multiple channels of data at a rate of 250 Hz. After accumulating 1000 data points, the input data are sent to the connecting cloud server for feature extraction of fish ECG. The results will start appearing on the application interface after at least 10 seconds of initial start of data acquisition in the form of dynamic graphs – 4 ECG channels of zebrafish. The user can disconnect the Zebra II at any time and save the raw data plus the exact time of acquisition through the application as a .txt file with a customizable name in the phone’s external storage as well as on the cloud server as a backup.



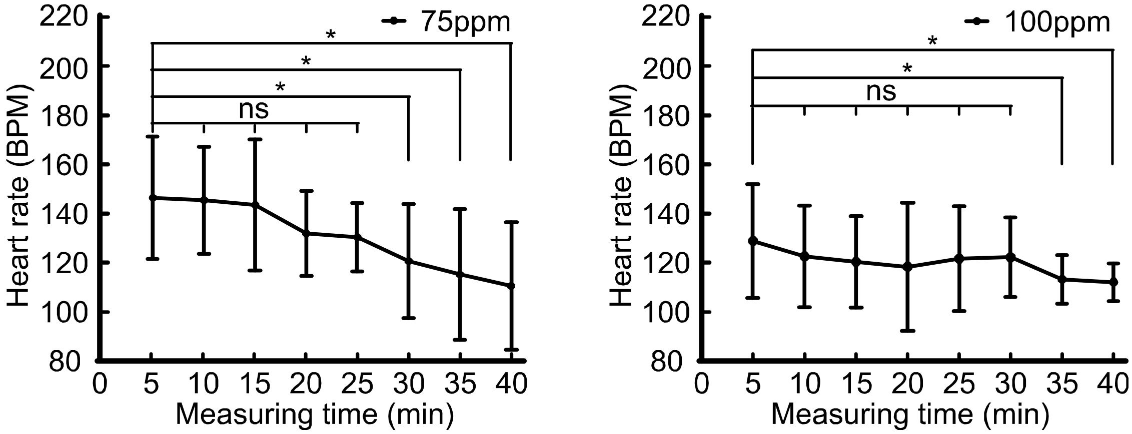
Supplementary figure 4. Characterization of electrode-skin impedance. (**a**) Timeline for experiment: Zebrafish were first anesthetized with 200ppm Tricaine in 3 minutes. Zebrafish were then loaded to each apparatus, followed by the tube system continuously providing low Tricaine concentration. The skin-electrode impedance was measured at 5 time points (*i.e.*, every 10 min for 40 min). (**b**) Averaged impedance magnitude (left side) of 5 time points and phase (right side) of the electrodes placed on fish skin (n=8 fish).



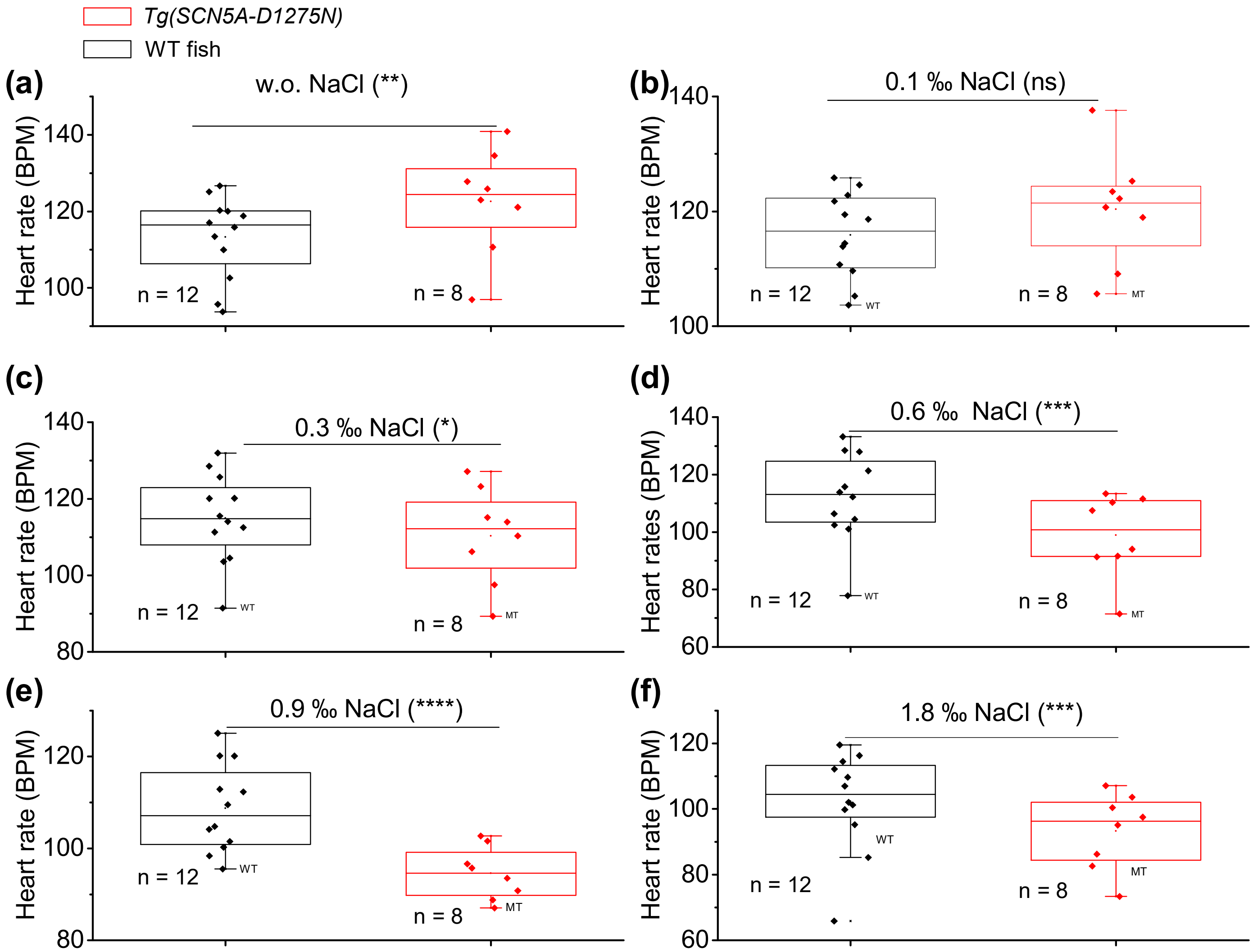
Supplementary figure 5. Comparison of performance between Zebra II system and iWorx system. The ECG measurement was conducted with two systems simultaneously (n = 8 fish). The collected data were then analyzed and compared in terms of correlation on time domain (**a**) and frequency domain (**b**). Correlation of heart rate (HR) (**d**) and QRS interval (**c**) extracted from ECG data collected by Zebra II and iWORX.



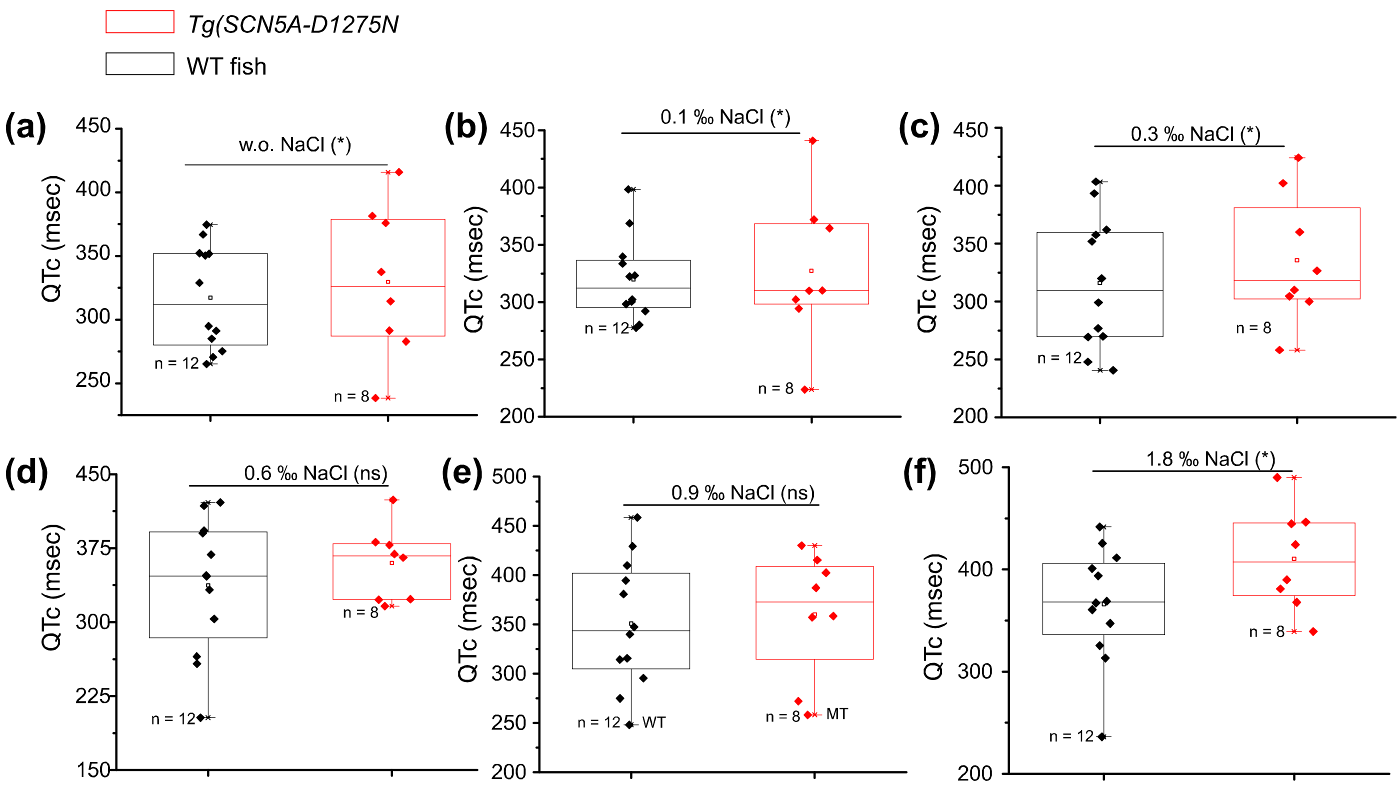
Supplementary figure 6. Compare the performance between Zebra II and Iworx with amiodarone treatment. Control group (n = 20 WT fish) and Amiodarone-treated group (n = 16 WT fish) were used to this experiment. The ECG data from two groups were acquired by the two systems and, and features such as HR (**a**), QTc interval (**c**), and QRS duration (**e**) were extracted from ECG data. The Bland Altman analysis was used to compare the agreement level between two systems in response to such features, including HR (**b**), QTc interval (**d**), and QRS duration (**f**). *p <0.05; **p < 0.01 (paired sample T-test). *ns* indicates not significant.



Supplementary figure 7. Characterization of heart rate variation with different Tricaine concentration. The experiment was conducted with n = 8 fish within 40 minute (min) long ECG measurement, showing the heart rate variation with two Tricaine concentrations in every 5 min. $p < 0.05$; $^{**}p < 0.01$ (paired sample T-test). *ns* indicates not significant.



Supplementary figure 8. Heart rate comparison between WT fish and *Tg(SCN5A-D1275N)* with different NaCl concentrations. HR value calculated with ECG data acquired from both WT fish and *Tg(SCN5A-D1275N)* mutant fish with the following treatments: without NaCl (**a**); with 0.1 ‰ NaCl (**b**); with 0.3 ‰ NaCl (**c**); with 0.6 ‰ NaCl (**d**); with 0.9 ‰ NaCl (**e**); with 1.8 ‰ NaCl (**f**). Overall, the HR in WT fish did not show significant difference among NaCl concentrations. In contrast, the in mutant fish showed significant changes with the HR decreasing in response to the increase of NaCl dose. p <0.05; **p < 0.01; **p < 0.001 (Student's T-test). *ns* indicates not significant.



Supplementary figure 9. QTc comparison between WT fish and *Tg(SCN5A-D1275N)* with different NaCl concentration. QTc interval calculated with ECG data collected from WT and mutant fish with the following treatments: without NaCl(**a**); with 0.1 ‰ NaCl (**b**); with 0.3 ‰ NaCl (**c**); with 0.6 ‰ NaCl (**d**); with 0.9 ‰ NaCl (**e**); with 1.8 ‰ NaCl (**f**). The significant difference showing in QTc value between two groups was found in 0.1, 0.3 and 1.8 ‰ NaCl. An increase in QTc value responding to the increase of NaCl level was found in both WT fish and mutant fish. $p < 0.05$; $^{**}p < 0.01$ (Student's T-test). *ns* indicates not significant.

Table S1:

Parameters	Numbers
Number of channels	4 (can be up to 8)
Bandwidth	1 Hz – 150 Hz
Input Range	±2.5V
Resolution	24 Bit
Sampling rate	250 Hz
Common mode rejection ratio (CMRR © 60 Hz)	100 dB
Input-Referred Noise	1 μV
Enclosure	Plastic
Dimensions	6.3''W x 4.8''L x 3.9''H

Table S2:

Parameters	Mean	STD	RSD (100*STD/mean)
Signal to noise ratio (SNR)	25.36	7.38	29.10
Heart rate	117.28	9.34	7.96
QTc interval	384.21	12.25	3.18
QRS interval	376.47	8.27	2.19