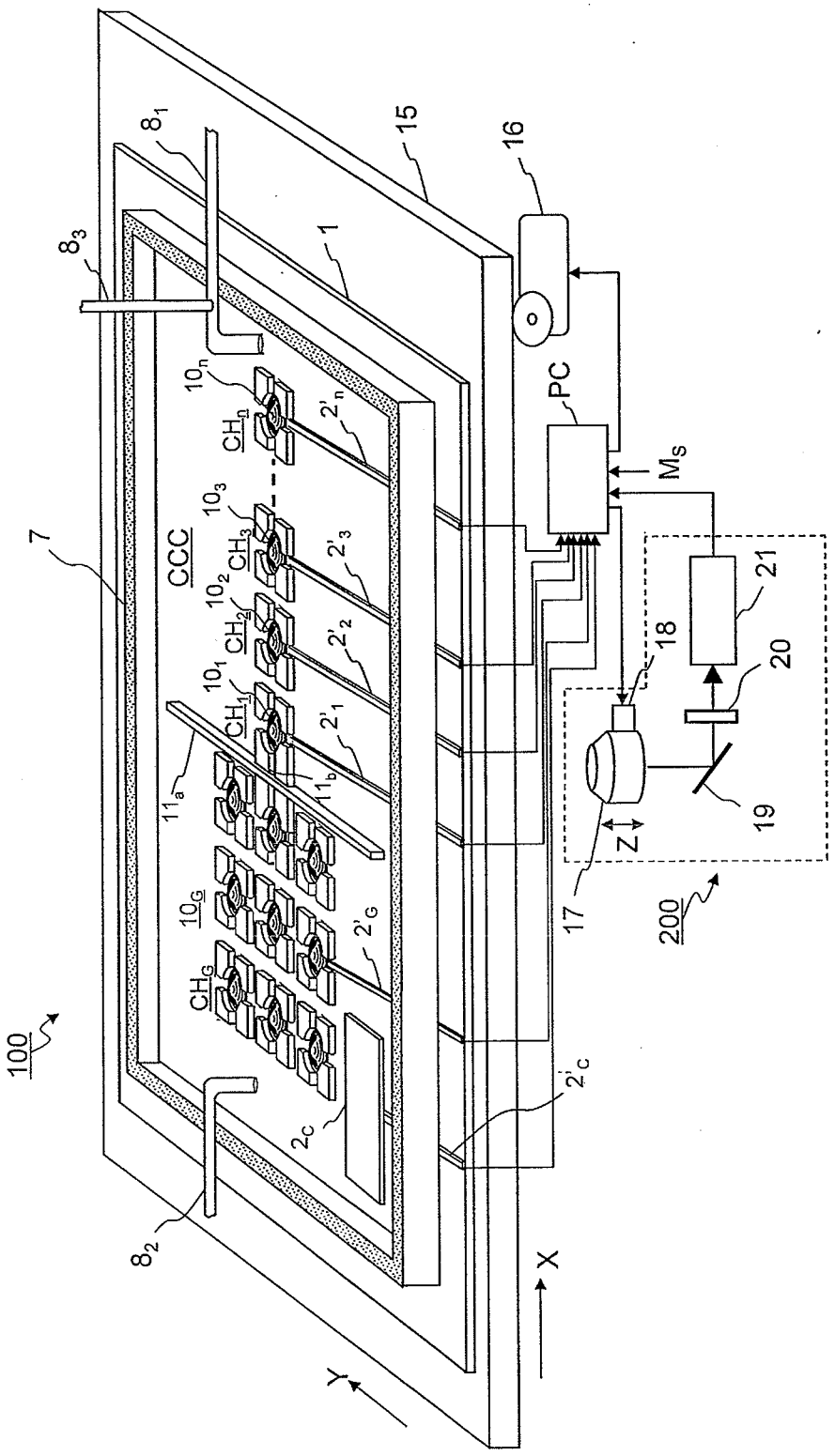
Fig. 1



100
7
CCC
8_1
8_2
8_3
1
15
16
PC
M_S
10_n
CH_n
$2'_n$
10_3
CH_3
$2'_3$
10_2
CH_2
$2'_2$
10_1
CH_1
$2'_1$
11_a
11_b
10_G
CH_G
$2'_G$
2_C
$2'_C$
X
Y
Z
17
18
19
20
21
200

Fig. 2

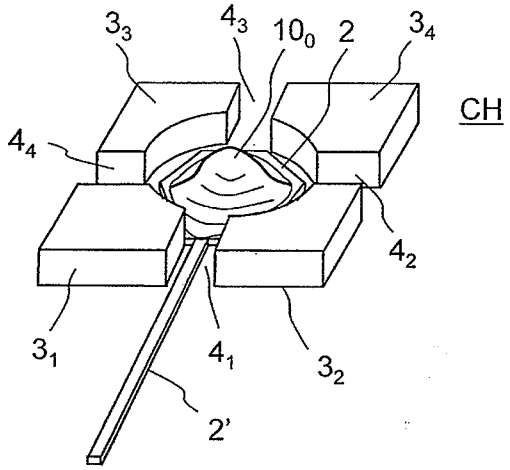

3_3
4_3
10_0
2
3_4
CH
4_4
4_2
3_1
4_1
3_2
2'

Fig. 3

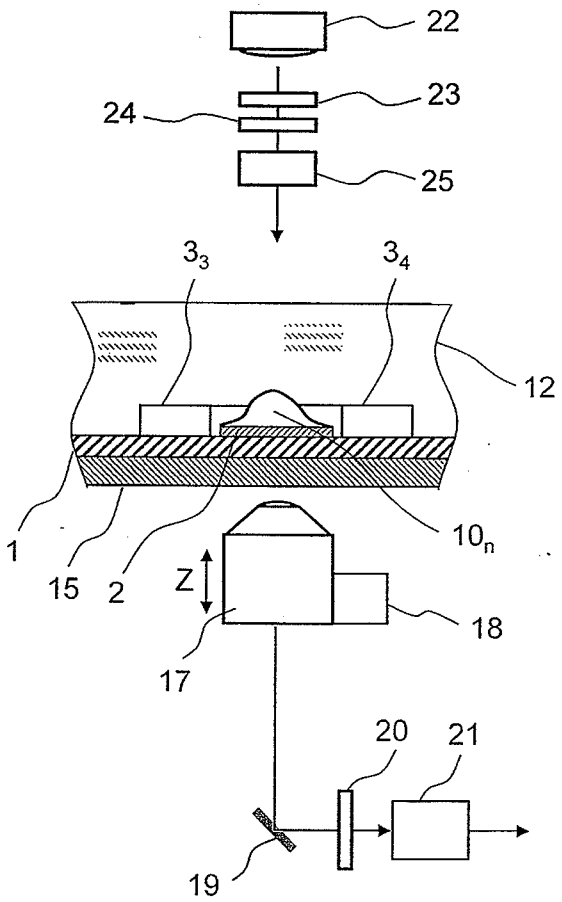

22
23
24
25
3_3
3_4
12
1
15
2
Z
10_n
18
17
20
21
19

Fig. 4

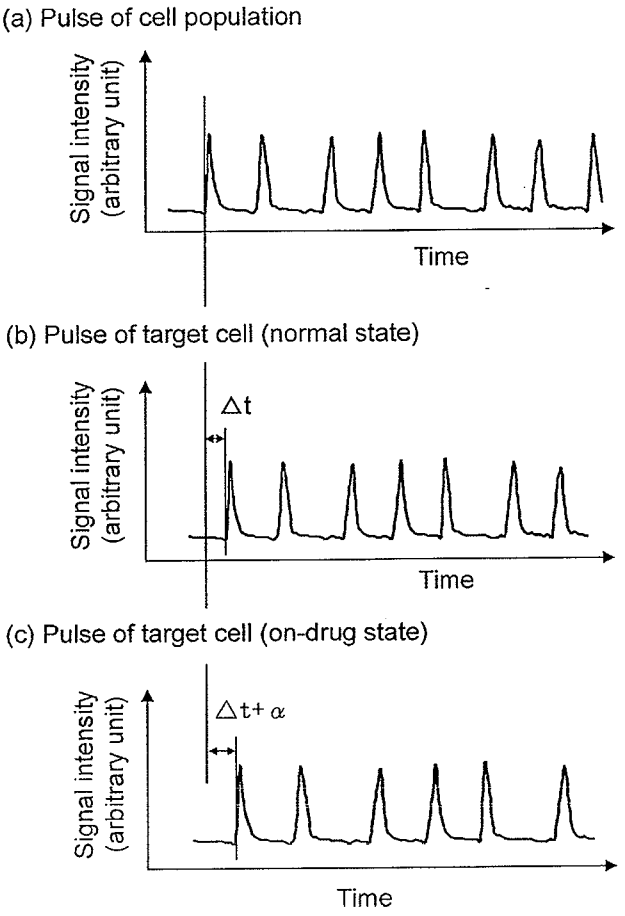
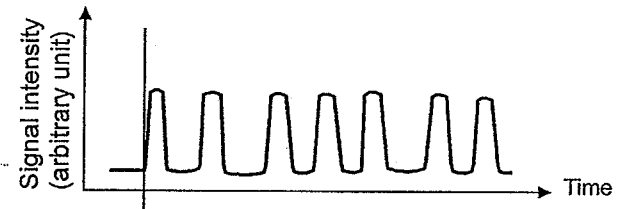
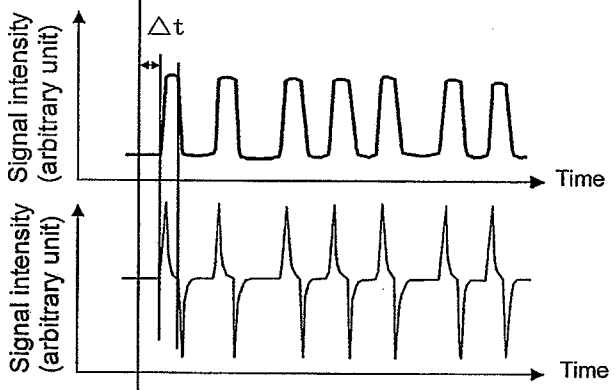

(a) Pulse of cell population
Signal intensity (arbitrary unit)
Time
(b) Pulse of target cell (normal state)
Signal intensity (arbitrary unit)
Δt
Time
(c) Pulse of target cell (on-drug state)
Signal intensity (arbitrary unit)
Δt+α
Time

Fig. 5

(a) Changes in volume of cell due to pulsation of cell population



(b) Changes in volume of cell due to pulsation of target cell (normal state)



(c) Changes in volume of cell due to pulsation of target cell (on-drug state)

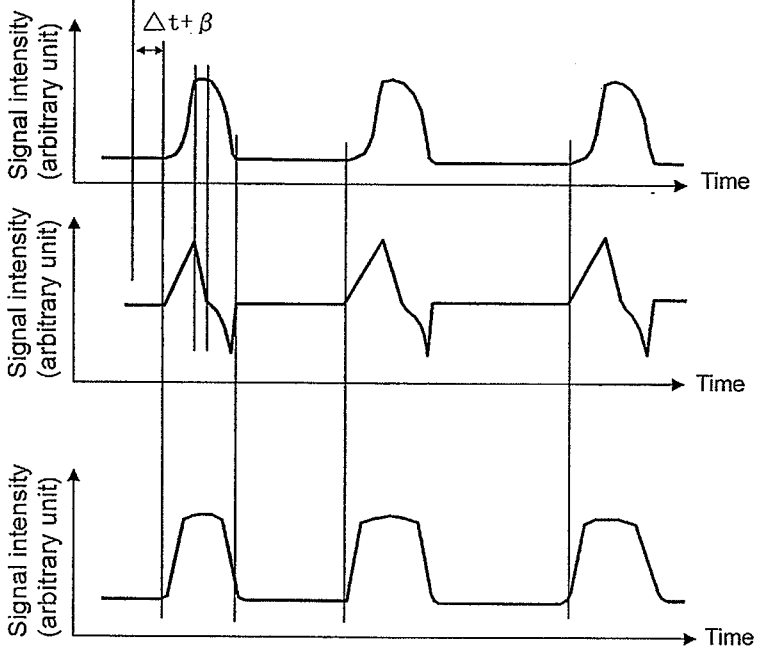
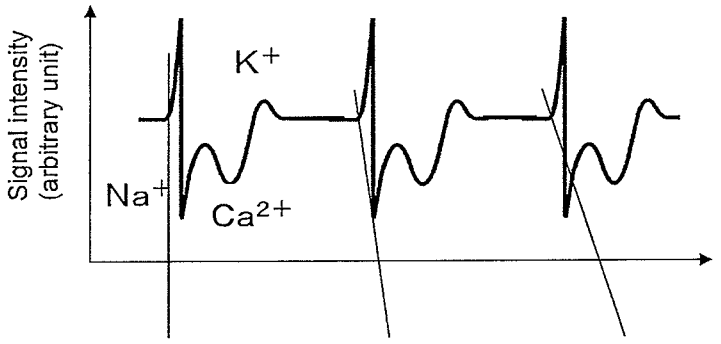


Fig. 6

(a) Changes in extracellular potentials of target cell (normal state)



(b) Changes in extracellular potentials of target cell (on-drug state)

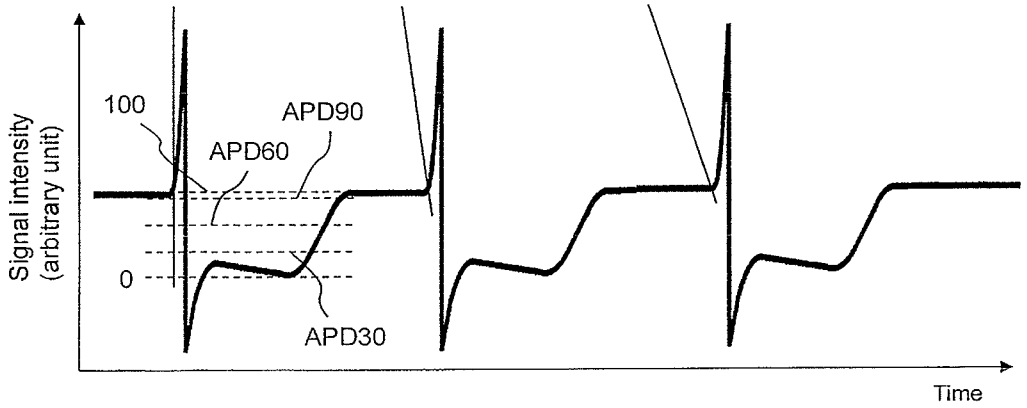


Fig. 7

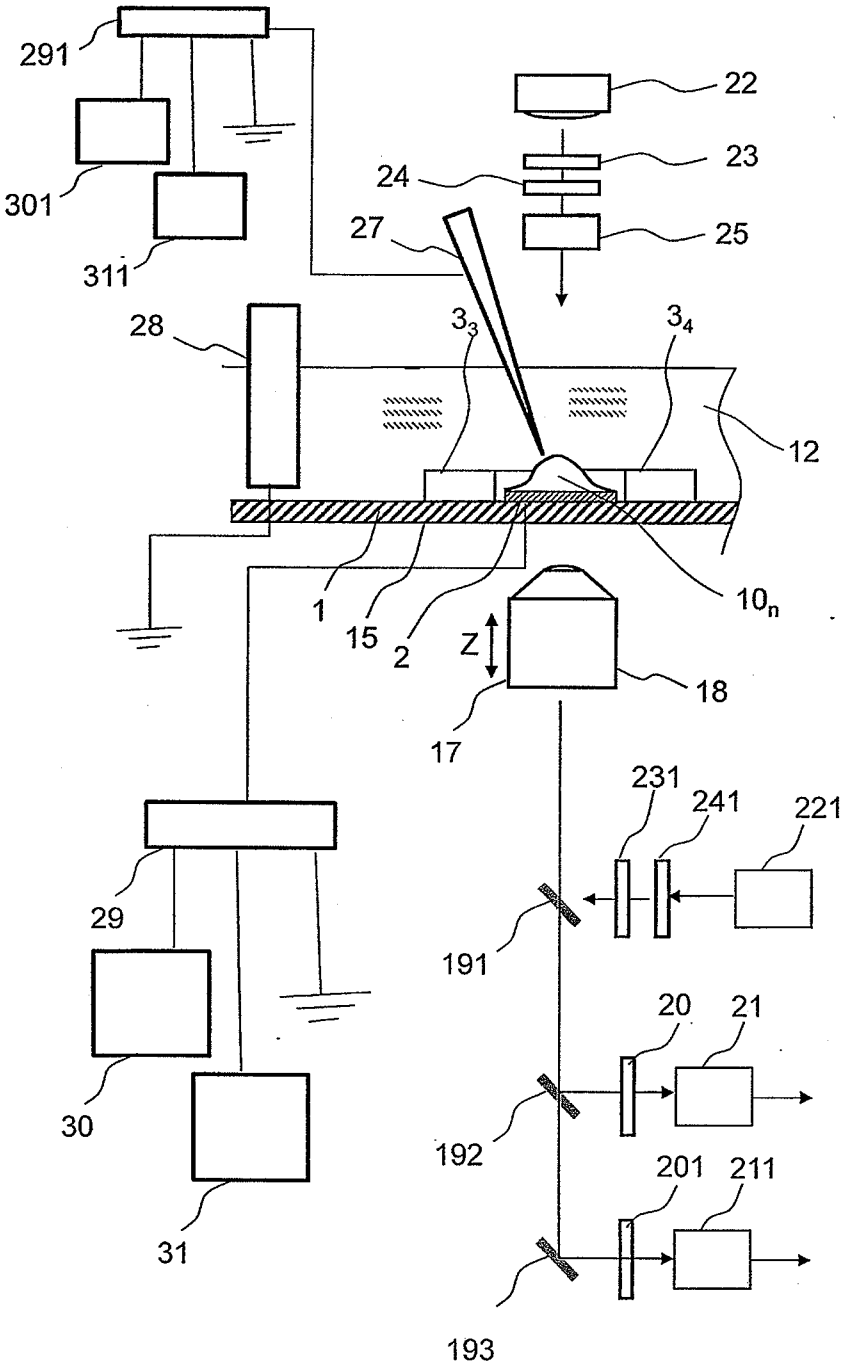

291
22
301
23
24
27
25
311
28
3_3
3_4
12
1
15
2
Z
10_n
18
17
231
241
221
29
191
20
21
30
192
201
211
31
193

Fig. 8

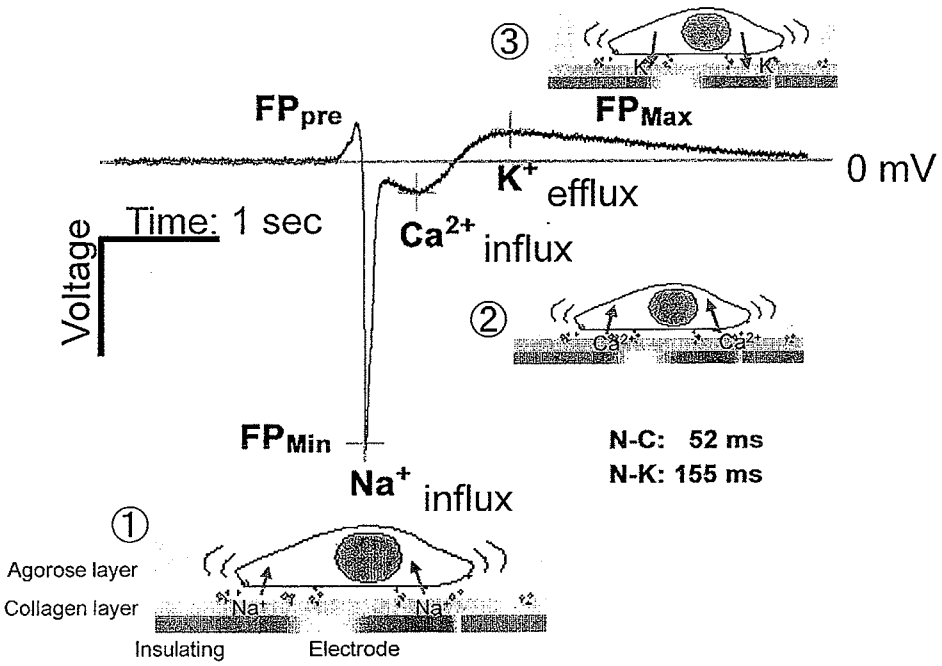

③
K+
K+
FPpre
FPMax
0 mV
K+ efflux
Time: 1 sec
Voltage
Ca2+ influx
②
Ca2+
Ca2+
FPMin
N-C: 52 ms
N-K: 155 ms
Na+ influx
①
Agorose layer
Collagen layer
Na+
Na+
Insulating
Electrode

Fig. 9

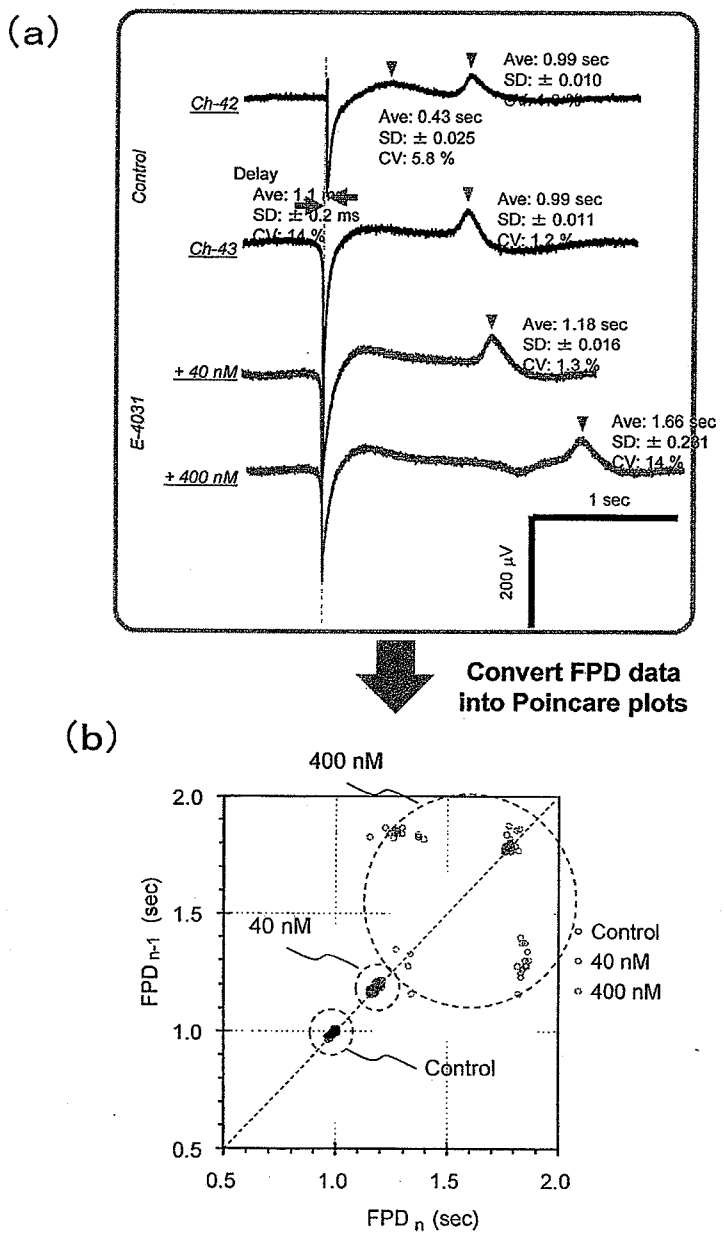
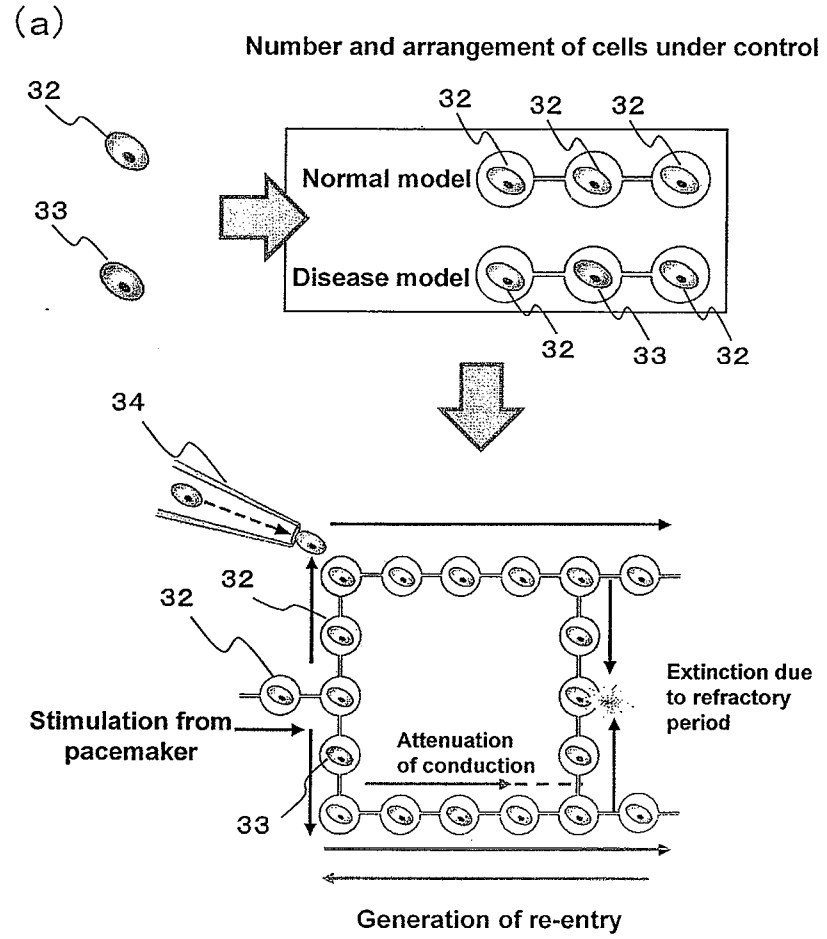

(a)
Control
E-4031
Ch-42
Ch-43
+ 40 nM
+ 400 nM
Ave: 0.99 sec
SD: ± 0.010
Ave: 0.43 sec
SD: ± 0.025
CV: 5.8 %
Delay
Ave: 1.1
SD: ± 0.2 ms
Ave: 0.99 sec
SD: ± 0.011
CV: 1.2 %
Ave: 1.18 sec
SD: ± 0.016
CV: 1.3 %
Ave: 1.66 sec
SD: ± 0.231
CV: 14 %
1 sec
200 µV
Convert FPD data
into Poincare plots
(b)
400 nM
40 nM
Control
Control
40 nM
400 nM
2.0
1.5
1.0
0.5
FPD n-1 (sec)
FPD n (sec)

Fig. 10


(a)
Number and arrangement of cells under control
32
33
32
32
32
Normal model
Disease model
32
33
32
34
32
32
Stimulation from pacemaker
Attenuation of conduction
Extinction due to refractory period
33
Generation of re-entry

(b)

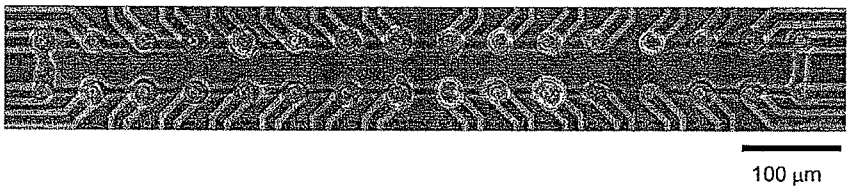
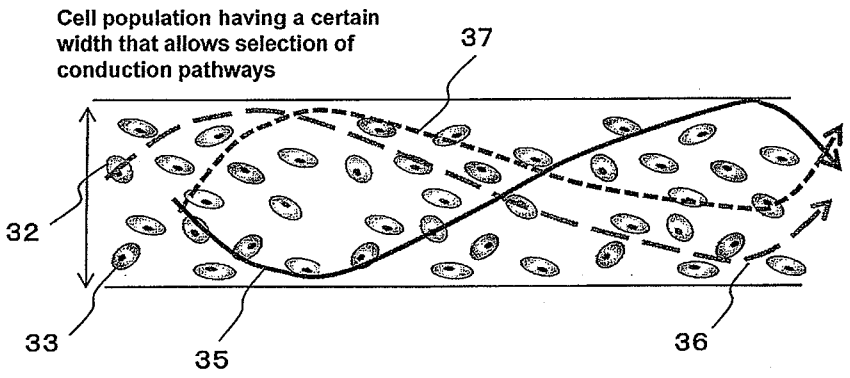
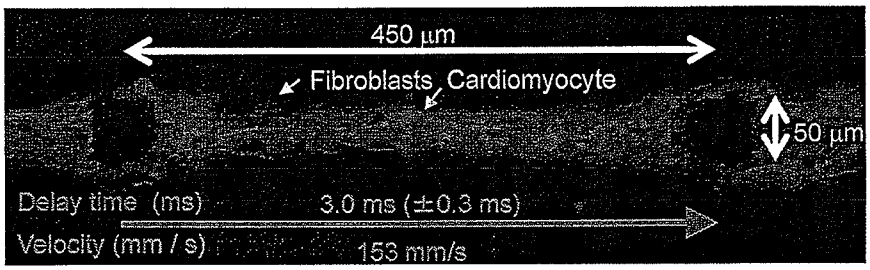

100 μm

Fig. 11

(a)



(b)



Chamber
Length: 450 μm
Width: about 50 μm

Total: 50 cells
Myocyte: 32 cells
Fibroblast: 18 cells

(c)

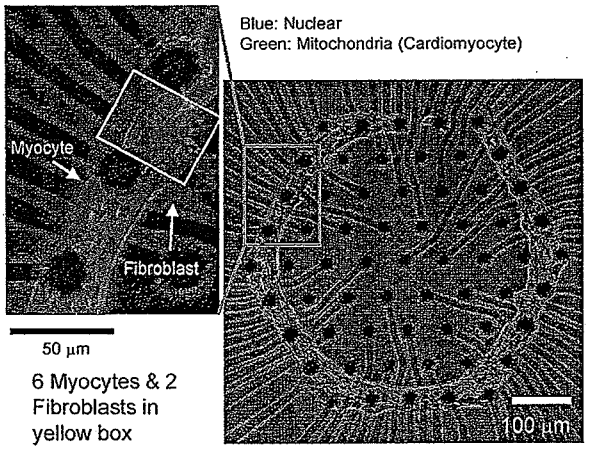
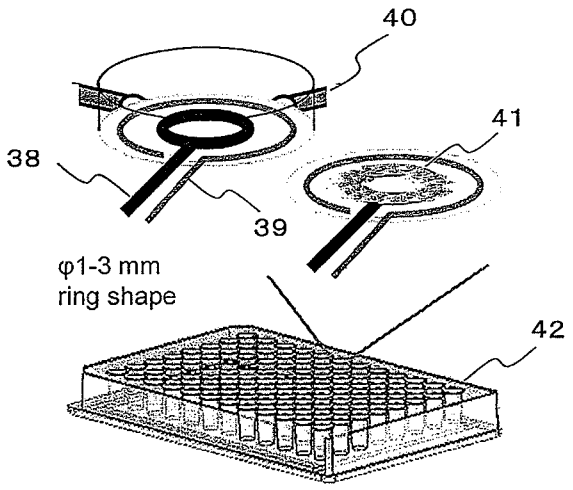


Fig. 12

(a)


40
41
38
39
φ1-3 mm
ring shape
42

(b)

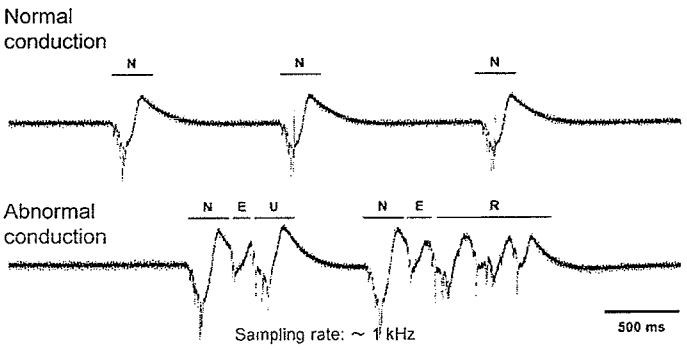

Normal
conduction
N
N
N
Abnormal
conduction
N E U
N E R
Sampling rate: ~ 1 kHz
500 ms

Fig. 13

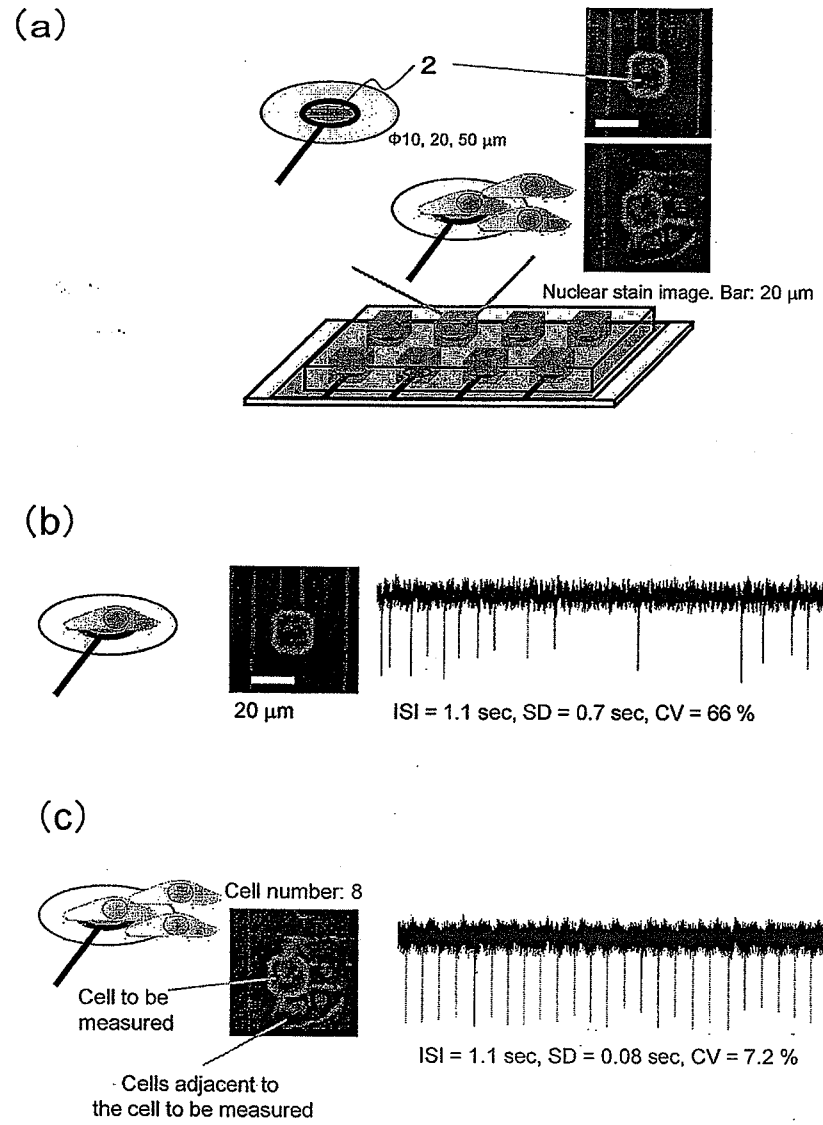

(a)
2
Φ10, 20, 50 μm
Nuclear stain image. Bar: 20 μm
(b)
20 μm
ISI = 1.1 sec, SD = 0.7 sec, CV = 66 %
(c)
Cell number: 8
Cell to be measured
Cells adjacent to the cell to be measured
ISI = 1.1 sec, SD = 0.08 sec, CV = 7.2 %

Fig. 14

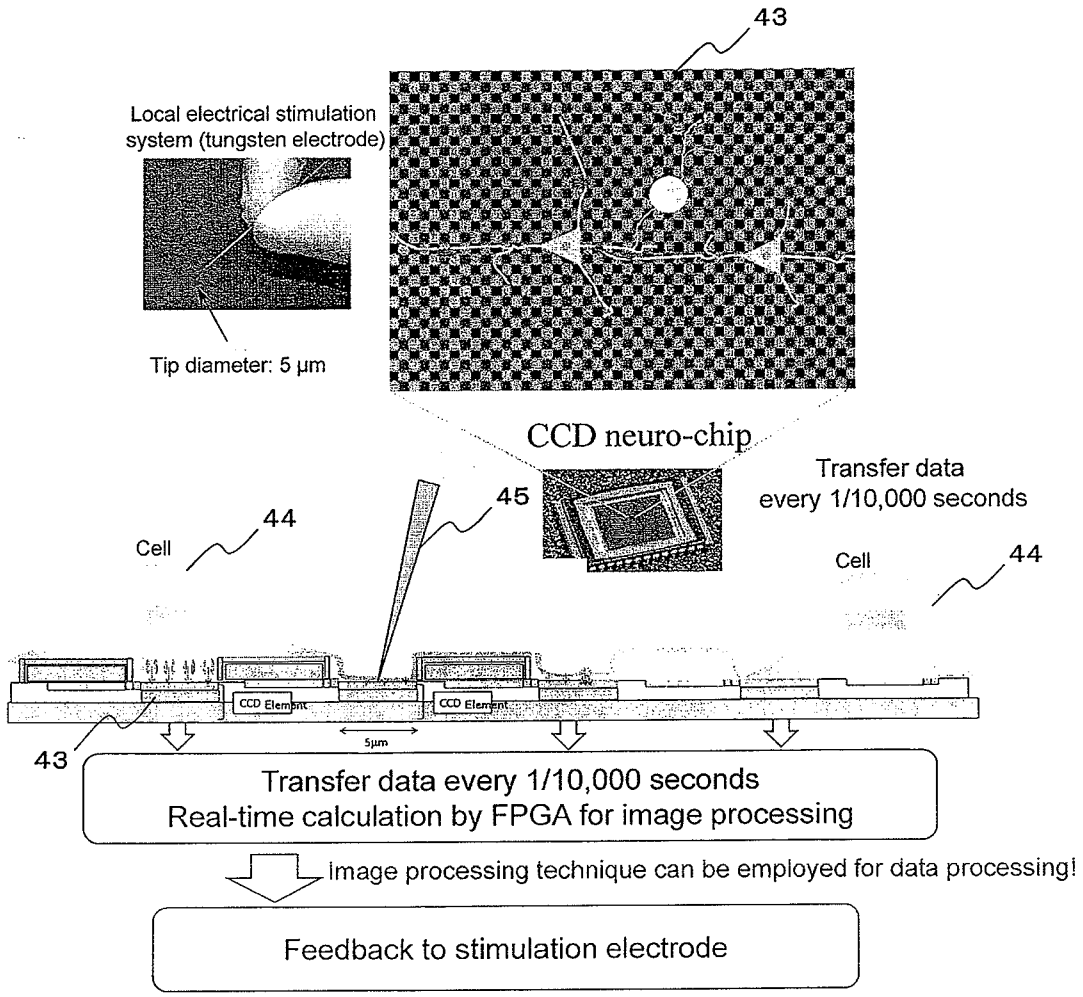
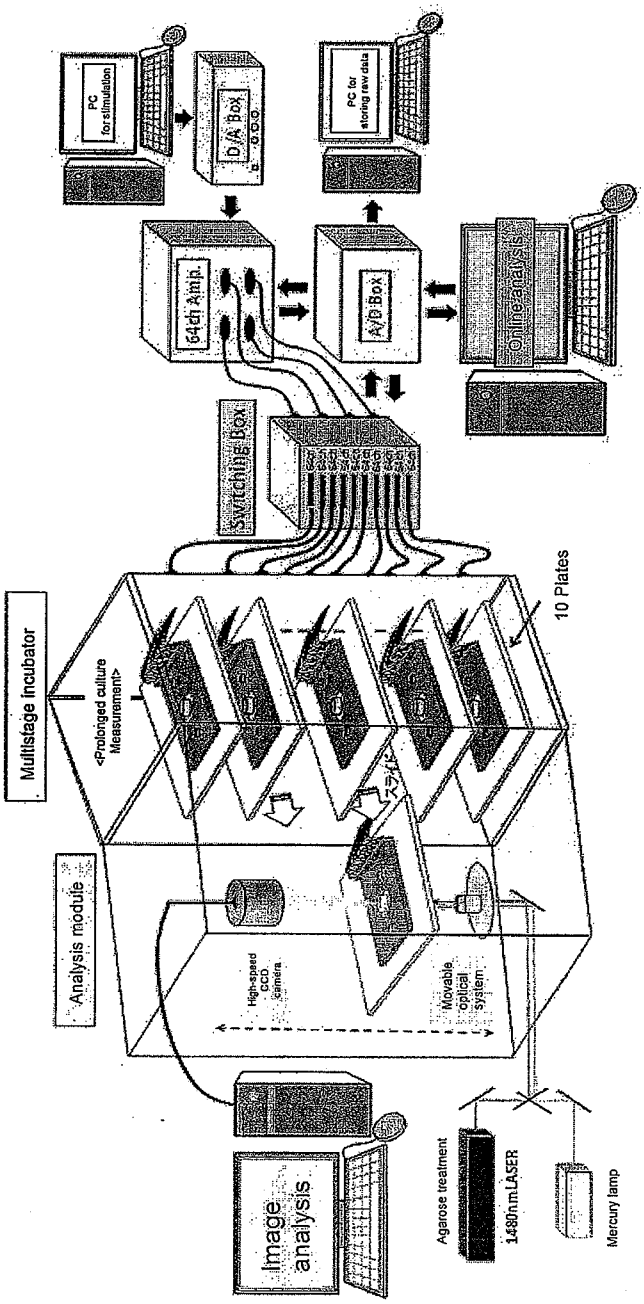
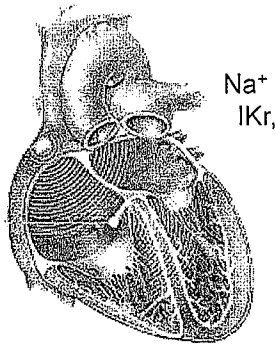

43
Local electrical stimulation system (tungsten electrode)
Tip diameter: 5 μm
CCD neuro-chip
Transfer data every 1/10,000 seconds
Cell
44
45
Cell
44
CCD Element
CCD Element
5μm
43
Transfer data every 1/10,000 seconds
Real-time calculation by FPGA for image processing
Image processing technique can be employed for data processing!
Feedback to stimulation electrode

Fig. 15


PC for stimulation
D/A Box
PC for storing raw data
64ch Amp.
A/D Box
Online analysis
Switching Box
10 Plates
Multistage incubator
<Prolonged culture Measurement>
Analysis module
High-speed CCD camera
Movable optical system
Image analysis
Agarose treatment
1480nm LASER
Mercury lamp

In vivo
(Individual level)



Na^+ ,Ca^{2+} Inhibitions
IKr, IKs

Corresponds to

Potential measurements
(Analysis of waveforms as changes in cellular potential)

Na^+ Inhibition

Corresponds to

Potential measurement
(Conduction velocity between adjacent cells)

Cardiac waveform
(abnormal cardiac rhythm)

Output of blood

Corresponds to

Optical measurement
(Change in shape of myocardial cell)

TdP (Torsades de Pointes)
Atrial fibrillation/Ventricular fibrillation model

Corresponds to

Myocardial cell network model
(Re-entry circuit)

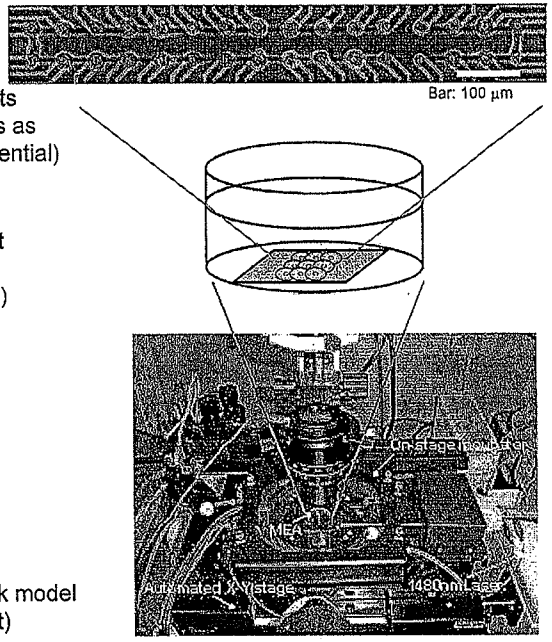
Cardiac hypertrophy model

Coresponds to

Myocardial cell network model
(Structural cell arrangement:
myocardial cells + fibroblast cells)

In vitro
(Cell network)

Na^+ ,Ca^{2+}
IKr, IKs



On-chip myocardial cell network
Optical/Potential measurement system

Fig. 16

Fig. 17

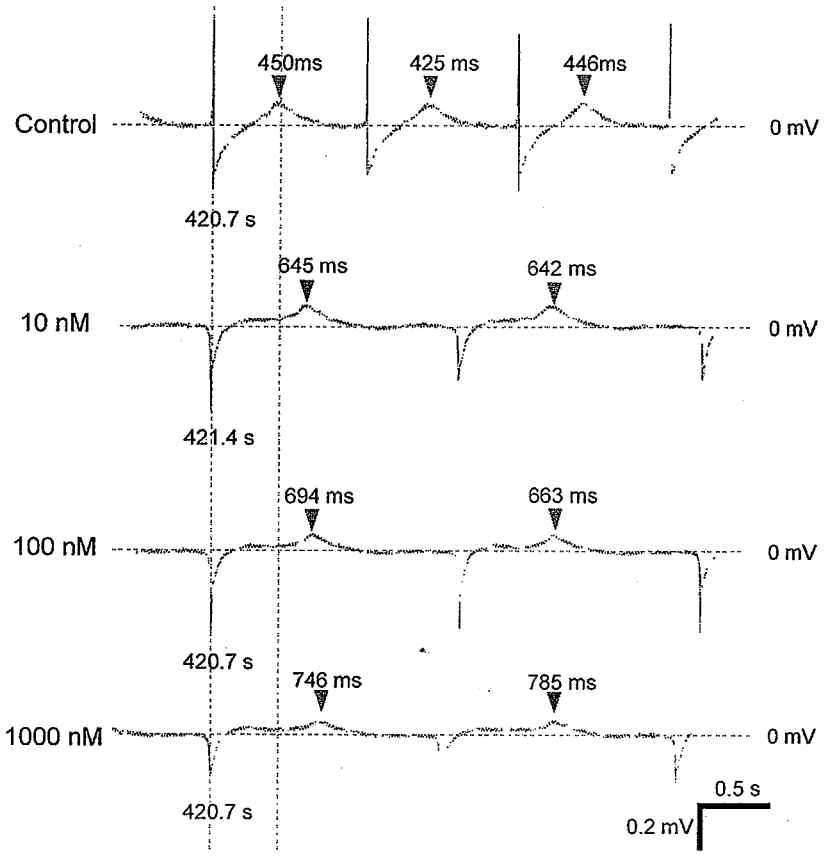

450ms
425 ms
446ms
Control
0 mV
420.7 s
645 ms
642 ms
10 nM
0 mV
421.4 s
694 ms
663 ms
100 nM
0 mV
420.7 s
746 ms
785 ms
1000 nM
0 mV
0.5 s
420.7 s
0.2 mV

Fig. 18

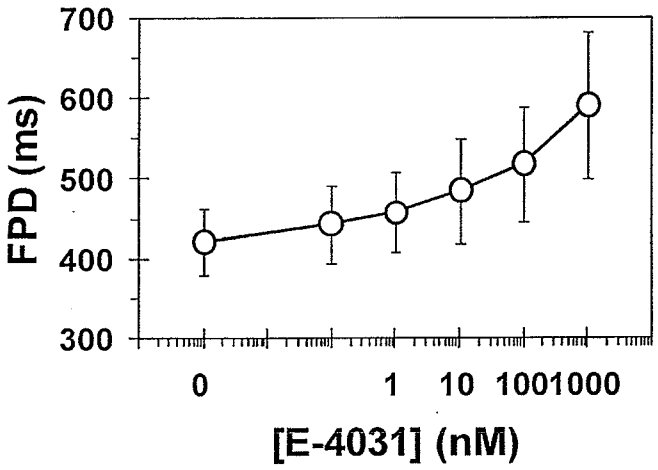
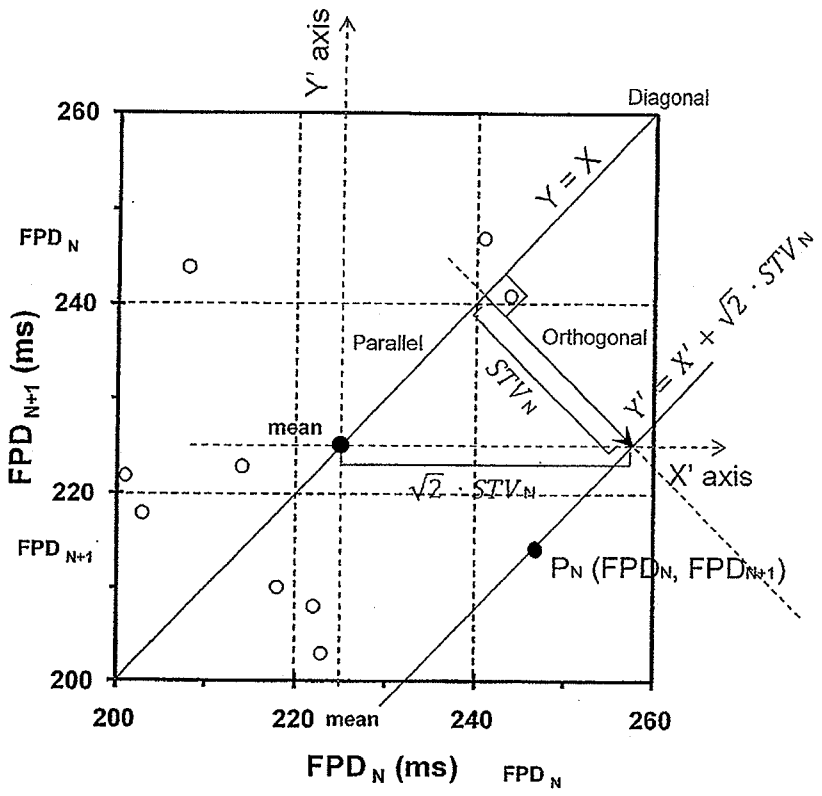


Fig. 19

(a)



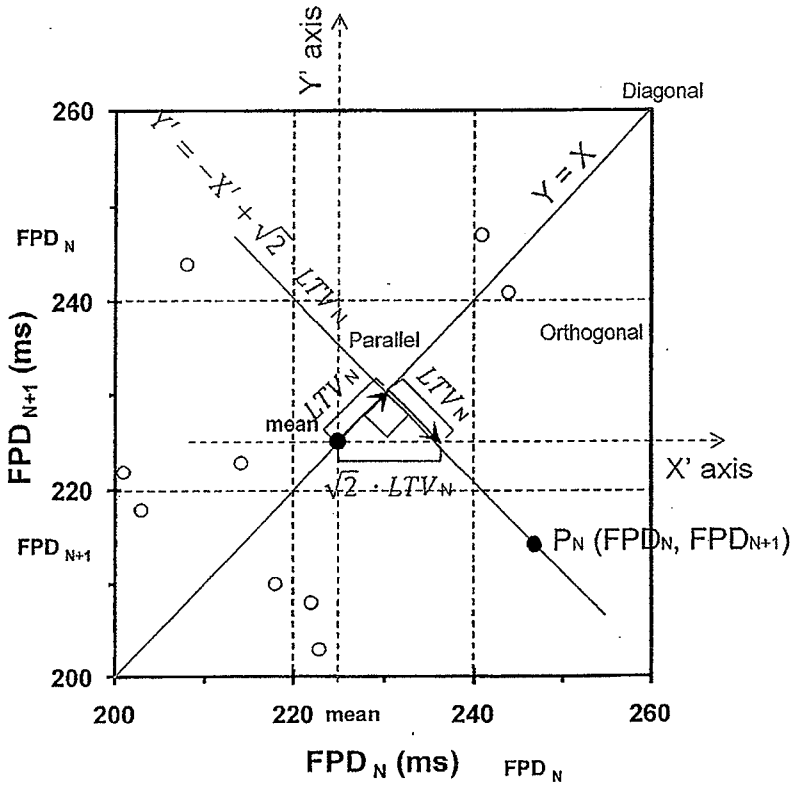
(b)

$$STV = \frac{\sum_{n=1}^{k} |(FPD_{n+1} - FPD_{mean}) - (FPD_n - FPD_{mean})|}{k \times \sqrt{2}}$$

$$= \frac{\sum_{n=1}^{k} |FPD_{n+1} - FPD_n|}{k \times \sqrt{2}} \quad \cdots \text{(Equation 1)}$$

Fig. 20

(a)



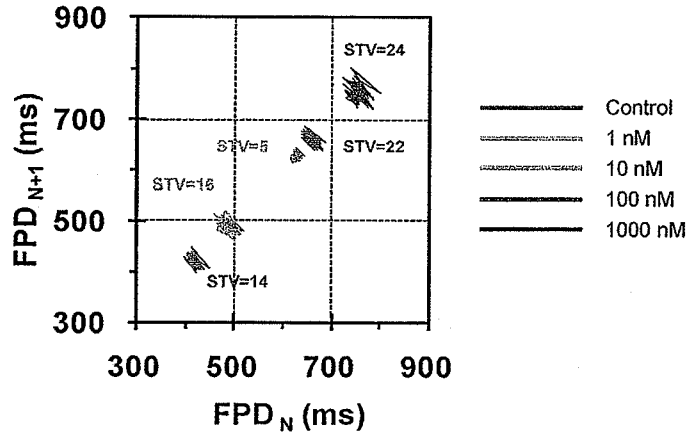
(b)

$$LTV = \frac{\sum_{n=1}^{k} \left| (FPD_{n+1} - FPD_{mean}) + (FPD_n - FPD_{mean}) \right|}{k \times \sqrt{2}}$$

$$= \frac{\sum_{n=1}^{k} \left| FPD_{n+1} + FPD_n - 2FPD_{mean} \right|}{k \times \sqrt{2}} \quad \cdots \text{(Equation 2)}$$

Fig. 21

(a)


900
700
500
300
300
500
700
900
FPD N+1 (ms)
FPD N (ms)
STV=24
STV=5
STV=22
STV=16
STV=14
Control
1 nM
10 nM
100 nM
1000 nM

(b)

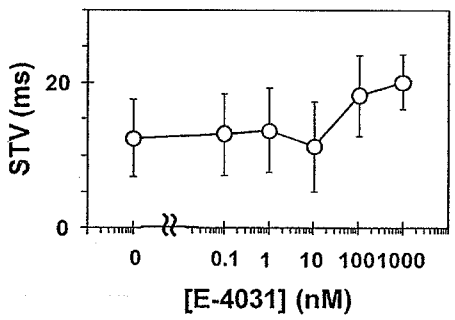

STV (ms)
20
0
0
0.1
1
10
100
1000
[E-4031] (nM)

Fig. 22

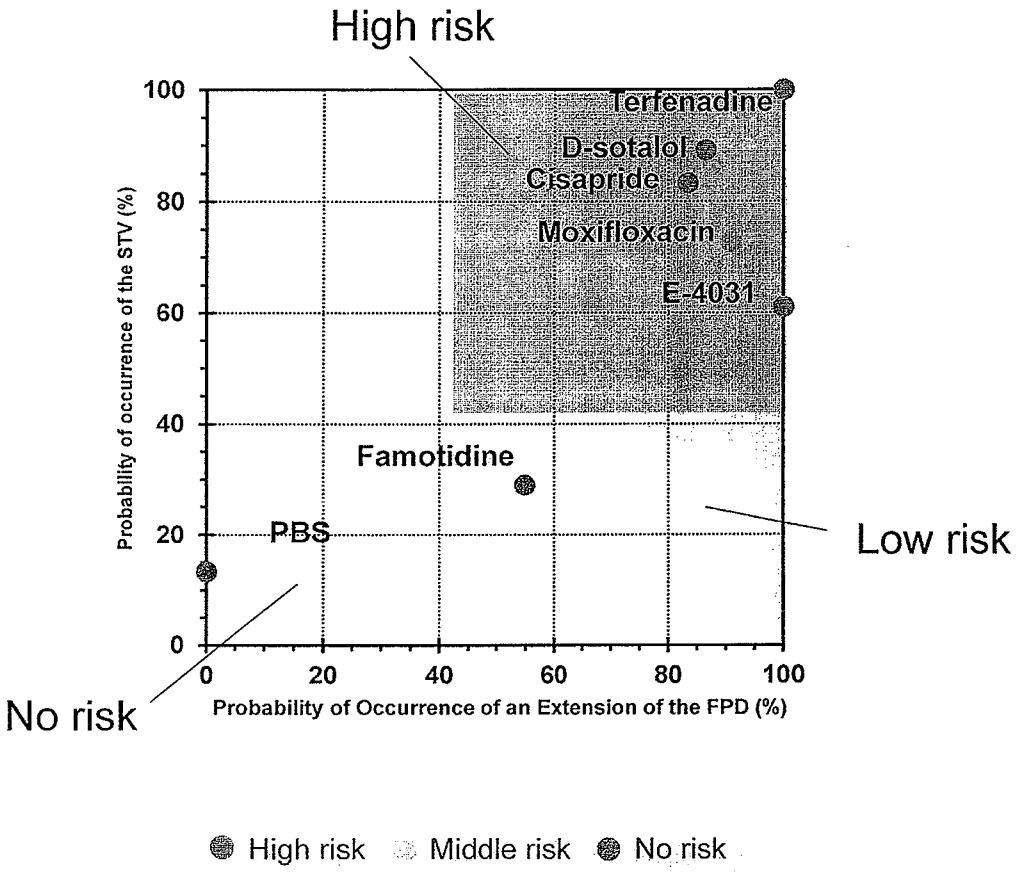
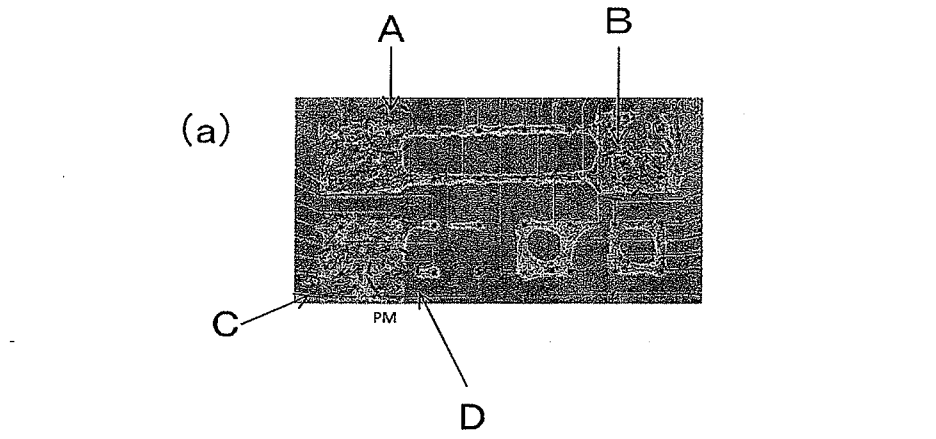

High risk
Terfenadine
D-sotalol
Cisapride
Moxifloxacin
E-4031
Famotidine
PBS
Low risk
No risk
Probability of occurrence of the STV (%)
Probability of Occurrence of an Extension of the FPD (%)
0
20
40
60
80
100
High risk
Middle risk
No risk

Fig. 23


(a)
A
B
C
D
PM

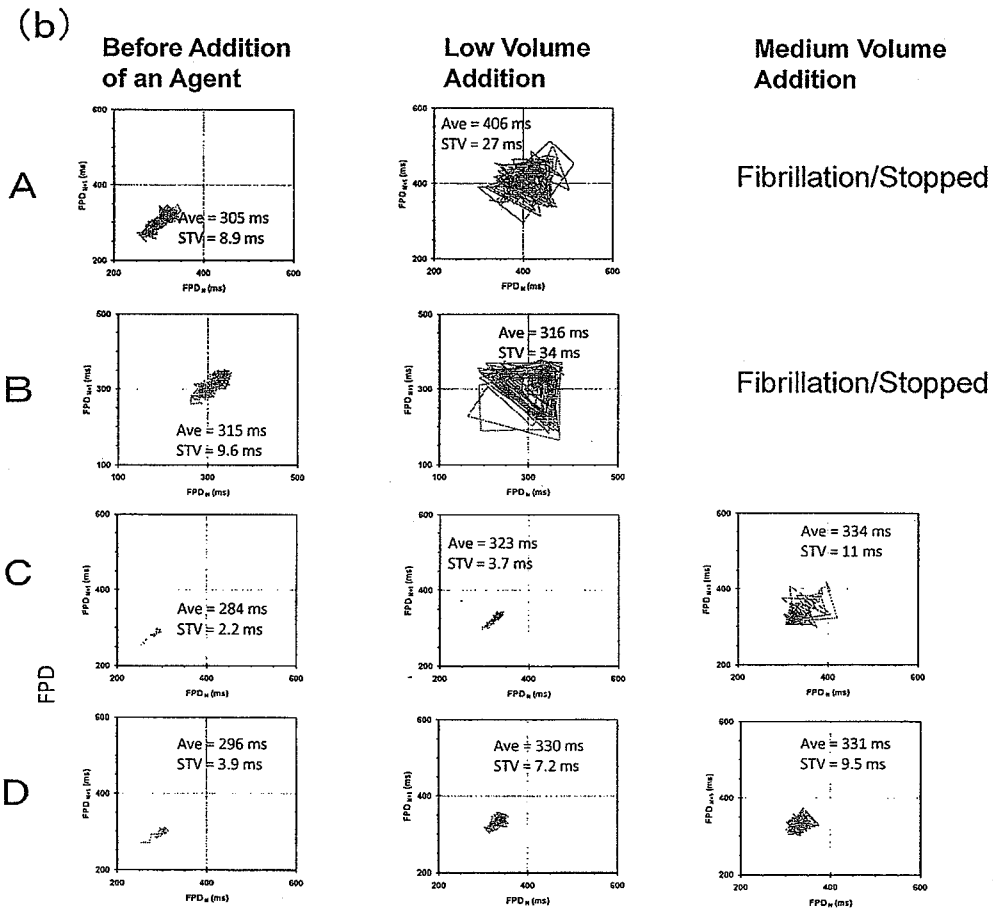
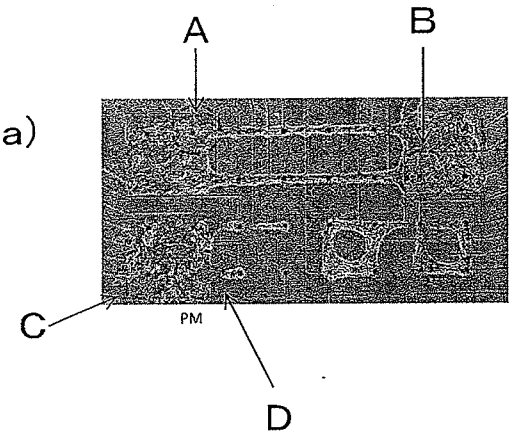
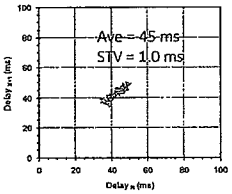
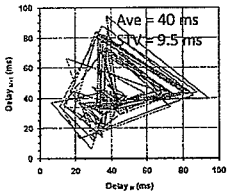
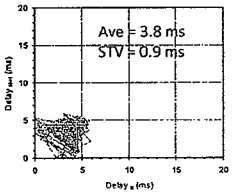
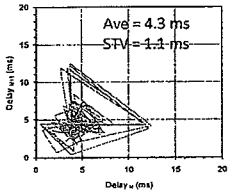
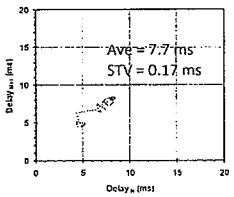
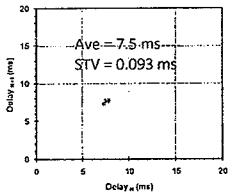
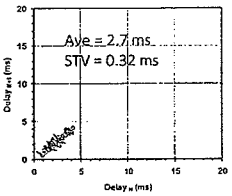
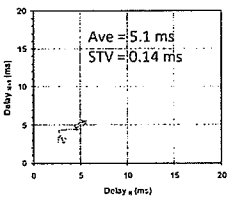
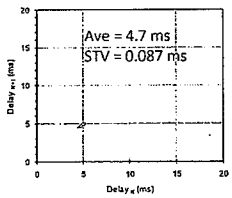
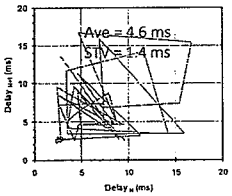

(b)
Before Addition of an Agent
Low Volume Addition
Medium Volume Addition
A
B
C
D
FPD
Ave = 305 ms
STV = 8.9 ms
Ave = 406 ms
STV = 27 ms
Fibrillation/Stopped
Ave = 315 ms
STV = 9.6 ms
Ave = 316 ms
STV = 34 ms
Fibrillation/Stopped
Ave = 284 ms
STV = 2.2 ms
Ave = 323 ms
STV = 3.7 ms
Ave = 334 ms
STV = 11 ms
Ave = 296 ms
STV = 3.9 ms
Ave = 330 ms
STV = 7.2 ms
Ave = 331 ms
STV = 9.5 ms

Fig. 24

(a)



(b)

	Before Addition of an Agent	Low Volume Addition	Medium Volume Addition
A			Fibrillation/Stopped
B			Fibrillation/Stopped
C			
D			

(c)

$$STV = \frac{\sum_{n=1}^{k} | V_{n+1} - V_n |}{k \times \sqrt{2}} \quad \cdots \text{(Equation 3)}$$

Fig. 25

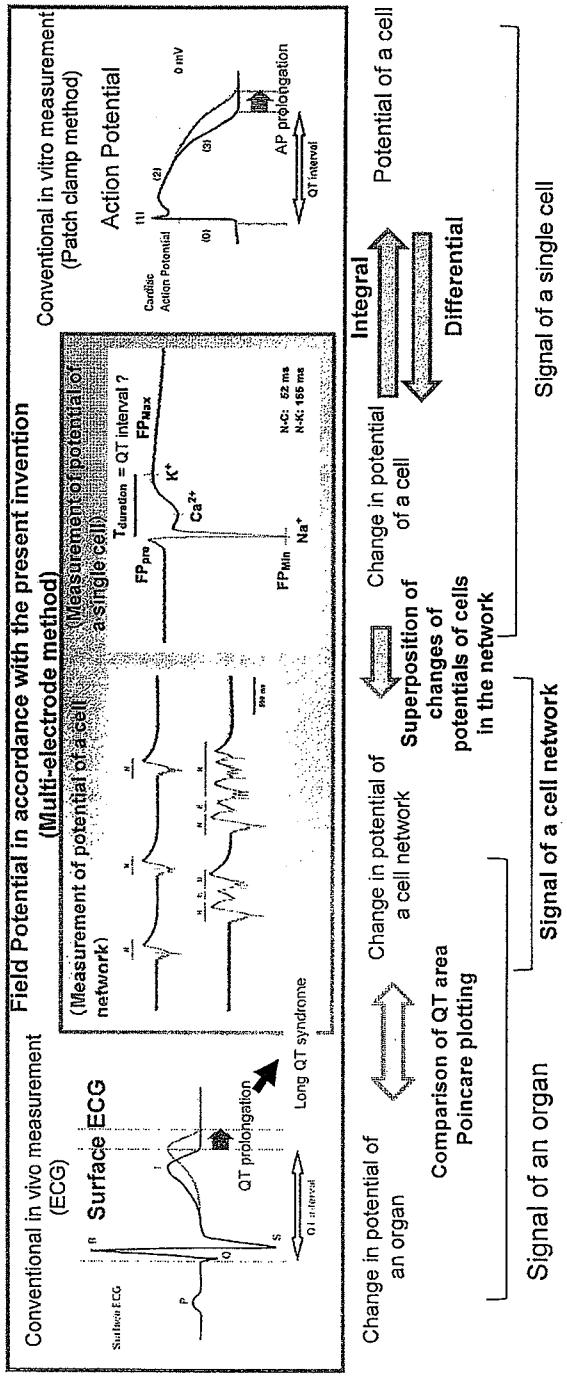

Field Potential in accordance with the present invention
(Multi-electrode method)
Conventional in vivo measurement
(ECG)
Surface ECG
QT prolongation
Long QT syndrome
(Measurement of potential of a cell network)
(Measurement of potential of a single cell)
Conventional in vitro measurement
(Patch clamp method)
Action Potential
AP prolongation
QT interval
Change in potential of an organ
Change in potential of a cell network
Comparison of QT area
Poincare plotting
Signal of an organ
Signal of a cell network
Superposition of changes of potentials of cells in the network
Change in potential of a cell
Integral
Differential
Potential of a cell
Signal of a single cell

Fig. 26

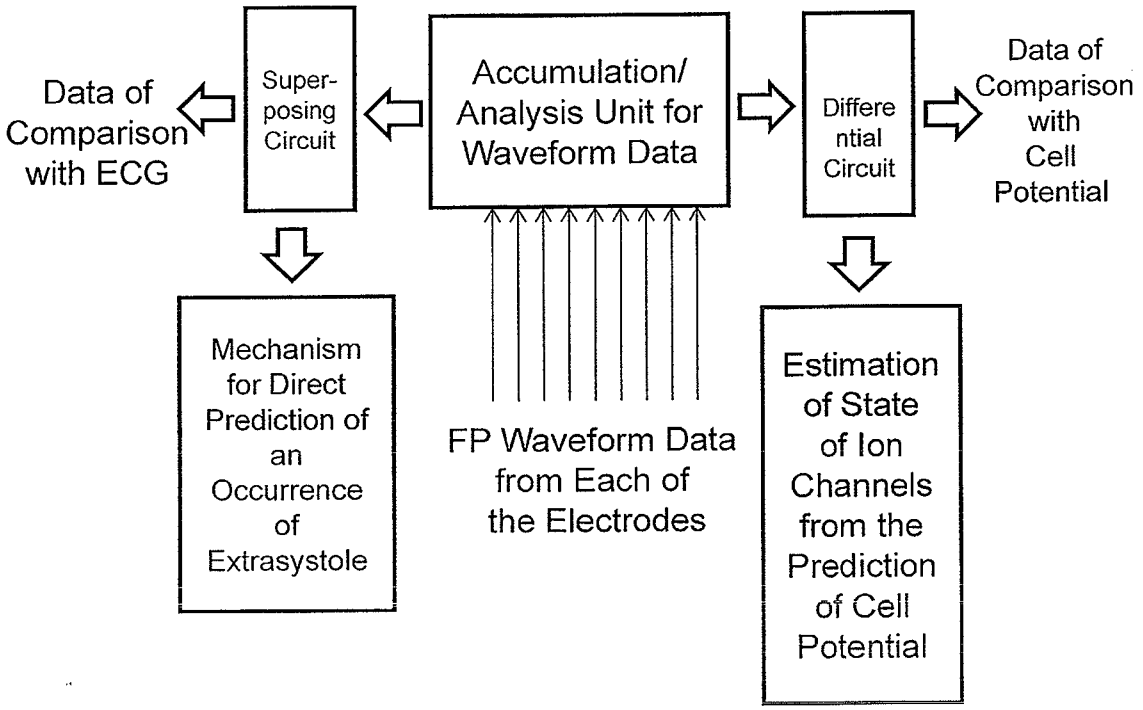

Data of Comparison with ECG
Super-posing Circuit
Accumulation/ Analysis Unit for Waveform Data
Differe ntial Circuit
Data of Comparison with Cell Potential
Mechanism for Direct Prediction of an Occurrence of Extrasystole
FP Waveform Data from Each of the Electrodes
Estimation of State of Ion Channels from the Prediction of Cell Potential

Fig. 27

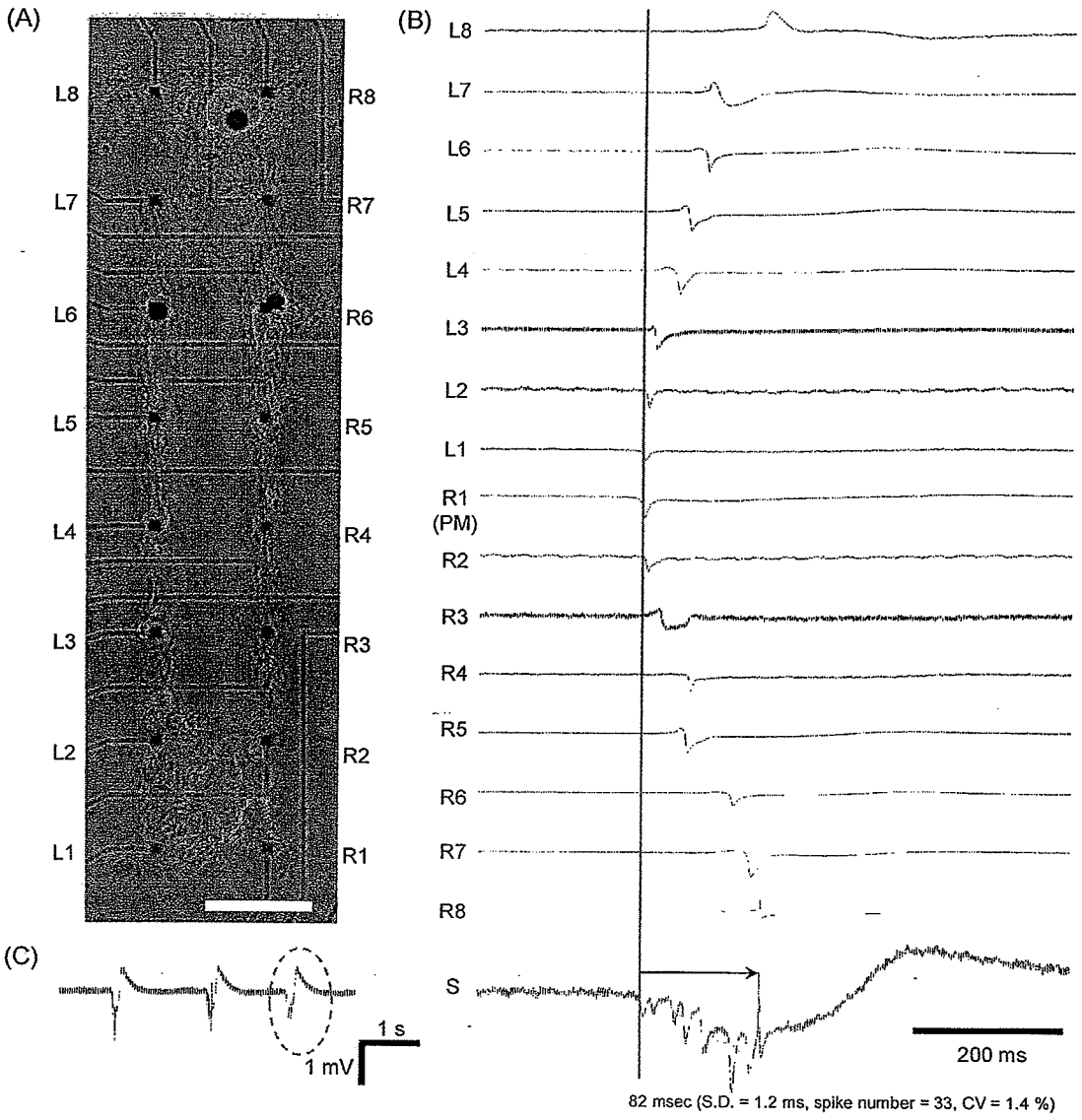

(A)
L8
L7
L6
L5
L4
L3
L2
L1
R8
R7
R6
R5
R4
R3
R2
R1
(B)
L8
L7
L6
L5
L4
L3
L2
L1
R1
(PM)
R2
R3
R4
R5
R6
R7
R8
S
200 ms
82 msec (S.D. = 1.2 ms, spike number = 33, CV = 1.4 %)
(C)
1 s
1 mV

Fig. 28

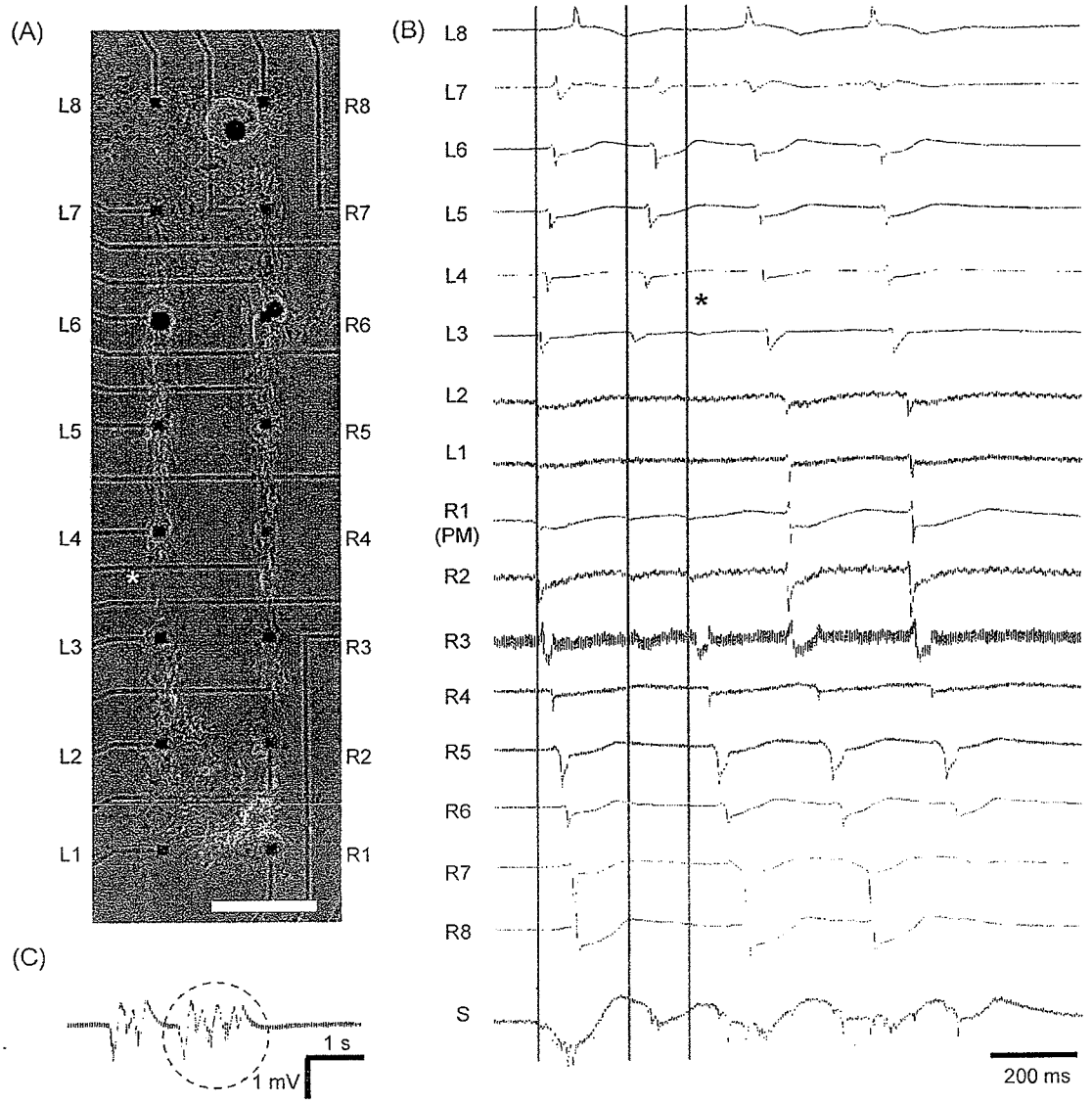

(A)
L8
L7
L6
L5
L4
L3
L2
L1
R8
R7
R6
R5
R4
R3
R2
R1
(B)
L8
L7
L6
L5
L4
L3
L2
L1
R1
(PM)
R2
R3
R4
R5
R6
R7
R8
S
200 ms
(C)
1 s
1 mV

Fig. 29

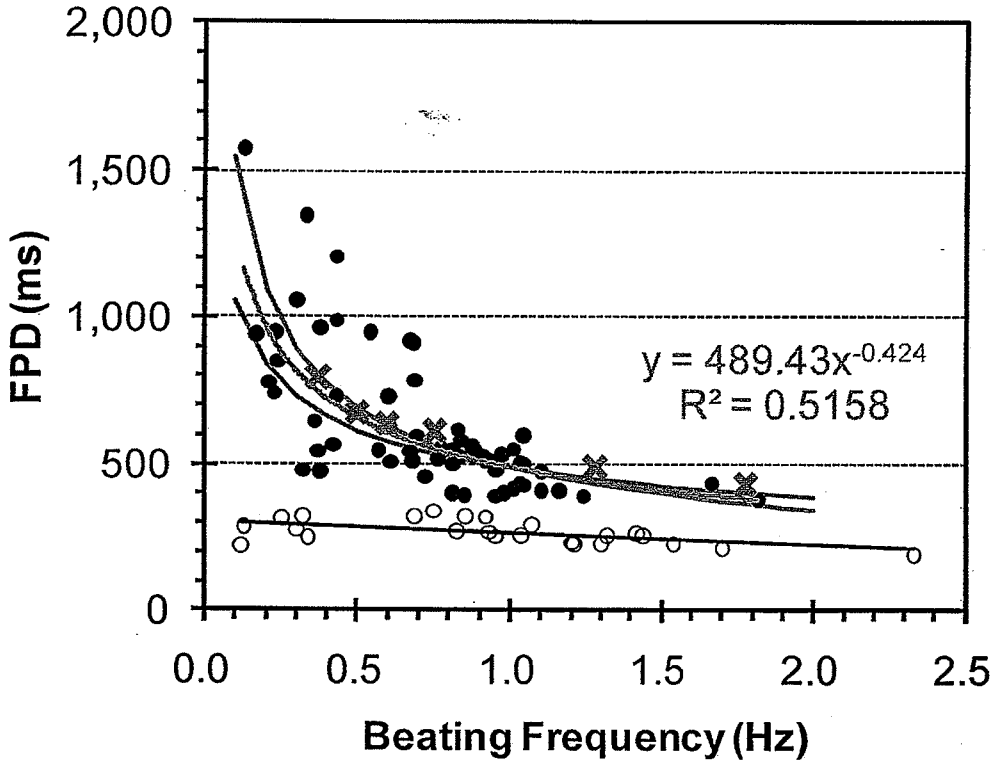

2,000
1,500
1,000
500
0
FPD (ms)
y = 489.43x-0.424
R² = 0.5158
0.0
0.5
1.0
1.5
2.0
2.5
Beating Frequency (Hz)

Fig. 30

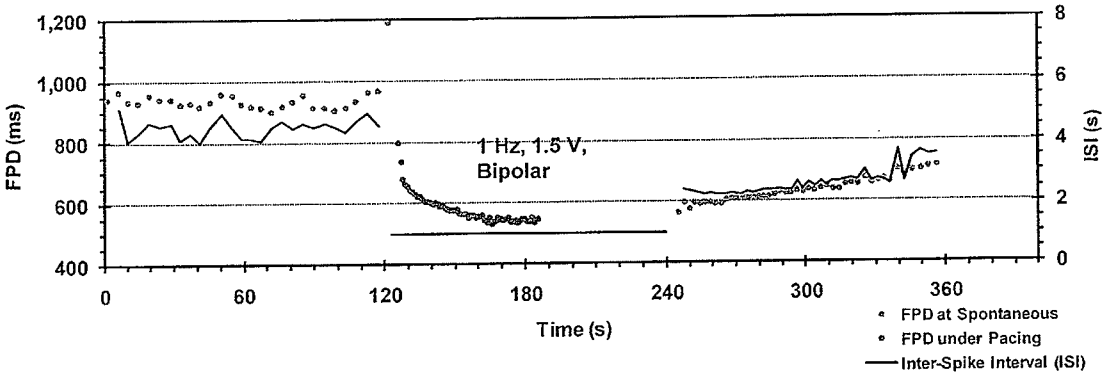
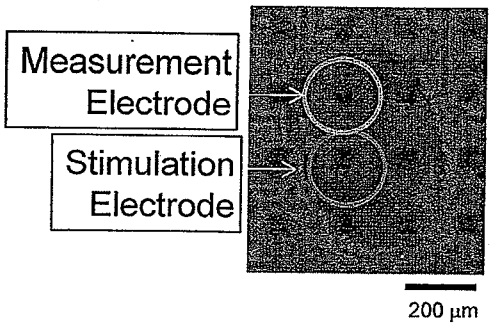

1,200
1,000
800
600
400
FPD (ms)
8
6
4
2
0
ISI (s)
0
60
120
180
240
300
360
Time (s)
1 Hz, 1.5 V,
Bipolar
FPD at Spontaneous
FPD under Pacing
Inter-Spike Interval (ISI)

Fig. 31

(a)


Measurement Electrode
Stimulation Electrode
200 μm

(b)

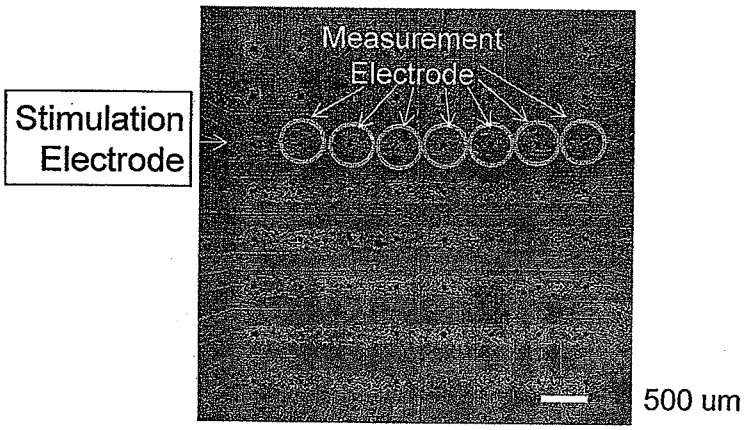

Measurement Electrode
Stimulation Electrode
500 um

Fig. 32

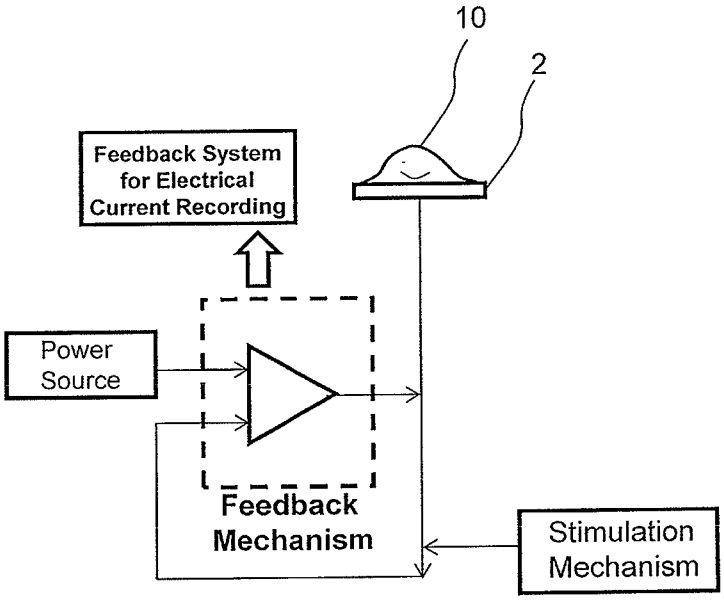

10
2
Feedback System for Electrical Current Recording
Power Source
Feedback Mechanism
Stimulation Mechanism

Fig. 33

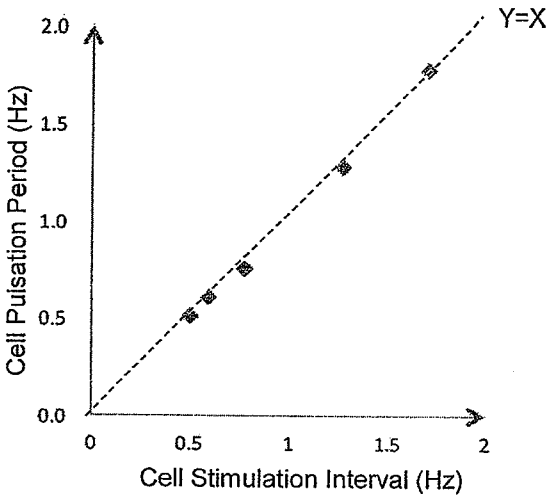

Y=X
2.0
1.5
1.0
0.5
0.0
Cell Pulsation Period (Hz)
0
0.5
1
1.5
2
Cell Stimulation Interval (Hz)

Fig. 34

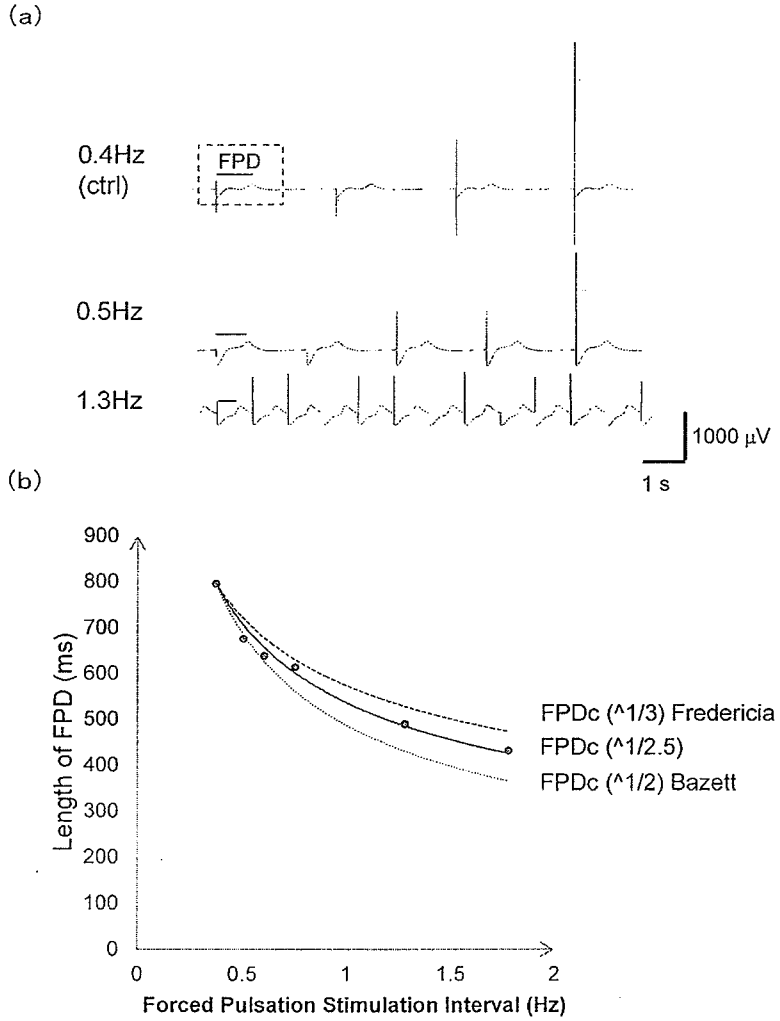

(a)
0.4Hz
(ctrl)
FPD
0.5Hz
1.3Hz
1000 μV
1 s
(b)
900
800
700
600
500
400
300
200
100
0
Length of FPD (ms)
0
0.5
1
1.5
2
Forced Pulsation Stimulation Interval (Hz)
FPDc (^1/3) Fredericia
FPDc (^1/2.5)
FPDc (^1/2) Bazett

Table. 1 Heart rate correction of beating frequency, field potential duration

	Beating Frequency				FPD			FPDc		
	Average (Hz)	S.D. (Hz)	C.V. (%)	Spike No.	Average (ms)	S.D. (ms)	C.V. (%)	Average (ms)	S.D. (ms)	C.V. (%)
0.4 Hz (ctrl)	0.37	0.01	1.5	110	795.3	13.9	1.7	536.7	9.7	1.8
0.5 Hz	0.50	0.00	0.0	94	675.6	6.6	1.0	513.7	5.0	1.0
0.6 Hz	0.60	0.00	0.0	142	638.5	7.5	1.2	521.8	6.1	1.2
0.8 Hz	0.75	0.00	0.0	189	613.7	8.6	1.4	547.8	7.7	1.4
1.3 Hz	1.28	0.00	0.0	253	489.4	11.5	2.4	540.2	12.7	2.4
1.8 Hz	1.78	0.00	0.0	306	431.2	9.1	2.1	542.8	11.4	2.1

Fig. 35

Fig. 36

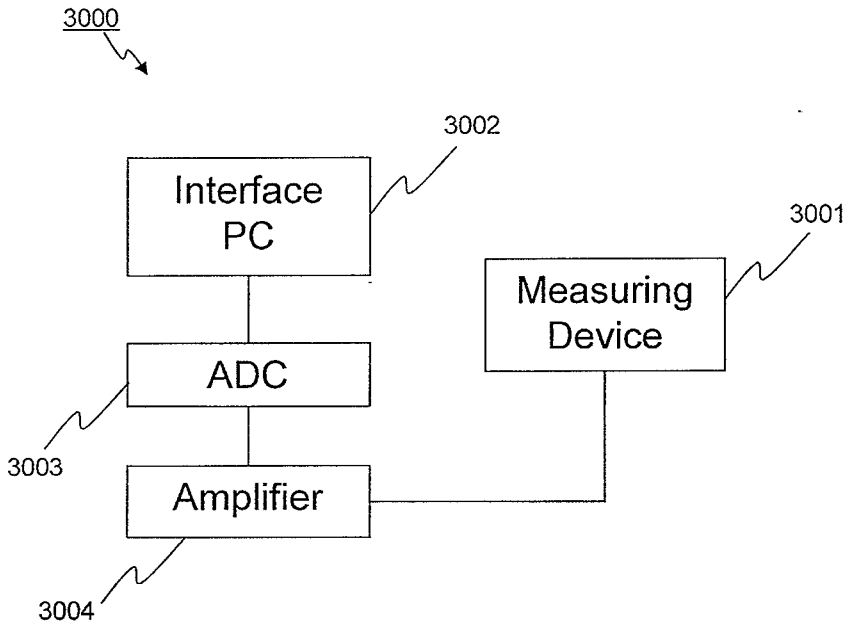
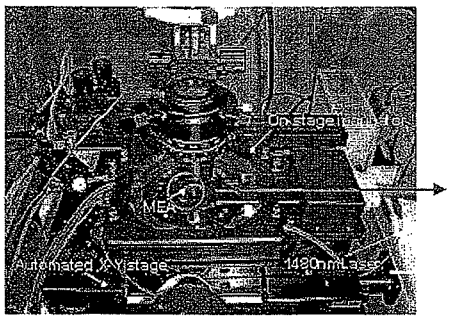
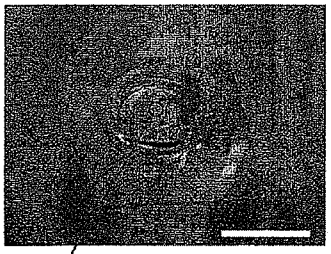

3000
3002
Interface PC
3001
Measuring Device
ADC
3003
Amplifier
3004

Fig. 37

A. Core of the system



B. MEA Chip



C. hES CM on MEA Chip

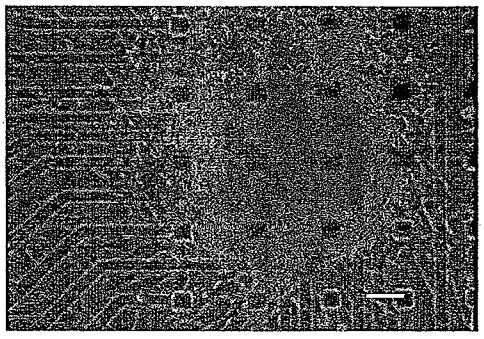


Fig. 38

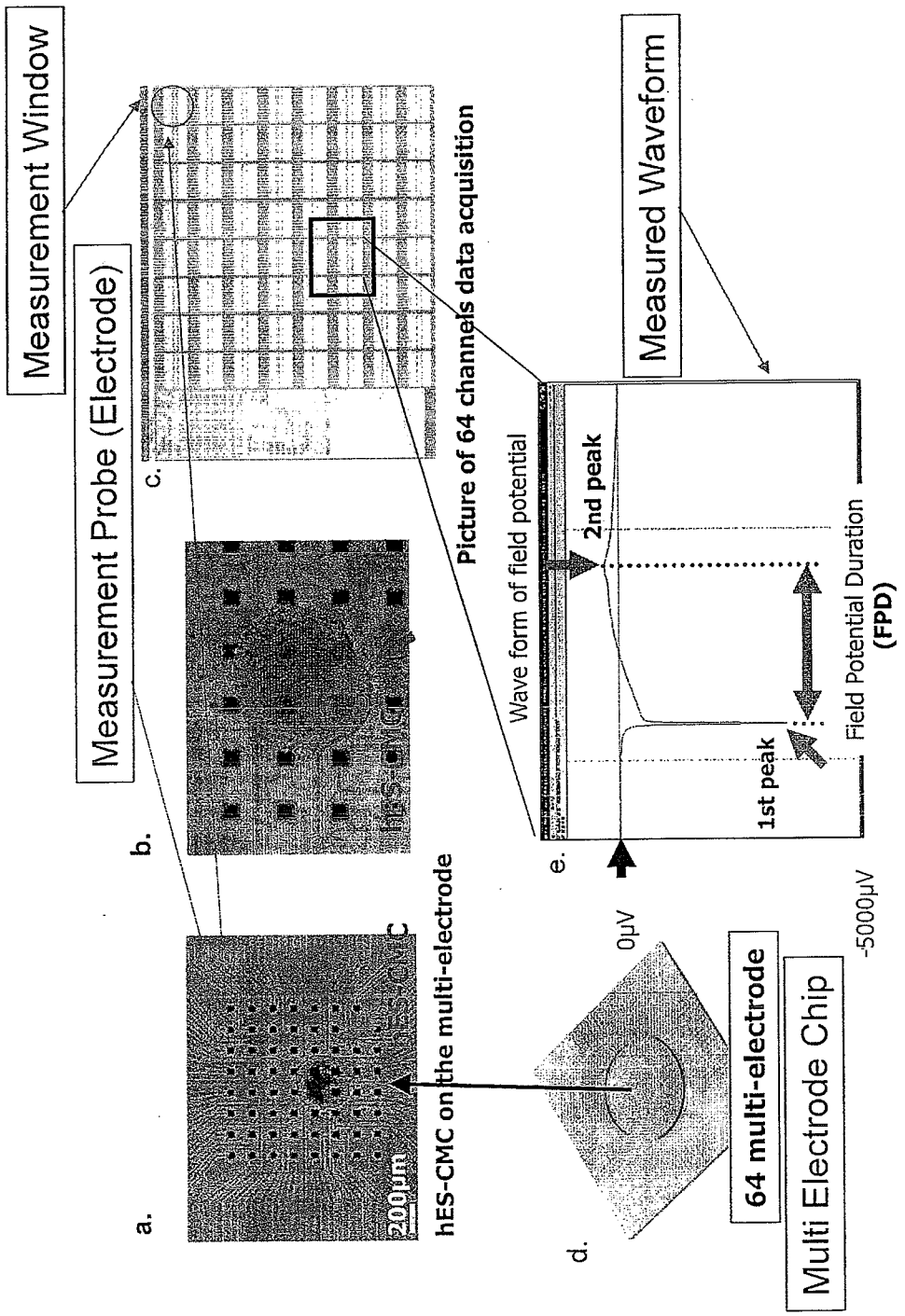

Measurement Window
Measurement Probe (Electrode)
a.
b.
c.
d.
e.
200μm
hES-CMC
hES-CMC on the multi-electrode
Picture of 64 channels data acquisition
64 multi-electrode
Multi Electrode Chip
0μV
-5000μV
Wave form of field potential
Measured Waveform
2nd peak
1st peak
Field Potential Duration
(FPD)

Fig. 39

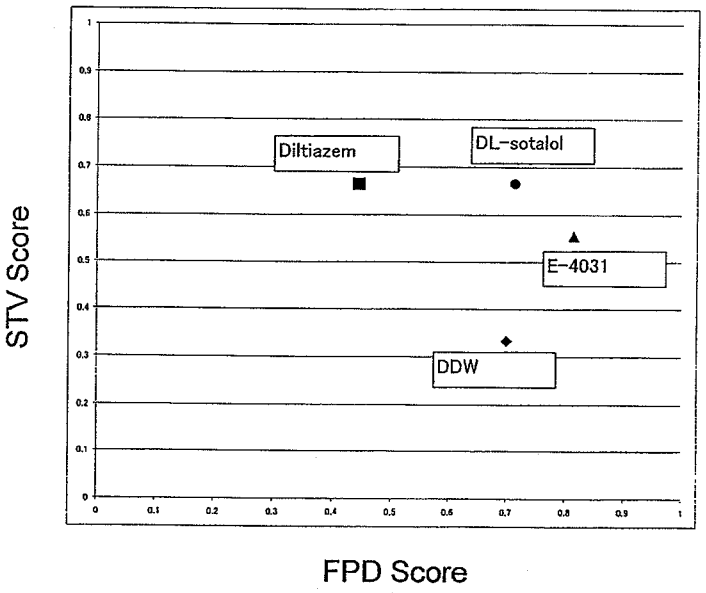

STV Score
FPD Score
Diltiazem
DL-sotalol
E-4031
DDW
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1

Fig. 40(a)

(a)

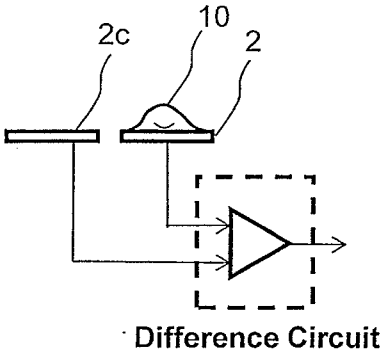


Fig. 40(b)

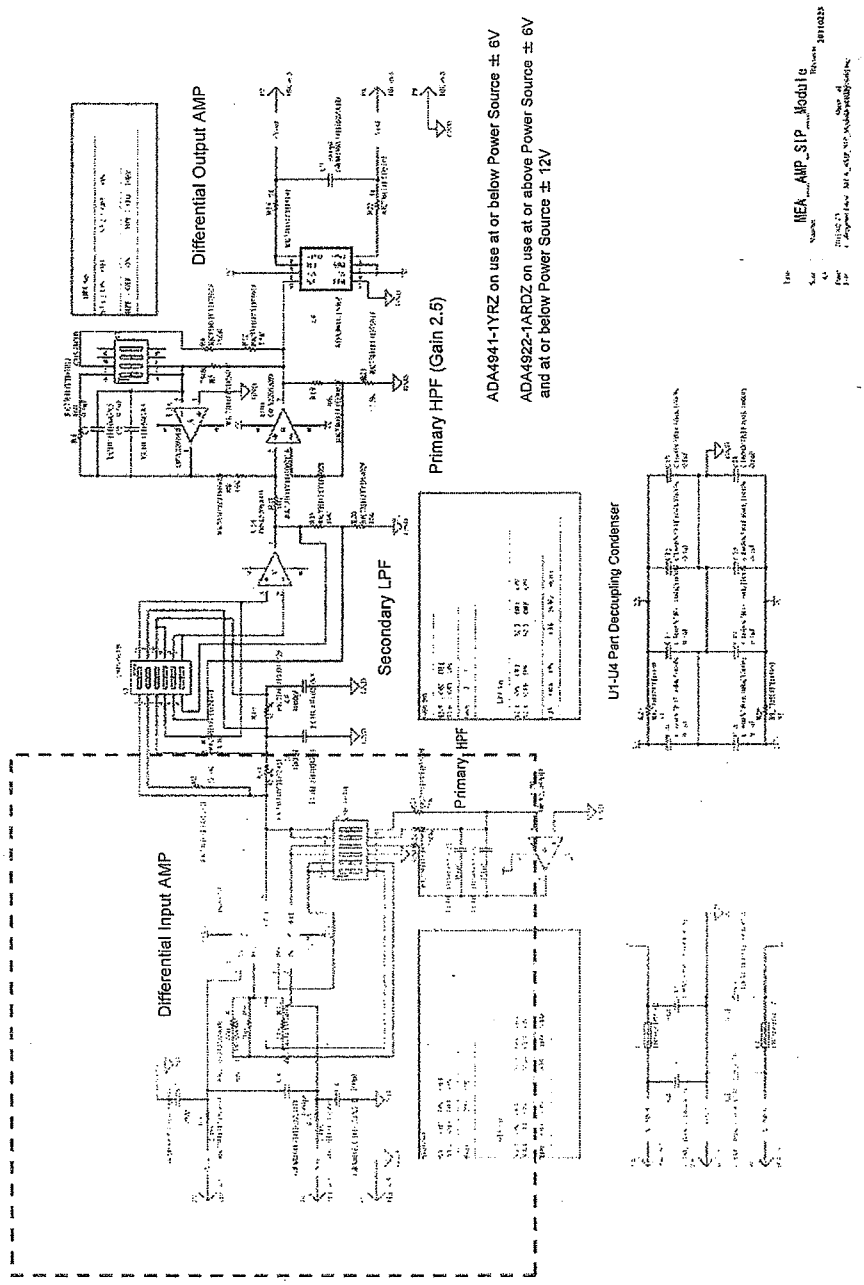

Differential Output AMP
Primary HPF (Gain 2.5)
ADA4941-1YRZ on use at or below Power Source ± 6V
ADA4922-1ARDZ on use at or above Power Source ± 6V
and at or below Power Source ± 12V
Secondary LPF
U1-U4 Pari Decoupling Condenser
Primary HPF
Differential Input AMP
MEA_AMP_SIP_Module

Fig. 40(c)

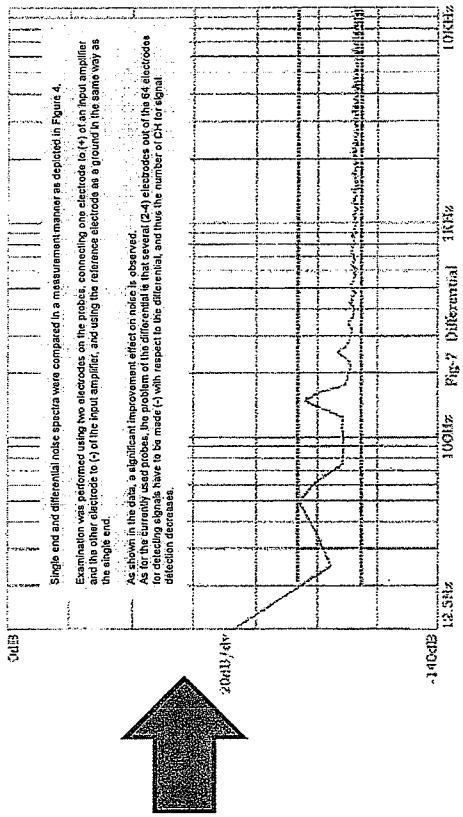

Single end and differential noise spectra were compared in a measurement manner as depicted in Figure 4.
Examination was performed using two electrodes on the probes, connecting one electrode to (+) of an input amplifier and the other electrode to (-) of the input amplifier, and using the reference electrode as a ground in the same way as the single end.
As shown in the data, a significant improvement effect on noise is observed.
As for the currently used probes, the problem of this differential is that several (2-4) electrodes out of the 64 electrodes for detecting signals have to be made (-) with respect to the differential, and thus the number of CH for signal detection decreases.
0dB
20dB/div
-140dB
12.5Hz
100Hz
1KHz
10KHz
Fig-7 Differential
0dB
20dB/div
-140dB
12.5Hz
100Hz
1KHz
10KHz
Fig-6 Single-ended

Fig. 41

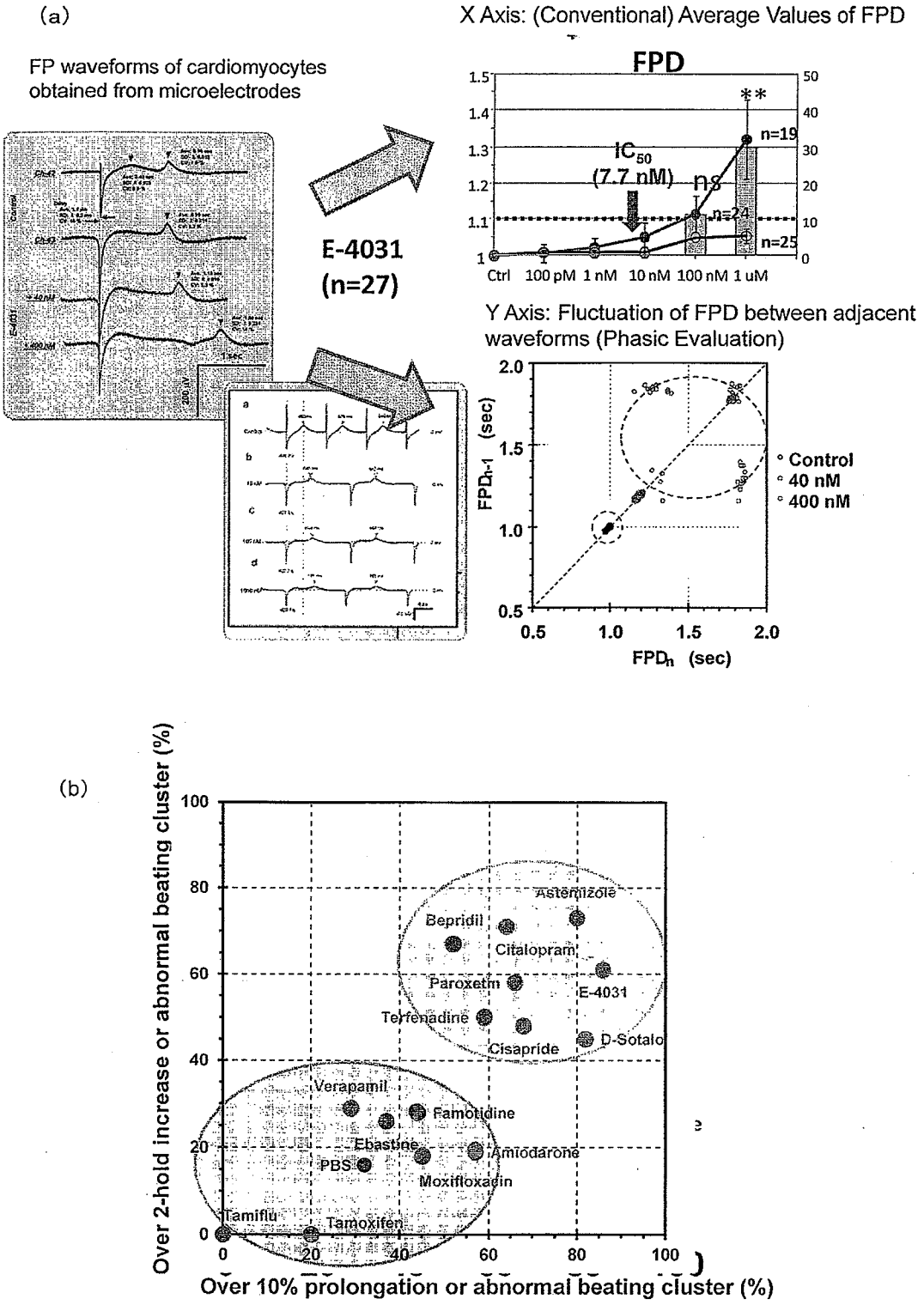

(a)
X Axis: (Conventional) Average Values of FPD
FP waveforms of cardiomyocytes obtained from microelectrodes
E-4031
(n=27)
FPD
IC50
(7.7 nM)
ns
**
n=19
n=24
n=25
Ctrl 100 pM 1 nM 10 nM 100 nM 1 uM
Y Axis: Fluctuation of FPD between adjacent waveforms (Phasic Evaluation)
FPDn-1 (sec)
FPDn (sec)
Control
40 nM
400 nM
(b)
Over 2-hold increase or abnormal beating cluster (%)
Over 10% prolongation or abnormal beating cluster (%)
Astemizole
Bepridil
Citalopram
Paroxetin
E-4031
Terfenadine
Cisapride
D-Sotalo
Verapamil
Famotidine
Ebastine
Amiodarone
PBS
Moxifloxacin
Tamiflu
Tamoxifen

Fig. 42

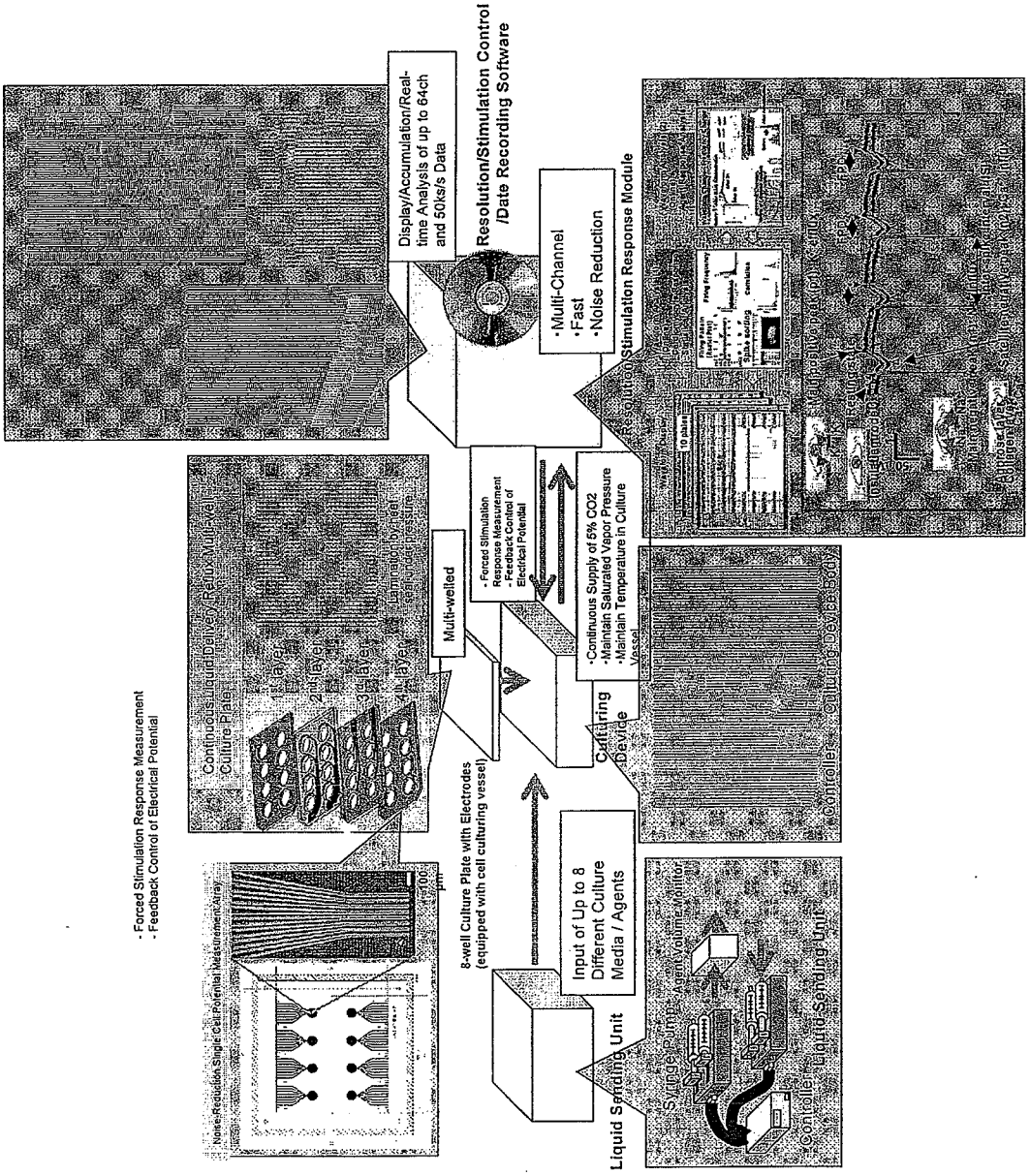

- Forced Stimulation Response Measurement
- Feedback Control of Electrical Potential
Noise-Reduction Single Cell Potential Measurement Array
100 μm
8-well Culture Plate with Electrodes (equipped with cell culturing vessel)
Liquid Sending Unit
Input of Up to 8 Different Culture Media / Agents
Culturing Device
Multi-welled
- Forced Stimulation Response Measurement - Feedback Control of Electrical Potential
•Continuous Supply of 5% CO2
•Maintain Saturated Vapor Pressure
•Maintain Temperature in Culture Vessel
Resolution/Stimulation Control /Date Recording Software
Display/Accumulation/Real-time Analysis of up to 64ch and 50ks/s Data
•Multi-Channel
•Fast
•Noise Reduction
Stimulation Response Module

Fig. 43

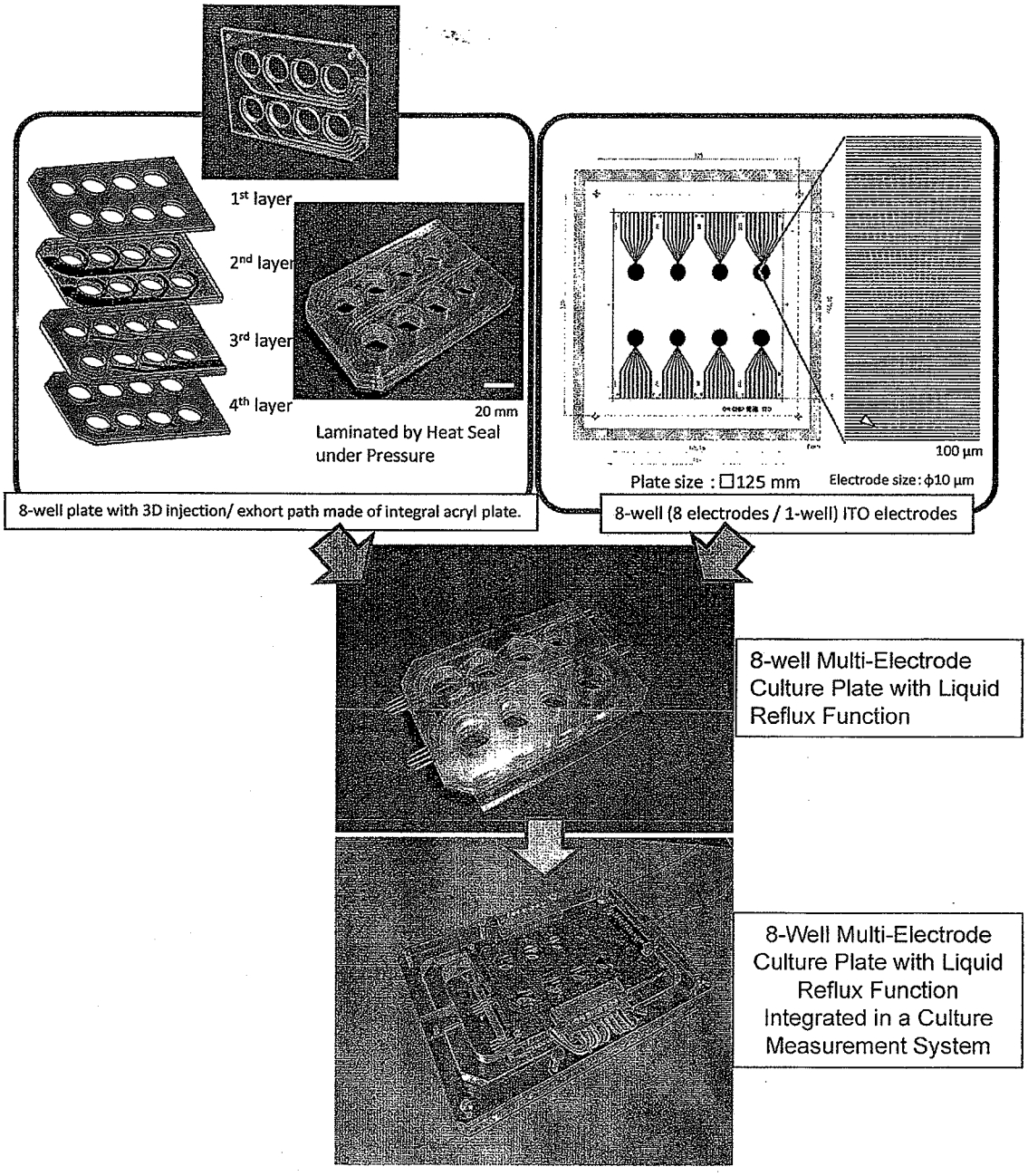

1st layer
2nd layer
3rd layer
4th layer
20 mm
Laminated by Heat Seal under Pressure
8-well plate with 3D injection/ exhort path made of integral acryl plate.
100 µm
Plate size : □125 mm
Electrode size: ϕ10 µm
8-well (8 electrodes / 1-well) ITO electrodes
8-well Multi-Electrode Culture Plate with Liquid Reflux Function
8-Well Multi-Electrode Culture Plate with Liquid Reflux Function Integrated in a Culture Measurement System

Fig. 44

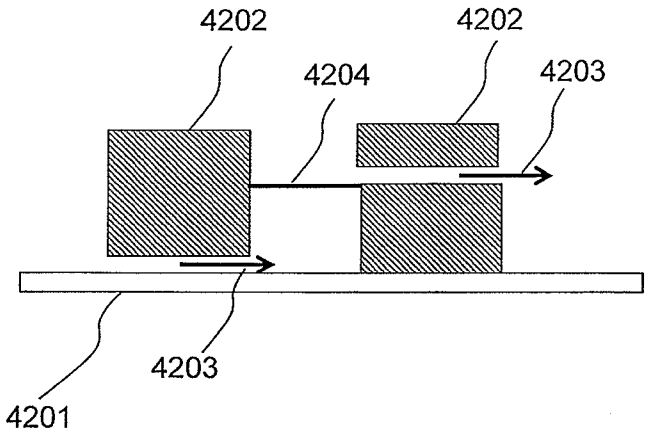


Fig. 45

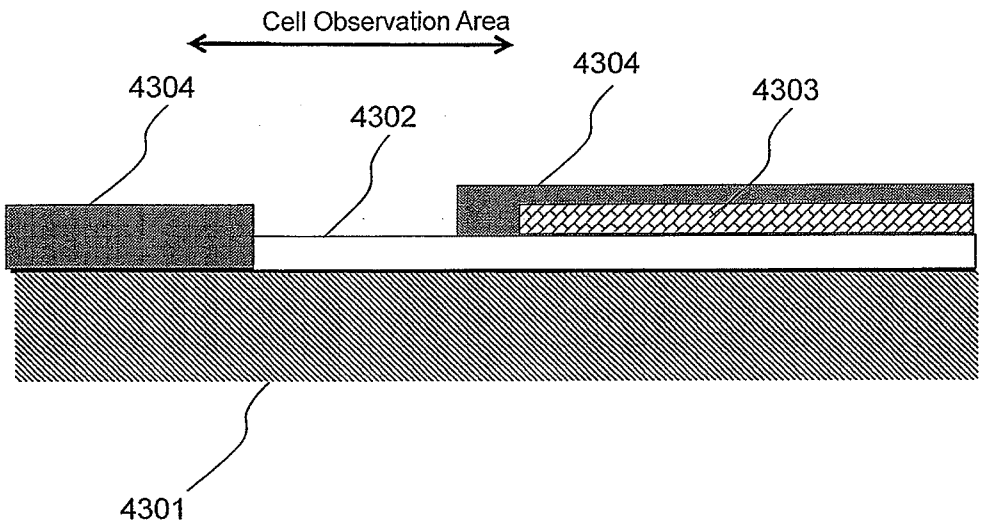
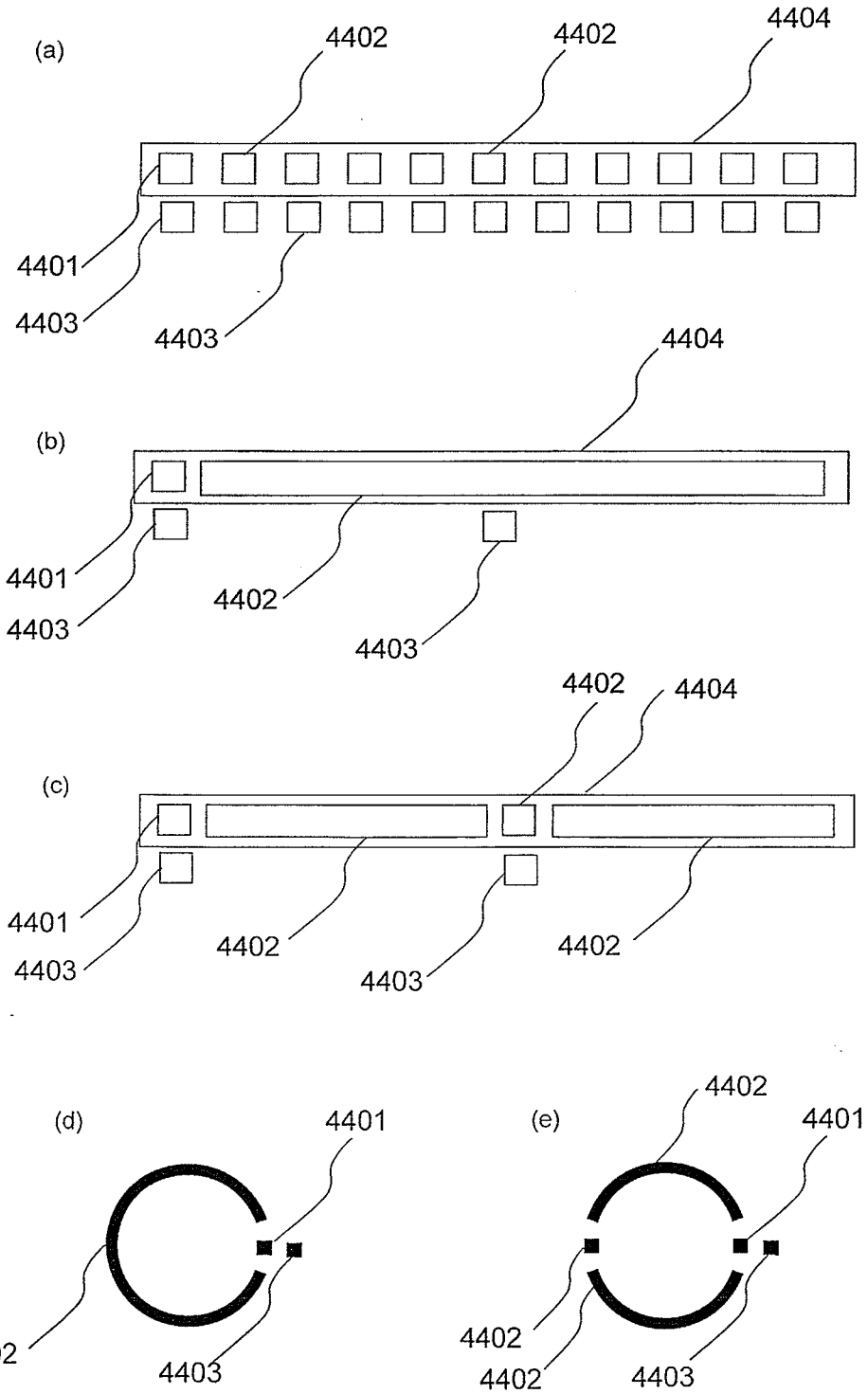


Fig. 46


(a)
4402
4402
4404
4401
4403
4403
(b)
4404
4401
4403
4402
4403
(c)
4402
4404
4401
4403
4402
4403
4402
(d)
4401
4402
4403
(e)
4402
4401
4402
4402
4403