Multi-TECC-24CH

Front view

- 6 channels (boards).

- Each channel has:
  - Inputs for electrodes of 4 wells.
  - Connector to monitor I and V on oscill.
  - Reset button
  - 2 LEDS to indicate activity of MTECC.

- In this picture the inputs are connected to 16 way connectors with flat cables that fit in the electrode manifold.

Back view

- USB connection to PC.
- Power input : 8 VDC – 3.5A.
- 2 inputs for logical signals. For instance a microswitch of the electrode manifold.
- 2 outputs (relay contacts)

Multi-TECC-96CH

- 24 MTECC boards with each 4 channels.

- Each boardl has:
  - Inputs for 4 wells.
  - Connector to monitor I and V on oscilloscope
  - Reset button
 - 2 LEDS to indicate activity of MTECC

Multi TECC PCB

IMPORTANT:
- Device is sensitive to static electricity.
- When handling the board, make sure that you are connected to GROUND.
- Do not put the PCB on surfaces that could have static electrical charges.
   For instance: plastic. Use pink, antistatic foil.

Features:

The Multi-TECC board has a main part with the microcontroller, SDRAM and several digital circuits to make communication with the I2C bus (connected on the backplane to a USB interface) and the **4 analog channels** on the PCB. The inputs of the analog channels are connected to the electrodes
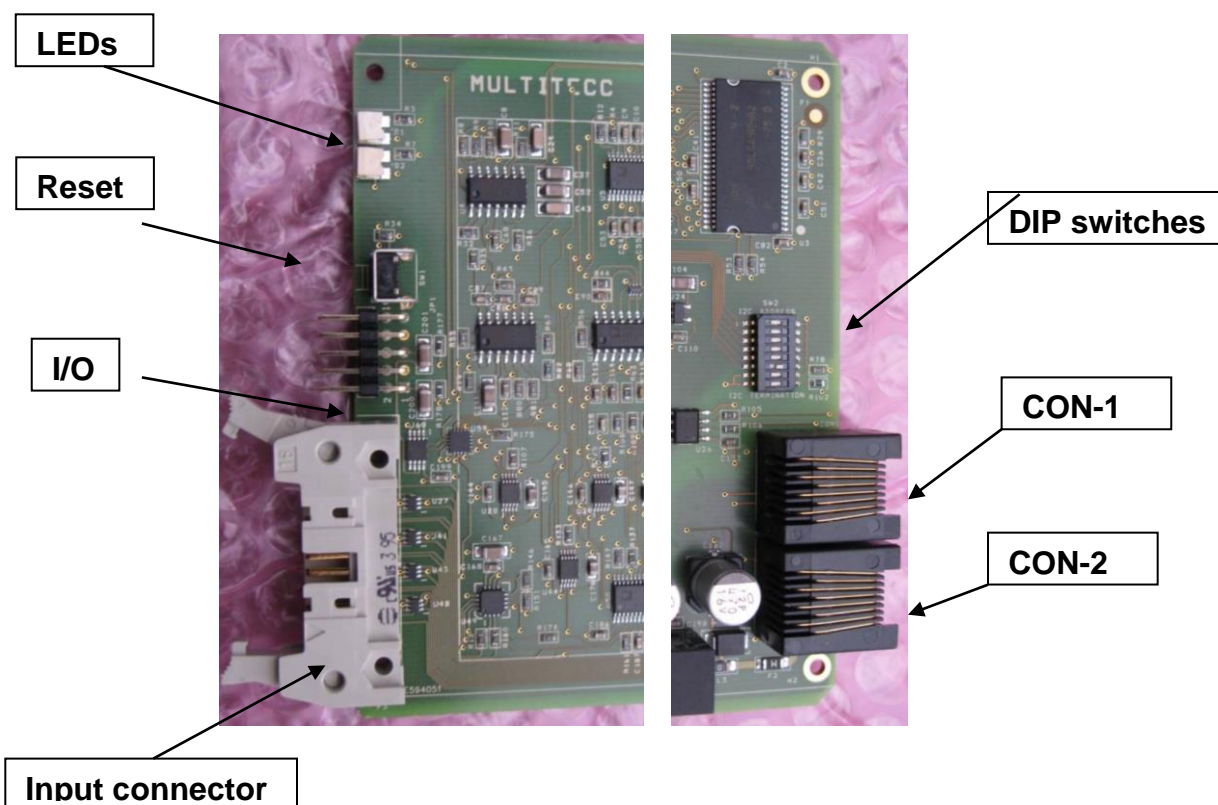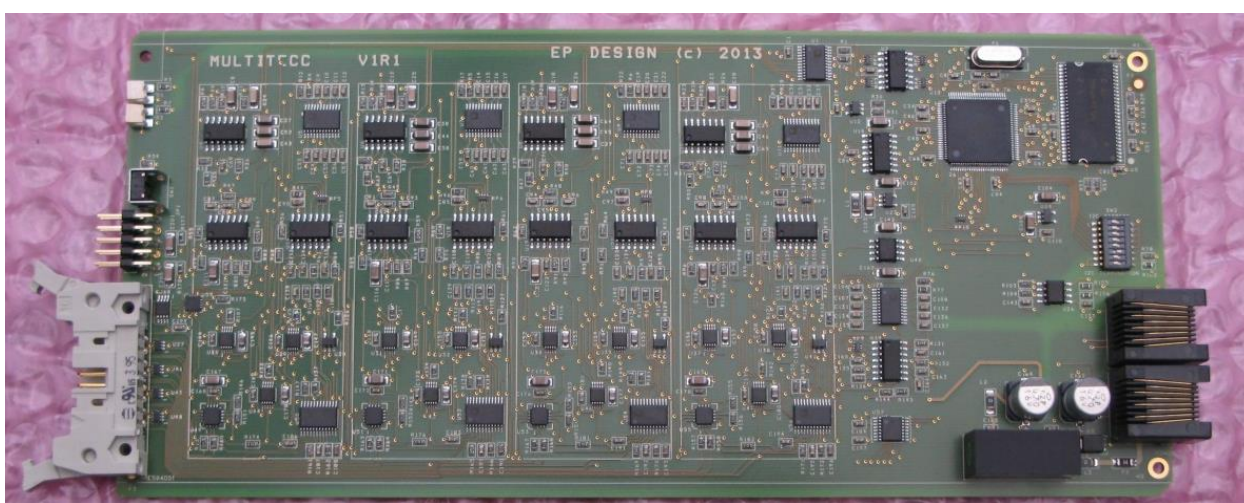
1 – Input connector (at front of PCB).

Current and voltage electrodes are connected to the clamp circuit with a 16 way IDC connector. Standard 16 way ribbon cable can be used for making the connection.

PIN #	Connection	
1	Ib4	I-basolateral - 4
2	Ia4	I-Apical - 4
3	Vb4	V-basolateral - 4
4	Va4	V-Apical - 4
5	Ib3	I-basolateral - 3
6	Ia3	I-Apical - 3
7	Vb3	V-basolateral - 3
8	Va3	V-Apical - 3
9	Ib2	I-basolateral - 2
10	Ia2	I-Apical - 2
11	Vb2	V-basolateral - 2
12	Va2	V-Apical - 2
13	Ib1	I-basolateral - 1
14	Ia1	I-Apical - 1
15	Vb1	V-basolateral - 1
16	Va1	V-Apical - 1

2 – Digital and analog I/O connector (10 way header at front).

PIN #	Signal	
1	I-Out	Monitor I
2	+5V	Power
3	V-Ref	DC voltage
4	GND	
5	V-Out	Minitor V
6	+3.3V	Power
7	Port	Processor port
8	PDI-Clock	Ser. port
9	Port	Processor port
10	PDI-Data	Ser. port

3 – Reset button.   Used to reset manually the microcontroller.

4 – LEDs GREEN and RED

5 – Digital and analog I/O connector (10 way header at front).

PIN #	Signal	
1	I-Out	Monitor I
2	+5V	Power
3	V-Ref	DC voltage
4	GND	
5	V-Out	Minitor V
6	+3.3V	Power
7	Port	Processor port
8	PDI-Clock	Ser. port
9	Port	Processor port
10	PDI-Data	Ser. port

DIP Switches

Are used to set **I2C slave address** and **I2C signals (SCL and SDA).**

Slave address is set in binary code.

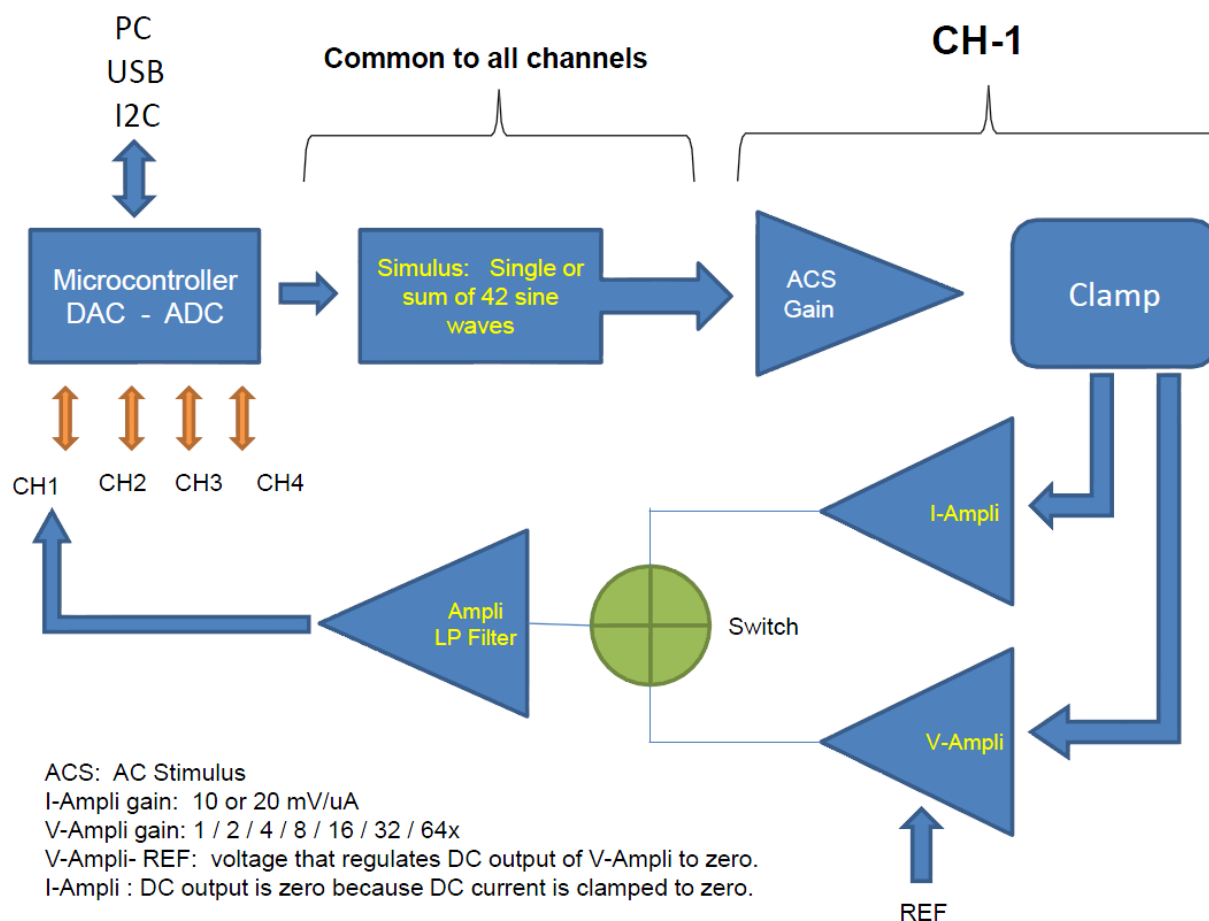In the example below, the slave address is set to 3.

		Example
8-9	SCL	Off
7-10	SDA	Off
6-11	Bit-0	ON
5-12	Bit-2	ON
4-13	Bit-3	OFF
3-14	Bit-4	OFF
2-15	Bit-5	OFF
1-16	Bit-6	OFF

CON-1 and CON-2

Pin #

1	**SDAP**
2	**GND-I2C**
3	**VCC-I2C**
4	**SCLP**
5	**PWS-Ext (6-9V)**
6	**PWS-Ext (6-9V)**
7	**PWS-GND**
8	**Not used**

General Layout.

PC
USB
I2C

Common to all channels

CH-1

Microcontroller
DAC - ADC

Simulus: Single or sum of 42 sine waves

ACS Gain

Clamp

CH1 CH2 CH3 CH4

I-Ampli

Ampli LP Filter

Switch

V-Ampli

ACS: AC Stimulus
I-Ampli gain: 10 or 20 mV/uA
V-Ampli gain: 1 / 2 / 4 / 8 / 16 / 32 / 64x
V-Ampli- REF: voltage that regulates DC output of V-Ampli to zero.
I-Ampli : DC output is zero because DC current is clamped to zero.

REF

DIGITAL POTENTIOMETERS

Digital potentiometers of type AD5271 20K are used to make important adjustments of the gain of different amplifiers in MTECC. The potentiometer has 256 possible discrete code settings ranging from 0 setting to 255.

1 - R_{AP} - Resistance in series with the membrane. R_{AP} is located at the apical side of the epithelium and is important to prevent oscillations of the clamp circuit. R_{AP} is used on each channel of the MTECC board.

2 – R_{SC} – Stimulus Control. Stimulus of each channel can be adjusted to obtain the requested peak-to-peak current (Ipp) of the sine wave current used to record the resistance. R_{SC} depends on Ipp and the gain of the current to voltage converter.

3 – R-IOG – Used to adjust the gain of the AC sine wave current (I_{AC}). R-IOG (current output gain) is automatically set by the MCU to obtain an optimal resolution of the analog to digital conversion. Setting of R-IOG is based in I_{PP} and the gain of the I to V converter (IV_{gain}).

4 – R-VOG – Used to adjust the gain of the AC sine wave voltage. (V_{AC}). Because IAC and VAC are successively recorded, a common amplifier and Digi-pot is used. The value of R-VOG is specified by the user in the RTX table.

AC voltage signal.

Output voltage of voltage input amplifier (INAMP) is additionally amplified. An amplifier with a digital potentiometer in the feedback is used for this purpose. The gain of this amplifier is denoted as $VOG = R_{VOG} / 1000$.
VOG is specified by the user in the RTX table.
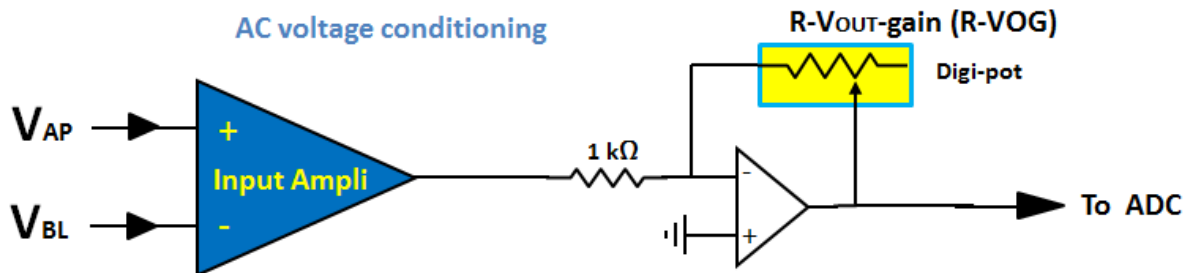
AC current signal.

Output voltage of current to voltage converter (I to V converter) is additionally amplified. An amplifier with a digital potentiometer (Digi-pot) in the feedback is used for this purpose. The setting of the Digi-pot is denoted as **R_{IOG}** . The gain of this amplifier is denoted as **$IOG = R_{IOG} / 1000$**. R-digipot is maximally 20k and accurate settings are obtained in the range of 2k and 20k. Consequently, IOG should be adjusted in the range of 2x to 20x.
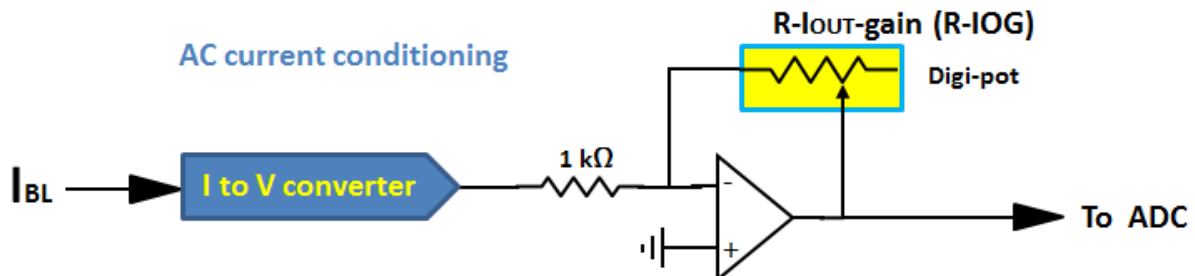
Taking into consideration following parameters, the optimal setting for IOG is calculated:

    1 – Maximum voltage range of signal applied to the ADC.
        Note that maximal value of V_{ADC} (V_{ADC}-max) = 2300mV.
    2 – Gain of an amplifier used after filtering the I_{AC} signal, which is 2x.
    3 – The requested peak-to-peak current (I_{pp}, in μA) in the RTX table.
    4 – The gain of current to voltage converter (IV_{gain}) in RTX table.

      IV_{gain} is either 10 or 20 mV/μA.

$V_{ADC} = 2 \times I_{pp} \times IV_{gain} \times IOG$

$IOG = V_{ADC}\text{-max} / (2 \times I_{pp} \times IV_{gain}) = 2300 / (2 \times I_{pp} \times IV_{gain})$

$R_{IOG} = 1000 \times IOG$

However the maximal value of R_{IOG} = 19900 and IOG is limited to a maximum gain of 19.9.

AC stimulus : Ipp

The stimulus that is used to measure the R_T is a sine wave current (I_{pp}). It is generated by the digital to analog converter (DAC) in the microcontroller. The output of the DAC (V_{DAC}) is filtered and scaled so that the requested **I_{pp}** value in the RTX table.   Scaling is done by

  1 - adjusting the voltage output of the DAC (V_{DAC}). V_{DAC} is a common
      source for the 4 channels on the MTECC board.
  2 - adjusting the signal for each channel is done with Digital potentiometer (R_{digi})
The V_{DAC} signal is attenuated with $R_{SC} = R_{digi}$ (Max 20k) at the feedback amplifier input of the clamp.

Stimulus Control
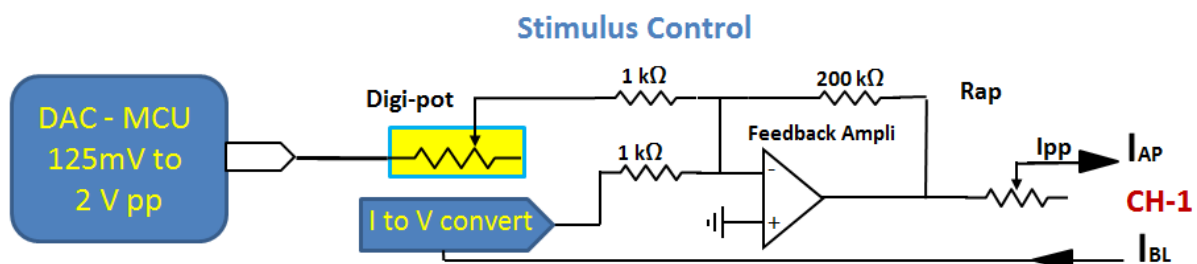
RTX table.

Parameters on each line:
Threshold RT,   INAMP gain,   Voltage Output Gain (VOG), I$_{PP}$, IV gain.

Parameters are separated by comma, OR, by TABs or blanc chars.
Length of table: 16 lines.   Line 16: not used,   put all values at line 16 = 0.
RT value of each line < RT of previous line.
Procedure:
- After reset or startup: first RT measurement is done with settings on 1st line.
- The recorded RT value (**RT-rec**) is used to select the line in the RTX table for the
   next measurement: line where **RT-rec > Threshold RT** is chosen.
- If overflows are detected: 1st line of RTX table will be used for next measurement.

Software (Check RTX Table Settings.EXE) is available to test correct settings to avoid overflows in amplifiers.

System RTX table.

This is the standard RTX table that remains in the code (memory) of the microcontroller. After reset, the software will use this table to set the hardware parameters (INAMP gain,   Voltage Output Gain (VOG), I$_{PP}$, IV gain) for recording Rt/Vt. The system RTX table has 8 lines that are used. The subsequent 8 lines are all zeros. For Firmware version 13 and higher:

RT-Treshold (Ohm)	Input V-gain	V-Output-Gain	Ipp (uA)	I to V gain (mV/uA)
30000	1	8	0.5	20
3700	4	8	1	20
240	8	8	5	20
27	16	5	30	10
0	0	0	0	0
0	0	0	0	0

User RTX table

This table can be loaded into the memory of the microcontroller. This table should have the same structure has the system RTX table and have 16 lines. The last line (#16) should have all zeros. It is highly recommended to use the software (Check RTX Table Settings.EXE) to determine optimal RTX settings for the expected range of Rt/Vt values.   Example of a user RTX table:

RT-Treshold (Ohm)	Input V-gain	V-Output-Gain	Ipp (uA)	I to V gain (mV/uA)
3700	4	8	1	20
240	8	8	5	20
27	16	5	30	10
0	0	0	0	0

Check RTX Table Settings.EXE : Example

Purpose: test correct settings to avoid overflows in amplifiers.

LIMITS: Overflows occur for VPP at ADC, V_{ADC} > 2350mV
          Low level V_{ADC} < 125mV are indicated as **warning**.

The **user interface** has 2 parts:
- **Input:**   **RTX Table parameters** that you want to verify.
          These are the values of one of parameters in one of the lines in
          the RTX table. Below we test the validity of parameters associated
          with RT_Treshold = 240 Ohm.

- **Output: The calculated parameters**.
     - **IOG**: Additional gain used to obtain sufficient resolution when recording
          the sine wave current (Iac). This gain is set with digital potentiometer.
          The IOG is determined on basis op IPP and the gain of the I to V
          converter (IVgain).   Acceptable values: 20 > IOG > 2.
          IOG should not be smaller than 2 because the digital potentiomer
          setting (R-IOG) becomes less accurate in this range.

- **V-ADC** : overflow will be shown when value is > 2350 because the range of the ADC is up to 2500. To obtain sufficient resolution the value should be > 125. Parameters VG, VOG, IPP and IVgain should be adjusted to avoid over or underflows.
  Vpp at ADC (in mV) is calculated as:

$$Vpp = 2 \times I_{PP} \times R_T \times INAMP_{Gain} \times VOG / 1000.$$

Make sure that V_{ADC} is in range of 125 to 2350.

- **V-MEM** : Peak to peak sine wave voltage over the membrane resulting from requested IPP. Depends on RT and Ipp. The user has to decide to which value the AC voltage over the membrane has to be limited.

$$V_{MEM} = R_T \times I_{PP} \times 1000 \text{ mV with RT in Ohm and Ipp in µA.}$$

- **I-ADC** : voltage resulting van I to V conversion and additional amplification with gain = IOG (additional gain Iac).

$$I_{ADC} = Ipp \times IV_{gain} \times IOG \text{ mV with Ipp in µA and IVgain in mV/µA}$$

Example:
1 – Test validity of parameters in line 2 of USER RTX table (RT-Treshold = 240)

RTX Table parameters

Threshold RT (Ohm)	240
INAMP (Voltage) Gain (VG)	8 ▼
Additional gain Vac (VOG)	8 ▼
Iac Stim (Peak-Peak) (IPP)	5.0
IV-Gain (mv/uA) (IVgain)	20 ▼

Calculated parameters

Additional gain Iac - IOG	10.0
VPP at ADC - V-ADC (mV)	132
VPP at membrane - V-MEM (mV)	1.2
IPP at ADC - I-ADC (mV)	1760

- V-ADC = 132 is still above the lower limit of 125 to obtain sufficient resolution of the ADC and thus a reliable accuracy of RT.
- V-MEM = 1.2mm. No problem for the cells!
- I-ADC = 1720 is still < 2350 (Max range of ADC).

2 – Now we want to verify of these parameters can be used over the entire range from RT=240 Ohm up to RT=3700 Ohm (RT-Treshold of 1st line).

RTX Table parameters

Threshold RT (Ohm)	3700
INAMP (Voltage) Gain (VG)	8 ▼
Additional gain Vac (VOG)	8 ▼
Iac Stim (Peak-Peak) (IPP)	5.0
IV-Gain (mv/uA) (IVgain)	20 ▼

Calculated parameters

Additional gain Iac - IOG	10.0
VPP at ADC - V-ADC (mV)	2042
VPP at membrane - V-MEM (mV)	18.5
IPP at ADC - I-ADC (mV)	1760

No overflows are taken place and voltage across the membrane (18.5mV) is still acceptable.   However, if we would have extended the 2nd range to 4400 Ohm,

overflow in the ADC will occur when probing menbranes with resistances in the range of 4310 up to 4400 Ohm.

RTX Table parameters		Calculated parameters	
Threshold RT (Ohm)	4400	Additional gain Iac - IOG	10.0
INAMP (Voltage) Gain (VG)	8	VPP at ADC - V-ADC (mV)	2429
Additional gain Vac (VOG)	8	VPP at membrane - V-MEM (mV)	22.0
Iac Stim (Peak-Peak) (IPP)	5.0	IPP at ADC - I-ADC (mV)	1760
IV-Gain (mv/uA) (IVgain)	20		

Important parameters related to the clamp circuit:

1 – Clamp LF: Set clamp amplifier in Low Frequency mode. This enables the user to select a low or high bandwidth of the feedback amplifier. For Rt and PD measurements, the LF mode should be chosen. When using the board for high frequency impedance measurements, HF mode should be chosen.

2 – IV LF: The current to voltage converter in the clamp circuit can de set to Low Frequency mode, reducing bandwidth to a few kHz. For Rt and PD measurements, the LF mode should be chosen. When using the board for high frequency impedance measurements, HF mode should be chosen.

3 – RAP: means: R-apical, or resistance in the current path of the clamp at the apical side. For this purpose, a digital potentiometer is placed at the apical side. It is variable from 1k to 20kOhm. The value of the potentiometer is automatically set at the lowest value possible, where the clamp does not oscillate. The firmware starts the reduction with an initial value of RAP that can be set by the user. Oscillation of the clamp is automatically detected by the firmware. The default threshold is 400, but can be set by the user.

4 – Oscillator threshold. A circuit in the clamp detects possible oscillations of the clamp. For this purpose, DC is removed from the output of the IV amplifier is and rectified. This signal is digitized and converted by the ADC. Due to background noise, the oscillator value is normally in the range of 150 to 200. Once, the clamp starts oscillating the value will become larger than 400, which enables us to detect oscillations of the clamp. Oscillations can be prevented by increasing the RAP, which lowers the bandwidth of the clamp. Also "Clamp LF" and/or "IV LF" can be enabled to reduce clamp bandwidth.

Points to notice:

1 – MTECC boards are powered by software. Power is turned OFF when stopping program (QUIT).
2 – The USB interface is powered through the USB connection of the PC.
3 – Note that when checking with Diolan ControlPanel: no slaves are seen when MTECC boards are not powered. Slaves can be seen after starting data acquisition software.

MTECC-24CH and MTECC-96CH :
Digital INPUT – OUTPUT ports: Located on backplane board.

OUT		IN	
1	2	1	2

OUT: relay contact (NO), max 3A.
IN: Logic input – **MAX 3.3Volt.**

I2C address setting:

The I2C address of the MTECC board is set with DIP switches located next to the RJ45 connectors:

The picture shows the setting for MTECC #6.
DIP switch 7 and 8 are the I2C termination switches. Only on the last board in a system these switch should be ON. <u>On all other board both need to be set OFF</u>.

In a TECC-24CH, setting the the termination switches on board #6, is an absolute requirment.

I2C adress setting:
least significant bit is next to the I2C termination switches (switch #7 and #8).

DIP switch number	Binary value.
6	1
5	2
4	4
3	8
2	16

In the above picture the I2C address is set to 6. It is the last board in the MTECC-24CH sytem

ADDENDUM Impedance

A – Recording software.

1 – Store MTECC_24CH_Manual_IMP.EXE and MTECC_24CH_Manual_IMP.UIR in program folder on C-drive: C:\\Z_MTECC_96CH_24CH. Make short-cut to EXE file.

2 – New features in User Interface:

- Command to perform the impedance analysis over a frequency range of 1 Hz up to 10 KHz.

- It is possible to monitor the analog signal of I and V. The channel of can by selected in the UI. A hardware circuit is needed to connect the 10 way header at the MTECC board with the oscilloscope.

Task sequence			
Operation		**N**	**Interval (s)**
Reset RTX	▼	1	
Record PD/Rt	▼	1	30
Record PD/Rt	▼	1	30
Record PD/Rt	▼	1	30
Record IMP	▼	1	60
Do nothing	▼		
Do nothing	▼		
Do nothing	▼		

- In the UI a panel for showing the impedance spectra is included. Spectra of the last recorded impedance spectrum can be displayed.

- When the program requests to move the electrode manifold to a new plate, there is a BEEP signal to alert the user (as in previous versions). In this program ALSO the relay at **OUTPORT #1** is closed. Normally the relay is in open condition. The closure of this relay contact can be used to communicate with the robot. Closure can be used to tell the robot to move to a new platform (plate). As soon as the electrode manifold is lifted, the **microswitch (MS)** is closed an OUTPORT #1 relay is opened. When the robot has put the electrode manifold on the next plate, the MS will be opened and the recording program will restart recording data. So, this feature can be used to link the data acquisition with a robot that can detect the closure of the relay at port #1.

- **RTX table**. The RTX table has 48 lines, 16 lines for each of the 3 frequency ranges.

- **Frequency ranges**.   Three overlapping ranges are used.

Range	ΔF	F_{max}
1	0.9537	40.06
2	15.2588	640.87
3	244.1406	10253.91

B – Plot software.

A program for plotting/inspecting the impedance spectra.
Layout is obvious.

C – Data file format.

It is an ASCII file with layout (can be edited in NotePad++):

1 – Two lines with general information, date, etc….

2 – One line of 126 floating point numbers with the frequencies used.
    FORMAT: 12 characters per frequency, with 2 digits after decimal point.

3 – Blanc line – empty line

4 – REAL parts of 1st record: 126 floating point numbers, 12 chars/number.

5 – IMAGINARY part of 1st record: 126 floating point numbers, 12 chars/number.

6 – Blanc line – empty line.

7 – REAL parts of 1st record: 126 floating point numbers, 12 chars/number.

8 – IMAGINARY part of 1st record: 126 floating point numbers, 12 chars/number.

9 – Blanc line – empty line.

ETC……..

Data can be imported in spreadsheet or other software package.

D – Fitting Software.

1 – Create folder on C-Drive: **Fit_Impedance**. - C:\\Fit_impedance
      This folder has the files used to fit the impedance spectra:
            - F1order.EXE
            - F2order.EXE
            - Fit_imp.ini
 F1order.EXE and F2order.exe are executables (programmed in FORTRAN 99) called by the fitting program (Fit.EXE) that has the user interface. The Fortran programs communicate with Fit.exe thought temporary files, "imp_temp_data.dat" and "imp_temp_results.dat" that are created by Fit.EXP and the Fortran executables. The user does not have to care about these files. However: the folder "Fit_impedance" is absolutely needed to create these files.

2 – Store the files Fit.EXE and Fit.UIR in C:\\Z_MTECC_96CH_24CH or equivalent directory where the EXE, UIR, LIB files are stored. Make a short-cut to Fit.EXE.

3 – Two models are supported:

 - One single RC network with depressed center. There 4 parameters:
      - Series (solution) resistance: R_s
      - Membrane resistance: R_m.
      - Membrane capacitance: C_m.
      - Depression of semi-circle described by: α.

 - A double membrane arrangement of 2 RC networks with depressed center.
      There are 5 parameters that can be determined: A, B, C, D and α.