Innovative Ussing Chamber system.

General features.

1 – Designed for experiments with epithelia cultured on Costar 6.5 mm single transwell filters.

2 –Simultaneous recording of transepithelial potential (PD), resistance (RT) and equivalent short-circuit current (I_{eq}) of four epithelial layers cultured on Costar filters.

3 – Filters are inserted in Lucite holders that are mounted in an anodized aluminum support. Temperature control of this support is provided by an electronic device operated at 24 VDC.

4 – The aluminum support is designed for two Lucite holders: one holder with filters with cells and a second one containing reference solutions for offset recording.

5 – The electrical parameters are recorded with Ag/AgCl electrodes that are mounted in an electrode manifold that can easily be positioned on the Lucite holders.

6 – The electrical parameters (PD, RT, I_{eq}) are simultaneously recorded with a four channel data acquisition system connected to a PC via an USB link.

7 – Bathing solutions of the cells can be gassed with humidified O_2/CO_2 mixtures.

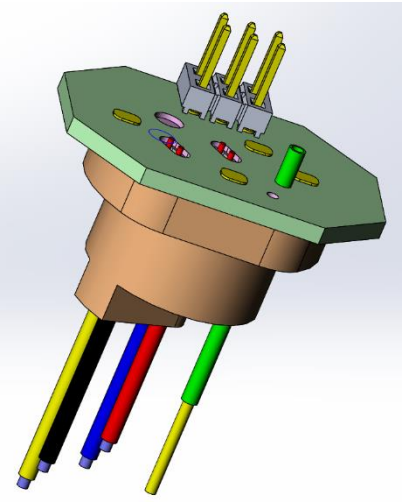
8 – Relative small bench area is required for this setup: approximately, W=30 cm; D= 50cm.

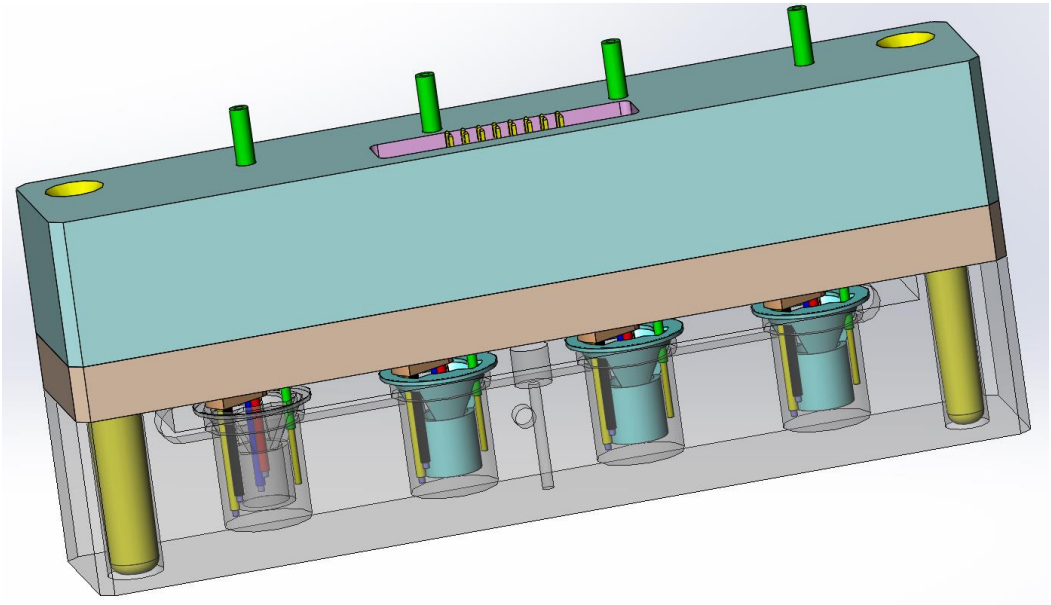
Electrode manifold – 4 channels.

Data are recorded with IV units that have 4 electrodes:

- Yellow: voltage – BL (Basolateral).
- Black: current BL.
- Blue: voltage AP (Apical).
- Red: current AP.

The electrodes are made with Ag/AgCl pellets.
Besides, the IV unit carries a small tubing used to aerate the basolateral compartment / solution.
Four IV units are mounted in a holder. In case of malfunction, they can be easily replaced individually.





- Filters are depicted in transparent mode. In this way electrodes in the wells can be seen.
    - Yellow: voltage – BL (Basolateral).
    - Black: current BL.
    - Blue: voltage AP (Apical).
    - Red: current AP.
- Connection of electrodes to data acquisition system: 16 way flat cable (not shown). Green tubings at the top are inlets for O_2/CO_2 gas mixture used for bubbling of basolateral bath.
- Two posts protect the electrode tips from misalignment during insertion in the wells. The diameter of the post on the right is 7mm while the diameter of the left post is 10 mm. A permanent Neodymium magnet is mounted in the tip of the 10mm posts. Its position matches with the location of a reed relay switch in the bottom of the heating block. During data acquisition, this feature enables us to detect the location of the electrode manifold.

Entire view of setup: Costar filters inserted in temperature controlled heating block and bottles for humidifying the O_2/CO_2 mixture.

This picture shows 2 heating blocks:

1 – Block for two Lucite holders for Costar filters or reference solution.

2 – Block with 4 holes (30mm diameter) holding the bottles for humidifying the O_2/CO_2 gas mixture.

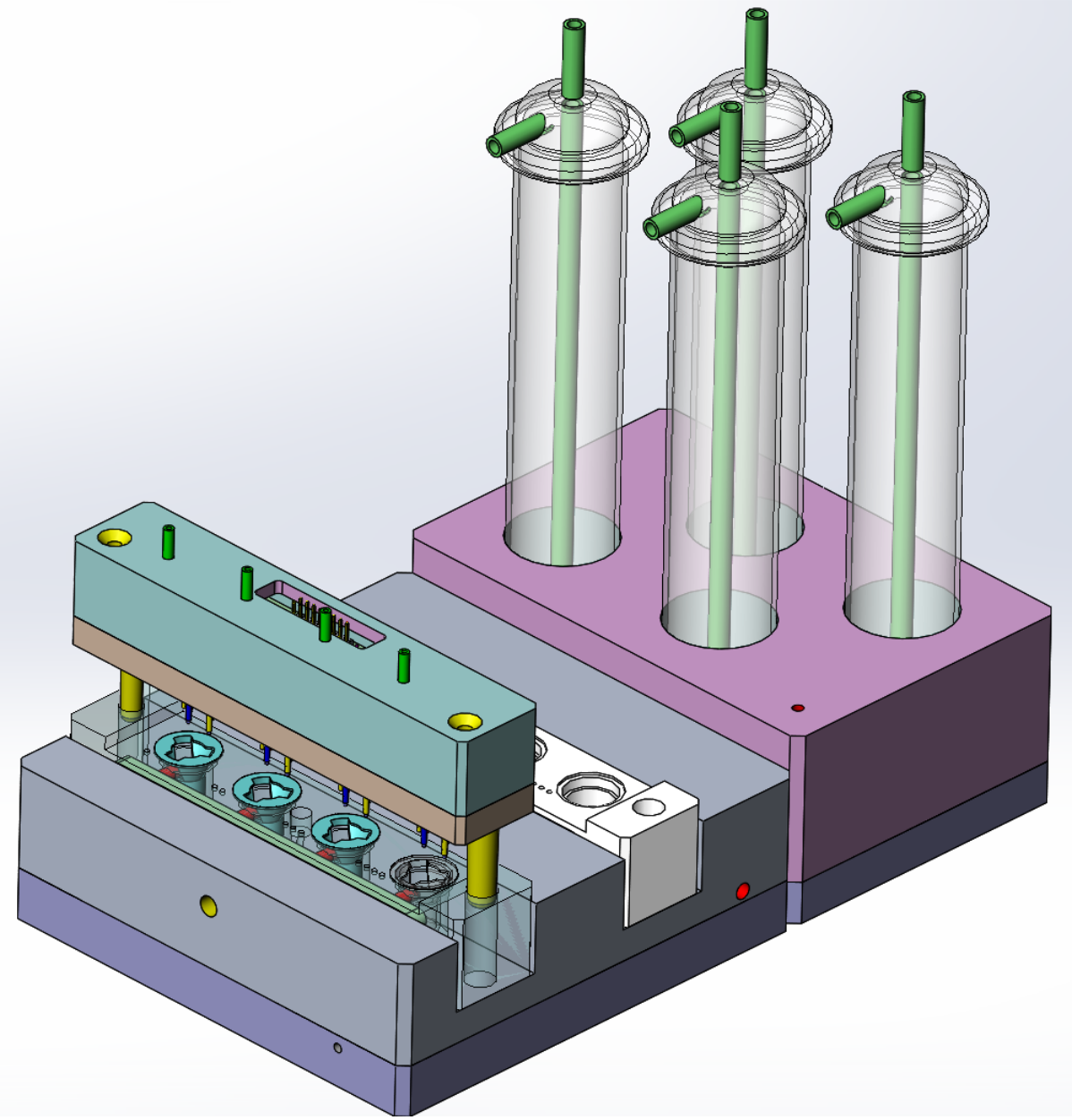
The heating blocks have separate temperature controllers that can be set in the range from room temperature up to 44° C.

Two Lucite holders are placed in the heating block:

1 – One with filters with epithelial cell layers that are investigated.

2 – The second holder is used to record electrode offset PD and to store the electrodes when adding compounds to the media in the filters.

Gas mixture is preheated in the heating block before it reaches the humidifying bottles.

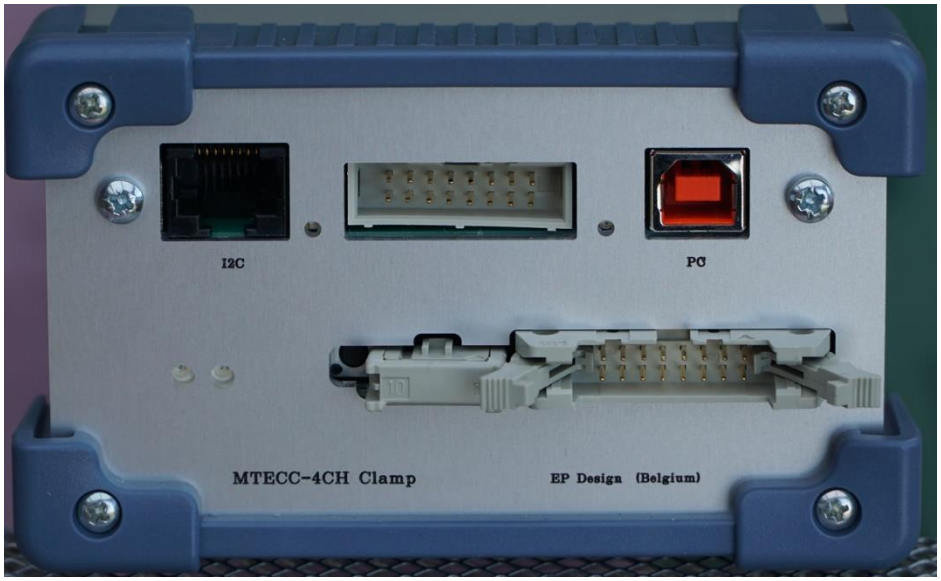


Data acquisition hardware.

Electrophysiological data are recorded with a single electronic board: MTECC.

- Analog Input: Lower located 16 way flat cable connector. The flat cable connects the four IV units with the MTECC.
- Digital input: Upper 16 way connector. Input from reed relays in heating block.

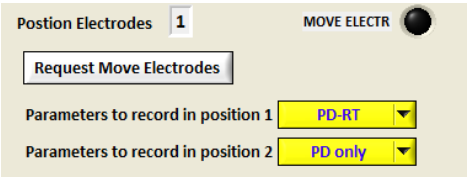
- USB input connector: connection to PC. Power is used from USB port.
- RJ45 connector: extension of I2C: connect with RJ45 input of MTECC board (in the back).

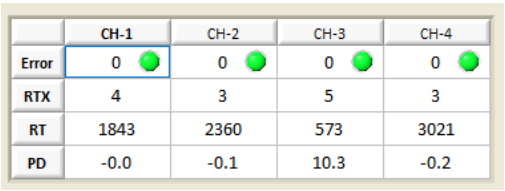


Recording RT, PD and Solution resistance (RS).

Main features:

- Recording of PD, RT and RS (solution resistance).
- RS is recorded with high frequency impedance analysis. For very leaky epithelial layers it is recommended to record and inspect the impedance curve. If the layer is not confluent, capacitance might not be detectable as part of semi-circle.
- Protocol file:
  1 - There is standard protocol file that continuously records PD, RT and RS.
  In the main panel the user can select the between PD, PD+RT or PD+RT+RS.
  2 - The user can make a protocol file that will be executed.
- The position of the electrode manifold is sensed with reed relays that are mounted in the heating block for the holder of the transwells. Using the standard protocol, the user can issue a "Request Move Electrodes". When hitting this button, the program will stop after the PD/RT recording is completed and an alert is issued to the user to execute the movement.

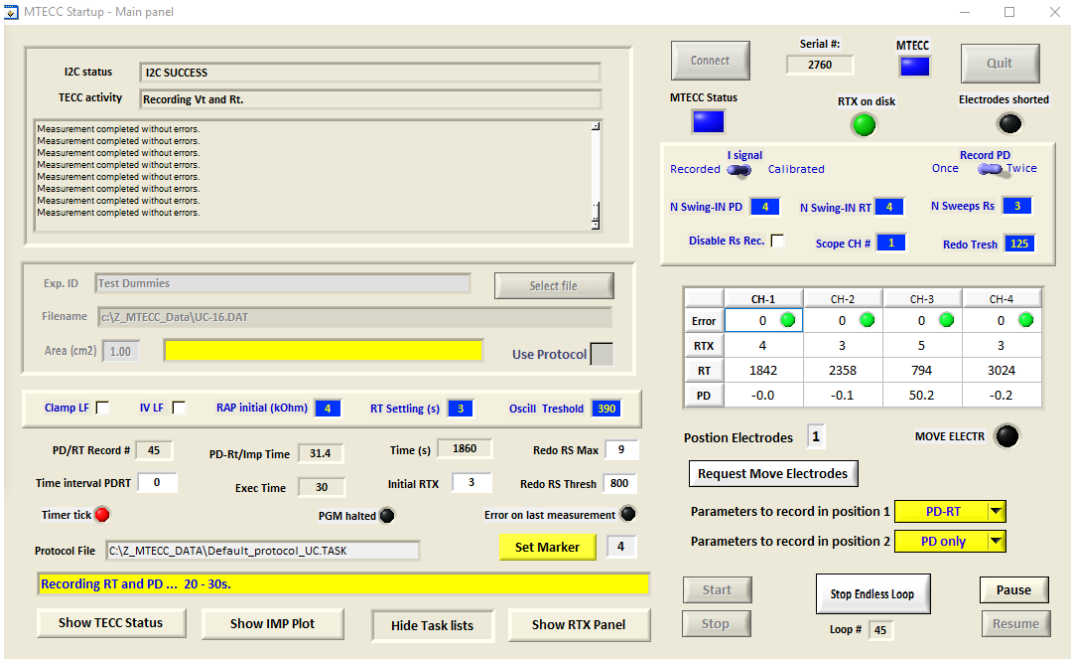




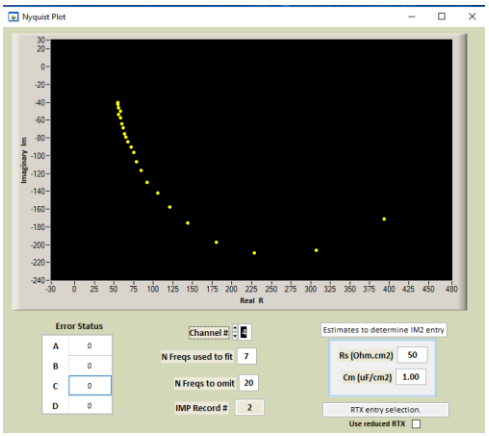
	CH-1	CH-2	CH-3	CH-4
Error	0	0	0	0
RTX	4	3	5	3
RT	1843	2360	573	3021
PD	-0.0	-0.1	10.3	-0.2

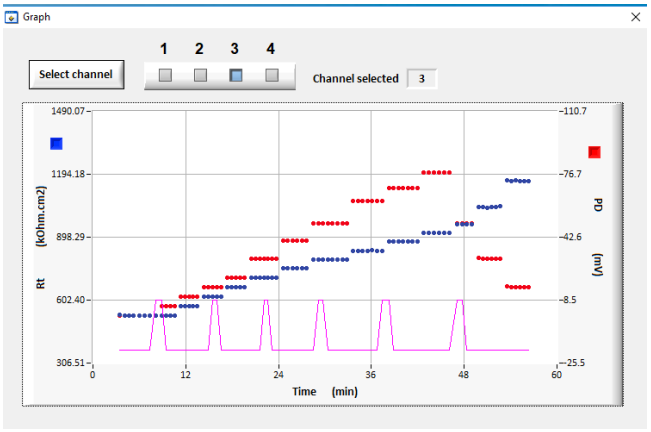
- Results are shown in a table in the main panel and data are stored in ASCII files on disk.
- Data traces can be seen in a graph for PD and RT. There is also a marker trace that will show the moments where the user hit the MARKER button the main panel.

Main Panel:



Example of high frequency impedance recording and Graph panel with RT-PD data and marker trace.





Designed by: EP Design (Belgium) Version: 30 March 2021.

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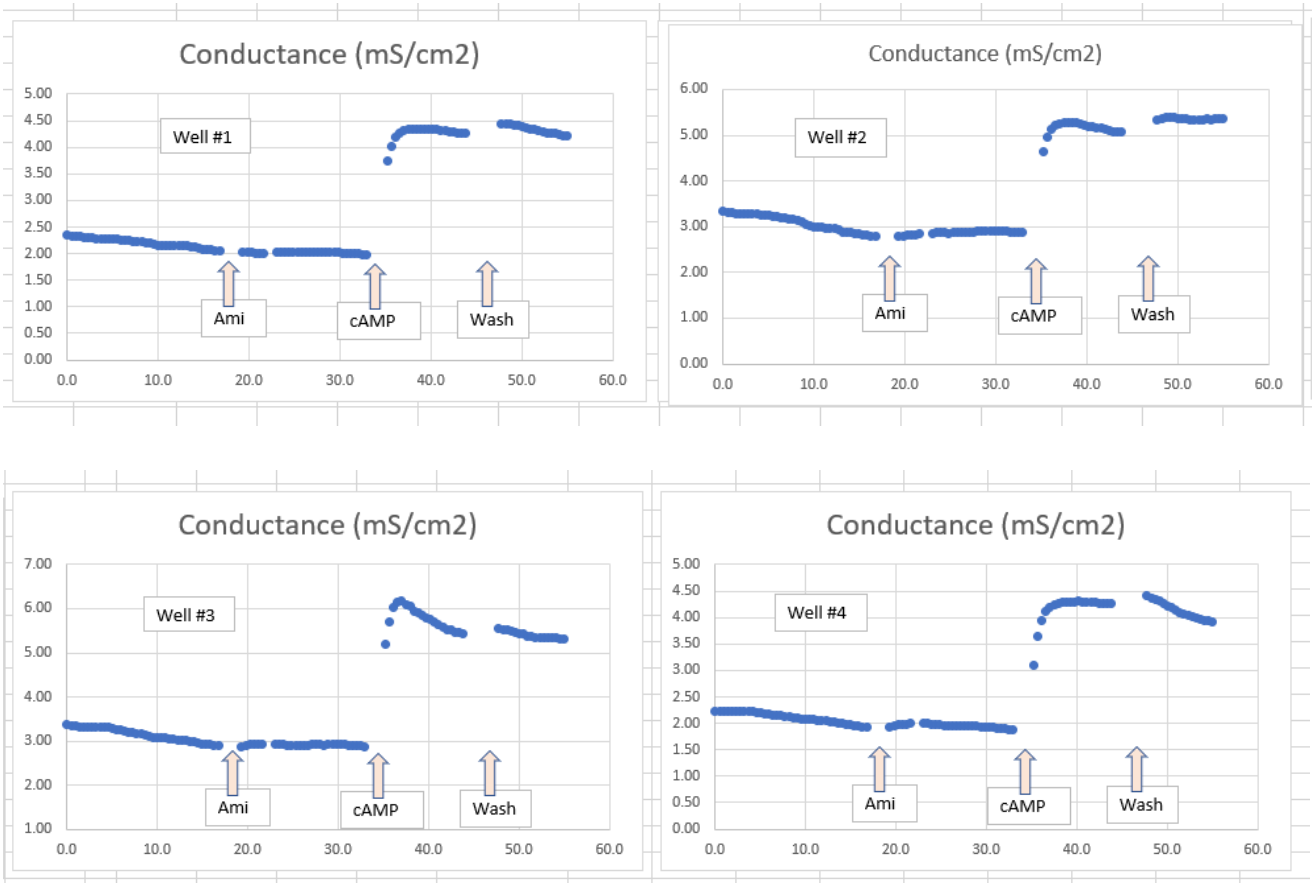
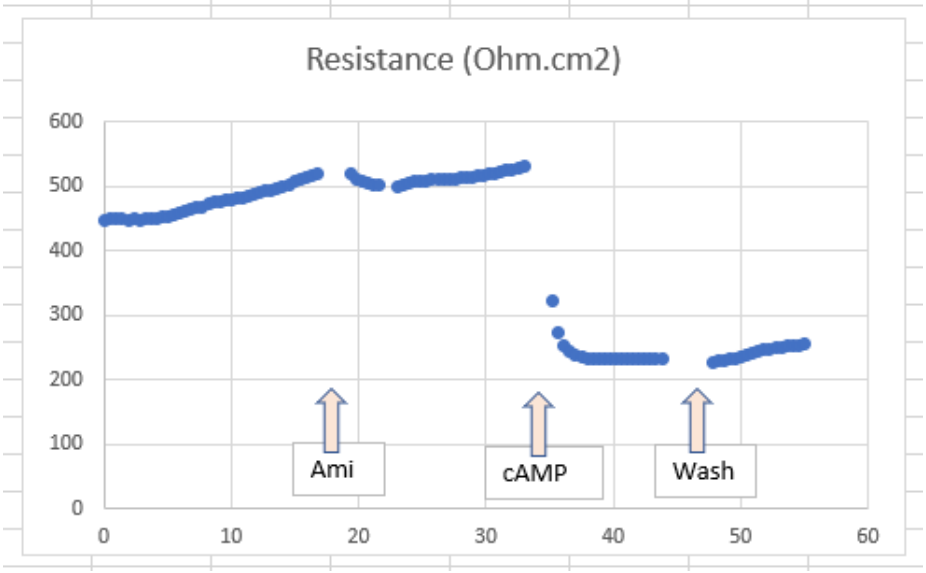
Records made with HBE cells.



Note: data stored in .RES file were imported in MS Excell and conductance was calculated from the RT values. The time course of RT of Well #4 is shown below.



Records from experiment made in laboratory of Prof. W. M. Weber (Münster, Germany).