Recording Rt and Vt with TECC.

1 – Vt recording.

The transepithelial potential is recorded with voltage electrodes and a differential amplifier. In TECC electronics, we use a programmable differential amplifier. Gain can be programmed to 1x, 2x, 4x, 8x, 16x, 32x, 64x and 128x. The microcontrollers looks for the best gain, which usually 16x or 32x, depending on the value of the potential difference across the epithelium. The potential is measured while the clamp is not closed: switch OPEN.

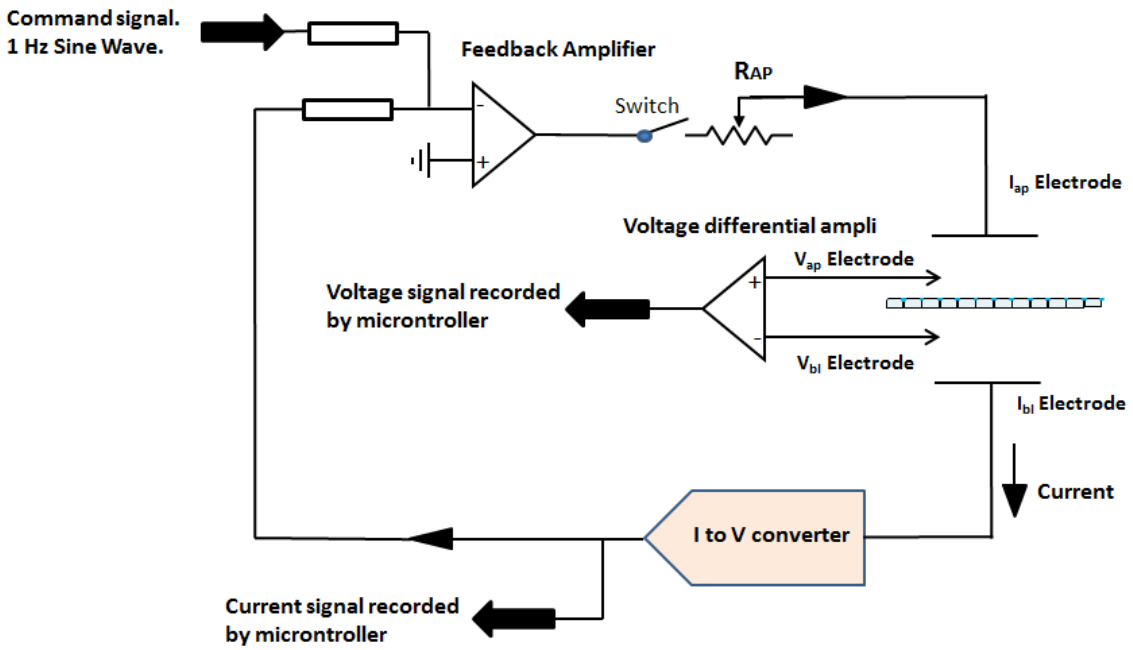
2 – Rt recording.

To record the transepithelial resistance we need to send a current through the membrane. Furthermore we need to record the potential difference elicited by this current (ΔVt) and the magnitude of the current sent through the membrane (ΔIt). TECC uses a sine wave current to record Rt. ΔVt is the amplitude of the voltage signal resulting from the current stimulus ΔIt. In order to be able to send currents with amplitudes, varying over a wide range, we use a current clamp circuit, shown below.

1 – Voltage is recorded with a differential amplifier. The output of the amplifier is connected to the microcontroller ADC (analog to digital converter), where it is processed.

2 – Similarly, the current is recorded with a I to V converter. The voltage output of this converter is also connected to the microcontroller.

3 – Because ΔVt and ΔVt are sine wave signals than can be accurately analyzed. Noise can be rejected by using filters and the software for processing the sine wave signals.



4 – How is the sine wave current generated? With current clamp feedback system it is easy to send a sine wave current through the membrane. The heart of the circuit is the feedback amplifier (FBA). The crucial point in the FBA is the MINUS input where different signals can be added. The gain of FBA is very high so the MINUS input should become zero, otherwise the feedback fails or results in an oscillation. So, with a stable clamp, the sum of the signals sent to the FBA will be zero: command signal (1 Hz sine wave from microcontroller) + output of the I to V converter. This means that signal at the output of the I to V converter should be a sine wave with opposite sign of the command signal (or phase turned 180°).

To stabilize the clamp we use a resistance (potentiometer) in the current path of the circuit. This resistance is located between the output of the the FBA and the apical current electrode: RAP. The microcontroller can select the optimal RAP value (for MTECC).

5 – Open and closing the clamp is done with an electronic switch that is under control of the microcontroller.