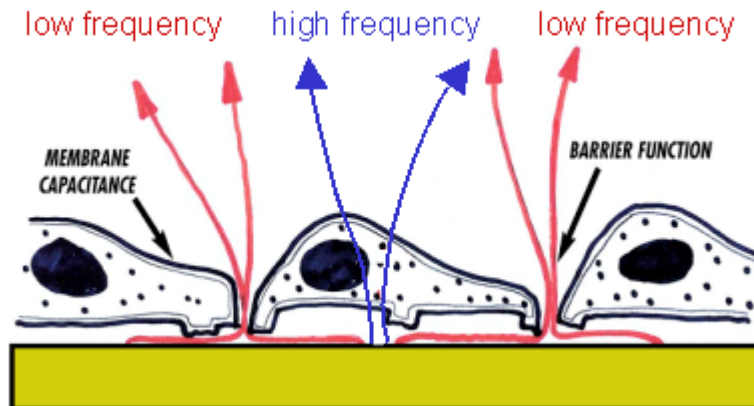


## What is the Significance of Using Different Frequencies During ECIS Experiments?

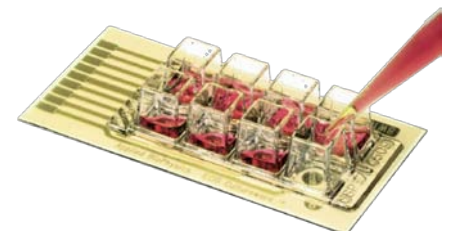
The figure below depicts the different current paths at high or low AC frequency:



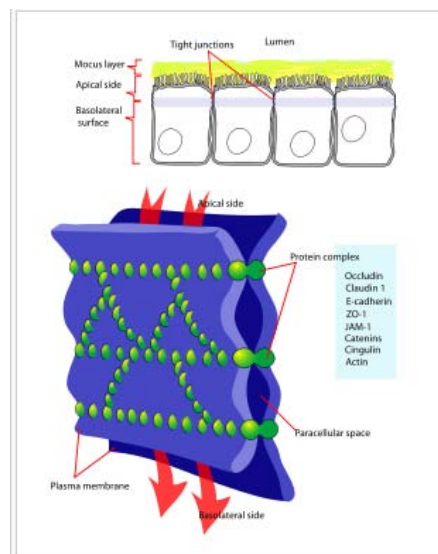
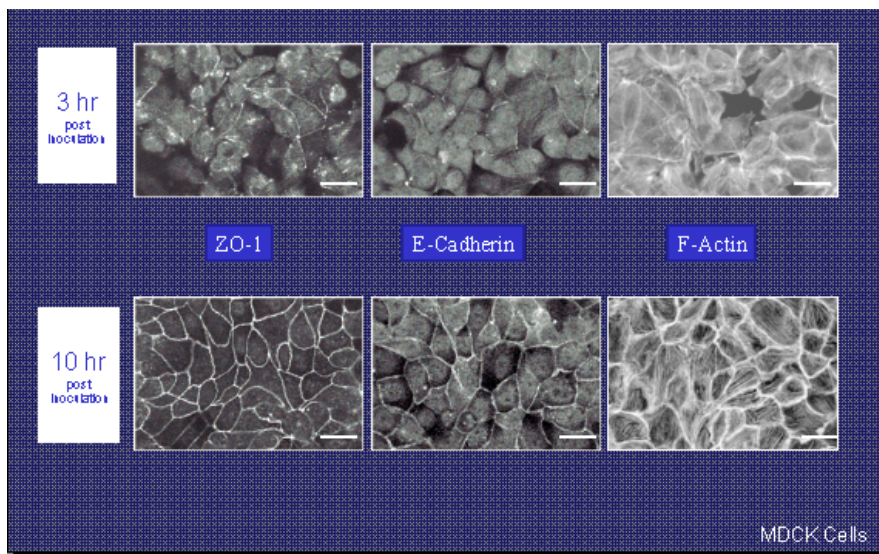
At low frequency, the current is able to easily go in and out of the cleft between the electrode and the basal cell membrane. A physical explanation is that the RC time constant of the resistive path and the capacitance of the gold electrode makes this region available to the current.

As the frequency increases to high levels (40,000 Hz), the current can no longer move effectively in this path. Instead the current which is maintained constant by the electronics, now couples capacitively through the cell's plasma membranes (blue arrows). Since these membranes are in series with the gold electrode's capacitance, they tend to block the gold and increase the impedance (in particular the portion of the impedance referred to as capacitive reactance).

Low frequency measurements are mostly detecting changes in cell-cell interactions (if it is a tight cell layer, mainly barrier function), whereas high frequency measurements mainly measure cell-substrate interactions.



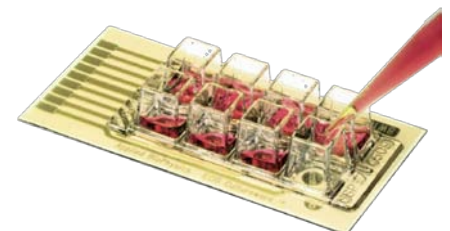
Below are six micrographs of MDCK\* cells that have been specifically stained to see the tight junction proteins (ZO 1, an occludin, and cadherin) as well as the organization of the actin micro filaments in the cytoskeleton.

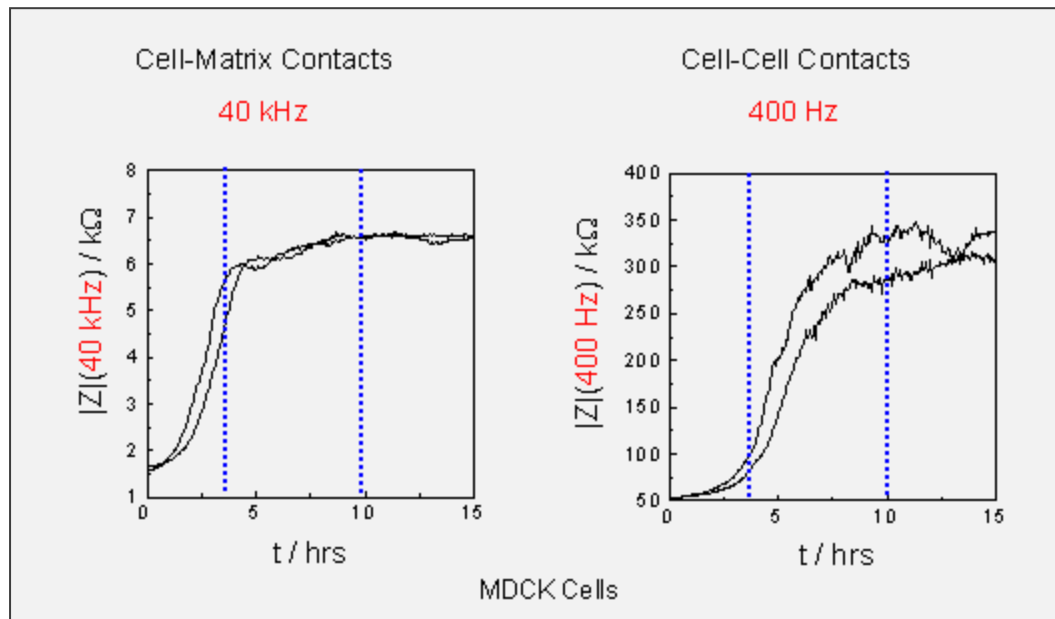


A diagram of a tight junction

Notice at 3 hours post inoculation the cells are covering the substrate, but it is not until  $\sim 10$  hours after inoculation we see the tight junction well formed as indicated by the organization of the stained proteins.

Now, what do we see with ECIS? Below are time course impedance measurements taken from the same cells at different AC frequencies as they attach, spread and organize on the ECIS electrode.





Notice the ECIS measurement at high frequency is seeing the coverage of the electrode (the cell-substrate interaction) and this curve reaches its plateau at approximately 3 hours post inoculation. The low frequency measurement, \*\*, however, does not reach a plateau until approximately 10 hours when the tight junctions are fully organized.

This is a good example of how ECIS measurements taken at different AC frequencies can distinguish distinct properties of cell monolayers.

\*Courtesy of Professor Joachim Wegener

\*\* The MDCK layer is extremely tight, with most cell layers, this measurement might be at 4,000 Hz where endothelial barriers show up very well.

