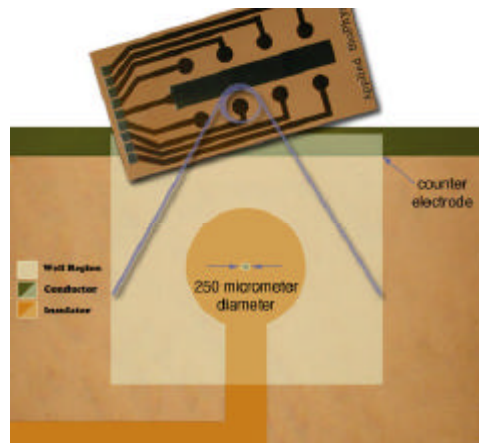


The ECIS core technology is based on a technique of measuring the change in impedance of a small electrode to AC current flow. The heart of the measurement is a specialized slide that has 8 individual wells for cell culturing. The base of the device has an array of gold film electrodes that connect the ECIS electronics to each of the 8 wells.

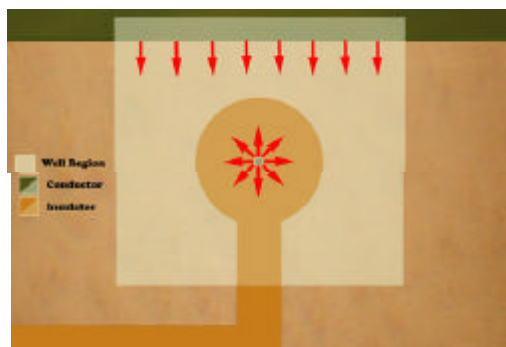


## 8 Well Electrode Array



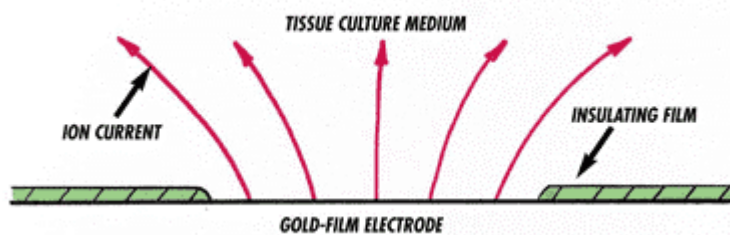
## Array with the wells removed showing more clearly the patterns of gold and insulating films

The current flows between a 250  $\mu\text{m}$  diameter electrode and a larger counter electrode using normal culture medium as the electrolyte.



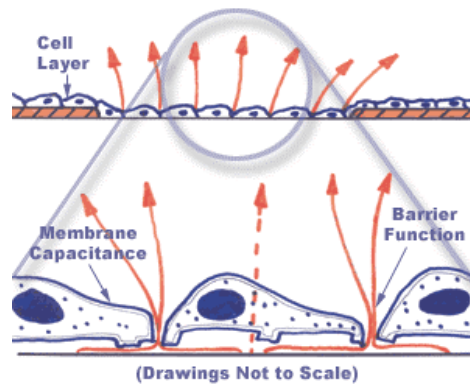
## Electrode top view indicating AC current flow between the small active electrode and the counter electrode

Without cells, the current flows unrestrained from the surface of the electrodes.



## Electrode cross section

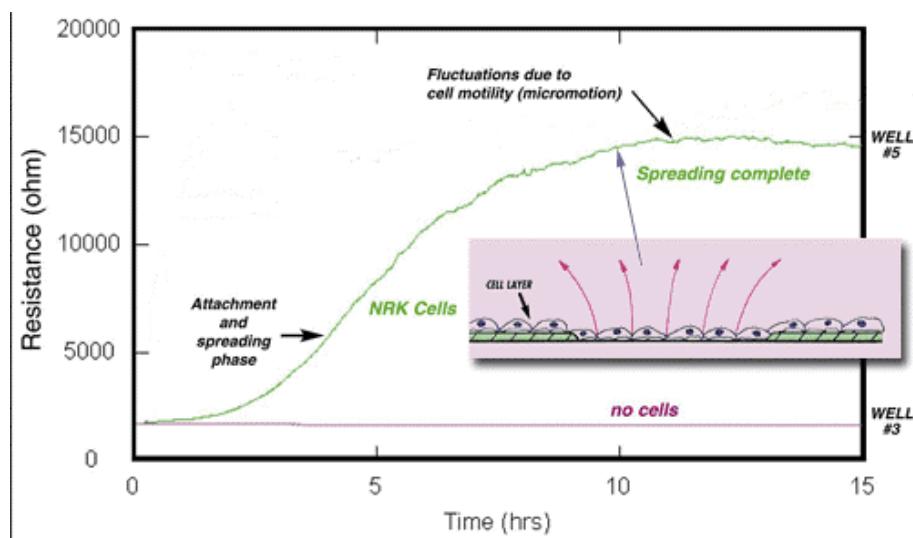
With cells attached and spread upon this region, the current must now flow in the spaces under and between the cells, as the cell membranes are essentially insulators.



This is a micrograph of the 250  $\mu\text{m}$  diameter gold electrode. The yellow area outside the circular electrode is an insulating film that defines the electrode perimeter. Both regions are excellent substrates for cell culture and essentially mimic the surfaces of normal tissue culture ware.

***Shown are some fixed and stained NIH 3T3 cells.***

At the start of the measurement the electrode has no cells attached to it and the resistance is about 2000 ohms. Upon inoculation, cells anchor and spread on the base of the well including the active 250  $\mu\text{m}$  electrode. With the presence of the cells, their insulating plasma membranes constrain the electrical current and force it to flow in regions beneath and between the cells. This convoluted current path causes large changes in the measured impedance. Although this is taking place at both the small electrode as well as at the counter electrode, the impedance of the small electrode is several hundred times larger, and so the contribution of the large counter electrode is a fraction of a percent and can be ignored. In addition to the overall increase in the impedance, small fluctuations can be easily observed, because the live cells continuously alter their morphology and hence the impedance. With the confluent cell layer in place, the resistance now has reached nearly 15,000 ohms. It is important to note that the AC current used in making these measurements (approximately 1 microampere) and the resulting voltage drops across the cells (a few millivolts) has no detectable effects upon them; the measurement is non-invasive.



This technique is a patented technology known as **ECIS**, an acronym for Electric Cell-substrate Impedance Sensing. Cell densities ranging from a heavy confluent layer to very sparse layers can be measured with this approach. The size of the electrodes restricts the maximum number of anchored cells that can be observed from 100 to 1000 cells (dependent upon the type of electrode array used in the instrument), but even a single isolated cell results in impedance changes that can be monitored