Choosing the ECIS® Measurement Mode

Most cell culture experiments involve changes that take place over minutes to hours; however, sometimes more rapid events are followed, e.g. signal transduction. The ECIS software offers three different modes for time course data collection:

- multiple frequency / time (MFT)

- single frequency / time (SFT)

- rapid time collection (RTC)

- Θ Note: A single timepoint frequency scan (FRC) is also available.

MFT Mode

For most experiments, the MFT mode is the best choice. This program will automatically measure eleven AC frequencies in each well selected from 62.5 Hz to 64,000 Hz. Data taken at different frequencies can reveal different aspects of cell behavior (morphology) and so this mode offers the greatest amount of information as to the behavior of the cell. Also the use of the ECIS® Model in data analysis requires that data be taken in the MFT mode.

The draw back of this mode is that for every switch of frequency and well, the system must wait a finite amount of time before it can make a measurement. This delay results in an average read time of each well of about 10 seconds. Thus in MFT mode each ECIS® 8 well array is read in about 80 seconds. For ECIS® 96 well plates the number of frequencies is reduced to seven to achieve a read time of approximately 10 minutes per plate.

Reducing Frequencies in MFT mode

To decrease the read time in MFT mode the number of frequencies obtained in MFT mode may be reduced. The trade off is that the accuracy of the ECIS® model is reduced as fewer frequencies contribute to its solution. In theory a minimum of three frequencies is required for fitting the ECIS® model, but in practice 5 is an absolute minimum for reliable results. To customize the frequencies acquired during MFT mode:

1. Select Set Frequencies from the Acquire menu.

2. Type in the frequencies desired in the pop-up window and push Okay

3. Select ◉ MFT and check that in parenthesis is the correct number of frequencies.

Some useful frequency sets are;

4000, 48000 Using these two frequencies allows for monitoring cell coverage of the electrode while observing changes in barrier function at the traditional frequency of 4000 Hz.

400, 4000, 40000 A three decade set that samples low, mid and high frequencies.

64, 640, 2000, 6400, 64000 A five frequency set for minimal frequencies and still provide reliable ECIS® modeling results.

SFT Mode

The SFT mode enables multiple wells to be monitored at a single frequency at a rate of 0.5 seconds per well. Data collected in this mode is focused on a single aspect of the cell's behavior. Since the read time of a single array is less that 5 seconds, this mode is ideal for reactions that take place over just a few minutes. In general the assays that require this rapid data acquisition is limited to Signal Transduction experiments where very quick changes in either barrier function or the cytoskeleton is followed.

For these experiments Resistance 400 - 4000 Hz is generally followed as it is the most informative. Resistance at 4000 Hz has widely been used to follow changes in barrier function of endothelial cells while Resistance at 400 Hz is more appropriate for the very tight barrier function of epithelial cells. Frequencies between 1000 and 4000 Hz are also very useful to monitor small morphology changes in non-barrier forming cells, as the current pathway at these frequencies probes the space between the cell and substrate.

RTC Mode

For the most rapid data acquisition, the RTC mode collects up to 512 samples per second but is restricted to monitoring a single well at a time and at a single frequency. The same frequency selection criteria as in the SFT mode apply but now much faster sampling rates can be achieved. For rates of 20 data points per second and below, data collection can be done until the os filesize is exceeded or the hard drive is filled. Rates faster than 20 datapoints per second can taken as long as they are powers of two (32, 64, 128, 256, 512). Data will be taken until an internal buffer of 16,384 points is filled.