## Measurement Techniques

Bioimpedance measurements, whether *in vivo* or *in vitro*, impose difficult requirements on the instrumentation, including[19,20] the necessity for wide operational frequency range, accurate four terminal measurement performance, safety when connecting to live subjects and the elimination of stray impedances due to cables and electrodes. In addition, some measurements are made even more difficult due to the non-uniformity and non-linearity of the materials themselves.

The specification of the Solartron 1294 Impedance Interface is achieved by the use of various techniques including 4-terminal connections to the sample, balanced generator and driven shield voltage measurement connections. In addition, the 1294 makes use of a sensitive multi-range current to voltage converter which allows the measurement of very low current levels which are commonly experienced in high impedance analysis or when using micro-electrodes.

The 1294 operates in conjunction with a frequency response analyzer (FRA), which provides the AC stimulus signal and correlation analysis of the output signals from the 1294. The use of correlation analysis is very important for the rejection of harmonics which result from non-linearities in the samples being measured.

The combination of instruments and associated software provides:-

- The ability to test in-vivo or in-vitro samples
- Impedance measurement range to >100 Gohm (2-terminal mode), 100Mohm (4-terminal mode)
- Wide frequency range 10µHz to >1MHz (IEC601 current limited output 100Hz - >1MHz)
- Increased measurement accuracy by the use of 4-terminal driven shield connections and balanced generator techniques
- Temperature control using external controller (e.g. LakeShore 340 / Oxford Instruments) and cryostat (e.g. Oxford Instruments)
- Flexible PC software allowing the control of complex experiments at a range of frequencies, stimulus levels and temperatures, and the ability to plot results in a wide variety of formats.

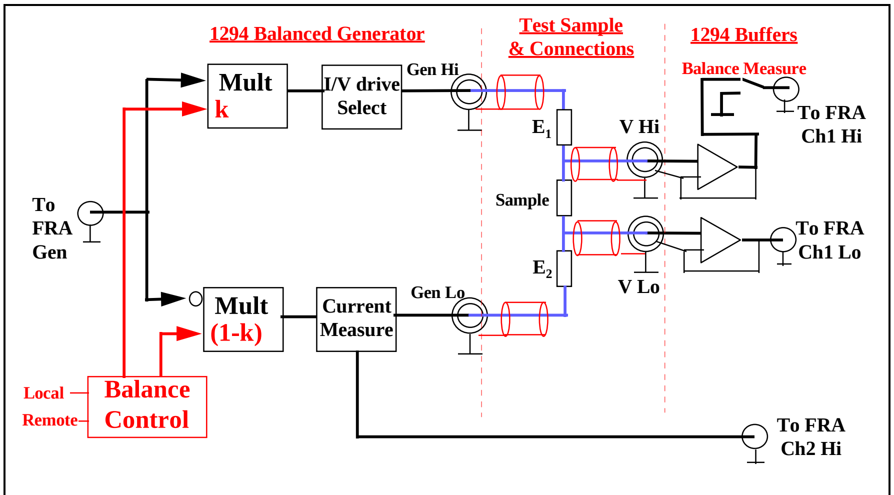


**Fig. 2: A schematic of the 1294 Impedance Interface**

The schematic (Fig. 2) shows some of the features which have been incorporated to provide accurate and reliable measurement of electrical impedance. The sample is positioned between V Hi and V Lo, either side are electrode impedances represented by $E_1$, $E_2$. These impedances are due to the sample connections which are present in many bioimpedance applications. There are also other stray impedances due to connections and the use of hydro gels (medical research), which may introduce imbalance in the electrode impedances which need to be considered and compensated.

The main features included in the 1294 are:-

- low capacitance voltage measurement inputs using driven shields
- balanced generator for better common mode rejection
- built in attenuator for low voltage / current outputs
- accurate and sensitive current to voltage converter
- bio protected sample connections conforming to IEC601

**Measurement techniques (4-terminal connections)**

For bioimpedance measurements, the electrodes which connect the instrumentation to the sample may be high impedance compared to the impedance of the sample itself. If conventional 2-terminal measurement techniques are used, there is no way to measure the impedance of the sample without including the impedance of the electrodes. This can lead to inaccuracies in the measurements, additional "features" on the impedance plots and to problems interpreting the data.

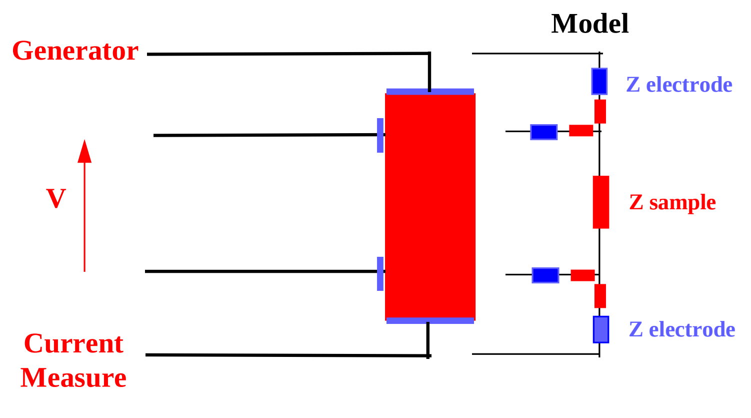


**Fig. 3: 4-terminal measurement**

The four electrode connection technique (fig. 3) uses separate electrodes for current stimulus and voltage measurement. This allows measurements of the sample on its own and minimises errors which would otherwise be introduced by the impedance of the current carrying electrodes. If 4-terminal techniques are employed, no current flows through the voltage measurement electrodes, which means that no voltage is dropped across these electrodes, leading to more accurate voltage measurements across the sample.

In addition, there may be localised disturbances where the current is injected into the sample (GenHi and GenLo). Four terminal techniques allow the voltage measurement electrodes to be placed well away from these disturbances in a region of linear electric field giving increased measurement accuracy.

**Driven shield connections to the sample**

Accurate measurements at high frequency are a specific problem due to errors introduced by input and cable capacitance. One technique which has been widely used to reduce the effects of capacitance is to position electrometer buffer amplifiers close to the sample. These buffers are able to drive the cable and input capacitance of the equipment and therefore reduce errors in voltage measurements. However, these external amplifiers require a power supply which usually involves extra cabling. In addition, where the temperature of the sample is to be varied, the accuracy of measurements may be effected since the buffers must be positioned close to the sample and are therefore subject to the same temperature variations.

An alternative solution is the use of driven shield cables. This technique replicates the signal waveform (which appears on the cable inner), onto the cable shield in order to minimise leakage current flow between the cable inner and the shield. Since no current flows between the cable inner and shield, the impedance appears to be very large and therefore the effects of the cable and input capacitance are minimised.

This method allows the high impedance buffers to be kept within the instrument. As an external power supply unit is not required for the electrometers, the cabling is reduced and the sample temperature may be changed without affecting the accuracy of the results.

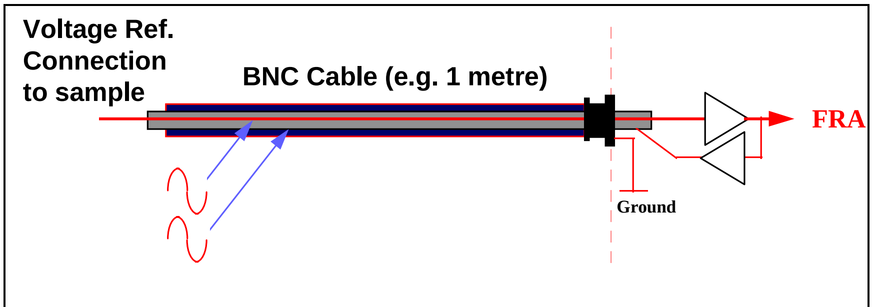


**Fig. 4: Driven Shields**

**Balanced Generator**

One problem associated with four terminal measurements on bio-materials is the requirement to study the impedance of the sample in the presence of relatively high electrode impedances (i.e. to reject the voltages across the electrodes in order to obtain accurate measurements of the voltage across the sample itself).

Figure 5 shows a typical measurement situation where the electrode impedance is ten times higher than the sample impedance which is required to be measured. In the example shown, the sample impedance is simulated by a 1Kohm resistor, and the electrode impedances are simulated by two 10kohm resistors. Using conventional measurement techniques, the AC stimulus voltage is applied to GenHi while GenLo is grounded, and the current through the sample and voltage drop across the sample (between V Hi and V Lo) are measured in order to compute the impedance of the sample.

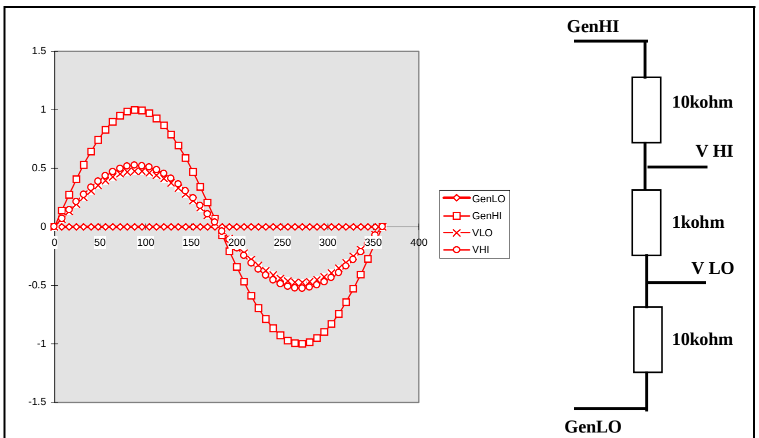


**Fig. 5: Standard signal analysis**

In this example, the FRA is required to measure a small voltage difference between two relatively high voltage signals which appear at V Hi and V Lo. For example, if 1 volt is applied between GenHi and GenLo, less than 50 mVolts appears across the sample (V Hi - V Lo) whereas the voltage on the V Lo measurement connection is approximately 500mV. The FRA is therefore required to measure a 50 mVolt “difference” signal in the presence of 500 mVolts which leads to errors which are referred to as “common mode” errors. In addition, the relatively high voltage on the V Lo connection causes some current which has passed through the sample impedance to leak to earth via the input / cable capacitance on V Lo instead of being measured by the current measurement circuit.

The use of a balanced generator (see Fig. 6) reduces errors due to common mode voltages and earth leakage allowing more precise measurements of the sample impedance. This is achieved by adjusting the GenHi and GenLo signals in order to provide a balanced stimulus to the sample which has the effect of making the voltage which appears at V Lo as close as possible to earth voltage, (i.e. zero volts).

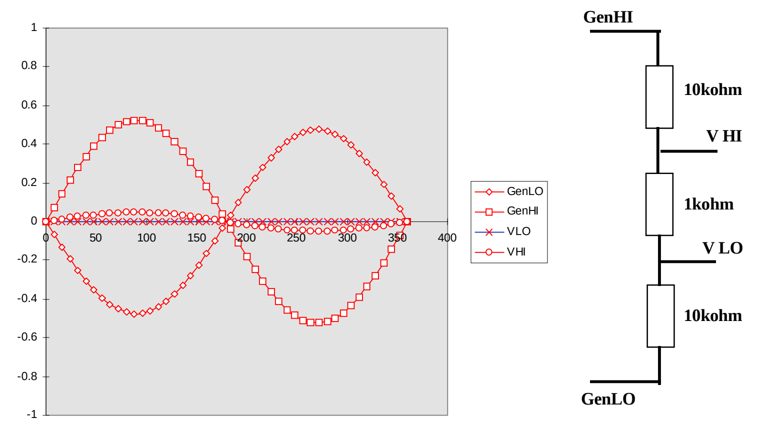


**Fig. 6: Signal analysis using a Balanced Generator**

The balanced generator can also cope with extremely difficult measurement situations where for instance the electrode impedances are not equal. This is also typical for bio-impedance measurements where it is difficult to obtain reproducable electrode contacts onto skin. In this case the signals on GenHi and GenLo are set to different voltage levels in order to again achieve zero volts on the V Lo connection.

**Software capabilities**

The software developed for use with this system is compatible with Windows 3.1 / 95/ NT4. It contains a variety of functions which allow maximum flexibility in terms of measurement and presentation of results, including:-

- Control of complex experiments involving multiple frequencies, stimulus levels and temperatures
- Presentation of results in a wide variety of formats including impedance, admittance, capacitance; with the ability to plot these as a function of frequency, stimulus level or temperature
- Multiple graph overlays allowing easy comparison of results taken at different frequencies or temperatures; or overlays of previously collected data
- Driver for Oxford Instruments and LakeShore Temperature controllers

**The future of bio-impedance measurements**

Electrical Impedance Spectroscopy is a non-invasive, non-destructive technique which gives a great deal of information about the properties of the materials under investigation. The new generation of bio-impedance analysis equipment with its advances in measurement technology allows more accurate four terminal measurements of a wide range of bio-materials to be obtained. The adverse effects of stray impedances and electrode impedance are minimised by the use of driven shield and balanced generator techniques.

The technology incorporated into the 1294 Impedance Interface will push the boundaries of measurement capabilities for biomaterial characterisation. The increasing range of applications provides an indication of the vital data that this instrumentation can provide in bio-materials analysis. The trend in biomedical and clinical research is to continue to open up further areas which will benefit from electrical characterisation techniques.

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