Hands on:
Bio-impedance measurements for pathological markers and diagnosis

Tissue bio-impedance is a potential bio-marker of some diseases. Between two electrodes, this quantity depends on:

- the physics of the metal-electrolyte junction,

- the nature of electrolytes of tissues (intra- or extra-cellular fluids) as well as membranes and organelles present on the paths of current lines, so as the extra-cellular matrix enabling cell-to-cell adhesion.

Few years ago, this lab was dedicated to cardiac fibrosis and built over research preliminary results adapted to and on master-grade students. However, due to a high percentage of student subject loss, ethical authorizations required for the lab are not given anymore. In this lab, we will focus on another pathological context: potato cooking!
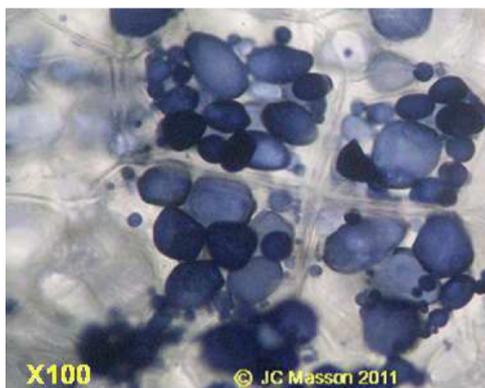
(a)	(b)

Figure 1: (a) Optical microscopy of potato tissue. Cellular membranes appear in grey, cells tend to have polygonal shapes. Colored dots are vesicles full of starch. The cells also contains a nucleus and organelles that does not appear on this image. (b) A typical pathology of potatoes: fourth-degree burns by prolonged exposition to liquid (water or fying-oil mostly). Comic speech bubble translation: *'5 more minutes!'*

More seriously, in this lab we will have a look on actual bio-impedance measurements using vegetables for obvious practical reasons. Like animals, vegetables are made of cells, with membranes separating electrolytes with different ionic concentrations. Bio-electric phenomena are similar between animal and vegetable tissues. If the potato is cooked, the effect of temperature

destroy the membrane integrity and the starch is dissolved in the resulting electrolyte. **The final objective is to identify an impedance model from a measurement to detect cooked potatoes from the state of the tissue (no more membranes, destruction of starch grains).**

1 Measurement hardware - rapid tutorial

In order to characterize the tissue we will performed a measure of the impedance over frequency, called **Electrode Impedance Spectroscopy** (EIS). All measurements will be performed with a board, called BIMMS, designed for research on bio-impedance purposes. This board embeds all the analog electronic front ends (AFE) required to perform impedance measurements with voltage (potentiostatic) or current (galvanostatic) stimuli, controlled by electro-mechanical relays. AFE are adapted for safe measurement of biological tissues (null net charge of the measurement stimuli). The board hosts a scope/generator, the Analog Discovery 2 (Digilent), in charge of AD/DA conversions. Details of the board and architecture are give in Figure 2.

1. We will work with potentiometric spectroscopy: stimulus in voltage, measure of current. Your first measurement will be in two points. Using the Figure 2, explain the path of the signal in the board.

2. Plug a resistor of known value between pins $V+$ and $V-$.

3. Open the script *EIS_measure.py*, just by reading the code, try to explain and comment main code blocs.

4. Run the script, check and validate results.

5. With this very first test:

   (a) Check the consistency of the measurement (is the value correct? what is the approximate error).

   (b) Knowing that board are calibrated, what is the bandwidth of measurement you can assess?
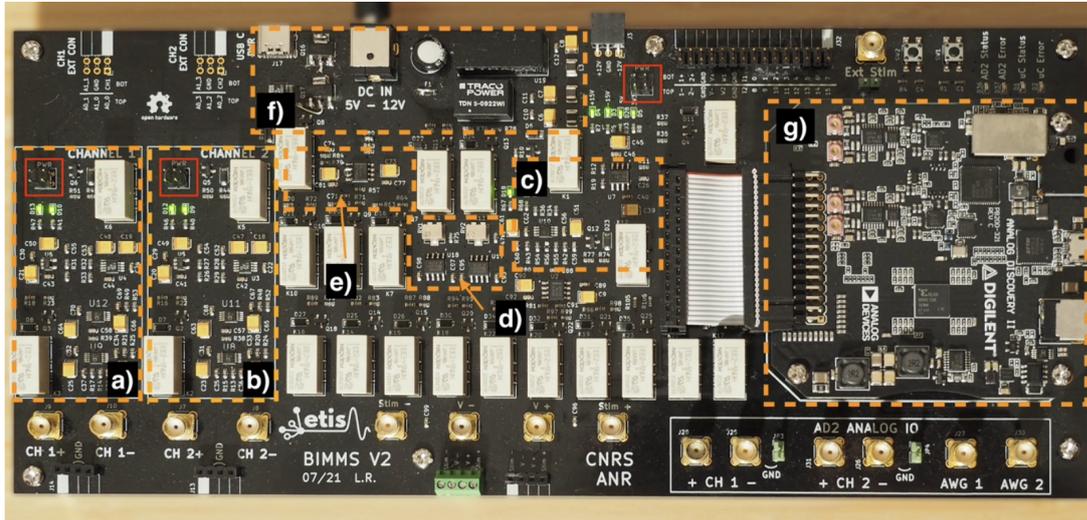
(a)
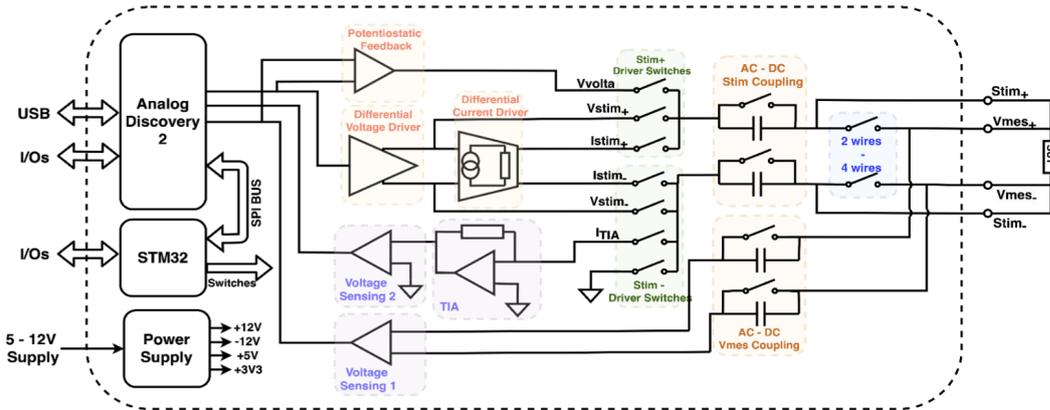
(b)

Figure 2: (a) Mains blocks of the system are highlighted in orange. a) and b) are respectively voltage readout channels 1 and 2; c) and d) are respectively the voltage stimulation circuit and the current source; e) the transimpedance amplifier; f) power supply section; g) Analog Discovery 2 PCB removed from its original enclosure and secured on top of the system. (b) Functional schematic of the proposed EIS system.

2 Electrodes characterization and configurations

We will now plug our electrodes to the board. Your setup contains for the moment 4 wires, respect carefully the color scheme from the board to the pins of your electrodes. For the moment we will only use two of the four electrodes: the two central ones. This configuration is called a **2-points configuration** and is illustrated in the following Figure:
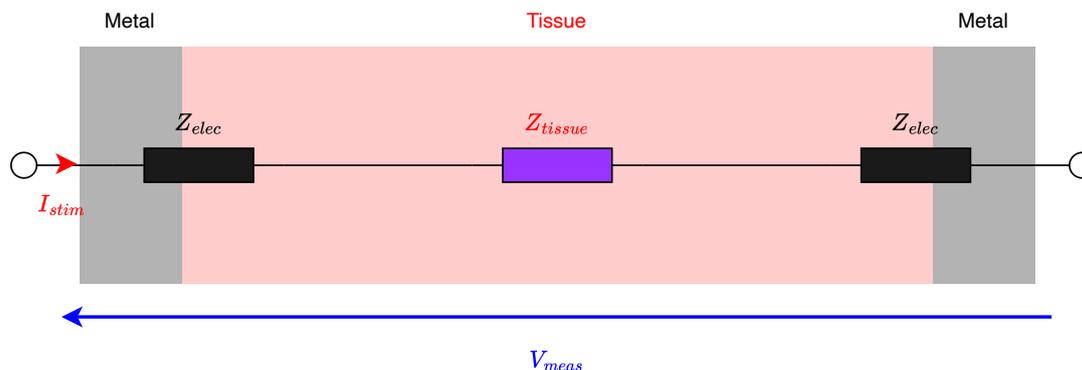
Figure 3: schematic view of 2-points measurements

In this case, the impedance measured is formed by the tissue and two electrolytes-metal junctions in series, so that:

$$Z_{measured} = \frac{V_{meas}}{I_{stim}} = 2Z_{elec} + Z_{tissue}$$

6. Plug the electrodes and **put the fork needles and only the needles** in demineralised water with half a tea-spoon of salt (NaCl mainly). We do have now electrodes in a simple resistive electrolyte.

7. With the script *EIS_measure.py*, make a measurement and check the curve.

8. Identify the bandwidth where you can observe: the electrodes, the electrolyte, parasitics.

9. Using the results, carefully measure the slope of the absolute value of **electrode impedance** and the approximate average value of the phase. **Comment your result**. Especially is it capacitive, resistive or neither one or the other?

10. **Repeat 2 or 3 times:** remove the electordes, add a teaspoon of salt and give a gentle mix. Place the elecrodes correctly and measure the impendance (change the name of recorded results accordingly).

11. Using the script *load_and_plot.py*:
    (a) load and plot one result,
    (b) modify the script to superimpose all measurements with different NaCl concentrations. Comment your result carefully.

It should be obvious for you now that the electrolyte, and further the tissue, are between metal-electrolyte junctions and parasitics impedances. **This is not the best solution to have a wide bandwidth evaluation of tissue impedance**. There is a technical solution to remove the influence of metal-electrolyte junctions from the measure. This solution relies on using separated pair of electrodes for stimulation and recording as illustrated in the next Figure:
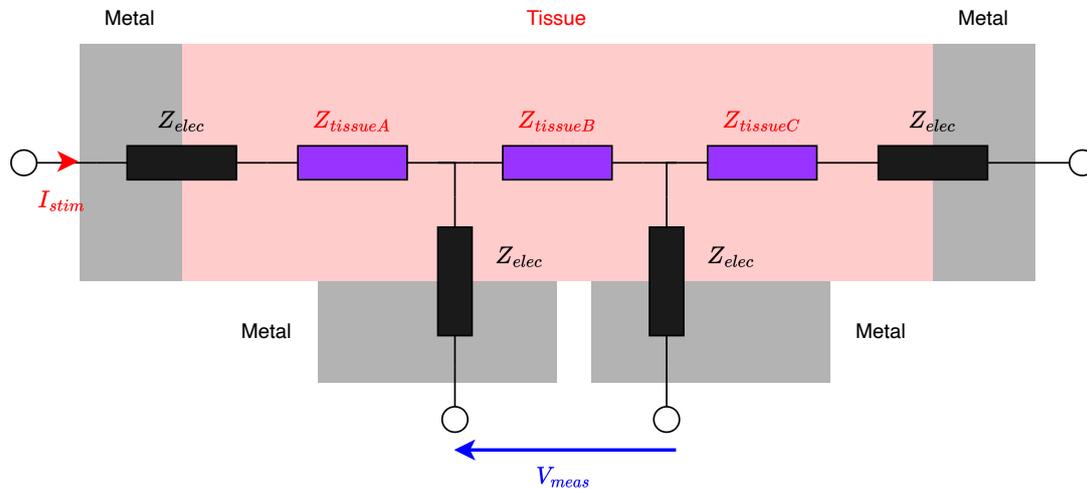
Figure 4: schematic view of 4-points measurements

In this so called 4-points measurement, if the voltage recording is performed with an infinite input impedance amplifier (perfect voltage amplifier), then no current is taken from the tissue on the central electrodes and:

$$Z_{measured} = \frac{V_{meas}}{I_{stim}} = Z_{tissueB}$$

Note that the measured impedance is not necessarily the Z_{tissue} from the 2 points configuration because of the geometry of the fork.

12. In the script *EIS_measure.py*, identify where something has to be changed for 4 points measurements.

13. with the fork in the NaCl electrolyte:

    (a) perform a 2-points measurement, save the results carefully;

    (b) perform a 4-points measurement, save the results carefully;

14. Using a copy of the script *load_and_plot.py*, plot the 2- and 4-points measurements on the same graph, compare and comment results.

3 Potato, there we go!

You do have now all the tools you need to measure the impedance of potatoes. All measurements will be performed on 4-points configuration as it highlights tissue properties in a wider bandwidth.

15. Take a raw (*sane* or not injured) potato, implant the electrode at a point were skin looks clean. Perform a measurement between 10^2 and 10^7 Hz.

We will first try to identify a model from this measurement. In first hypothesis, the impedance can be modeled by extra- and intra-cellular media resistivity, separated by capacitive membrena as depicted in the next Figure:
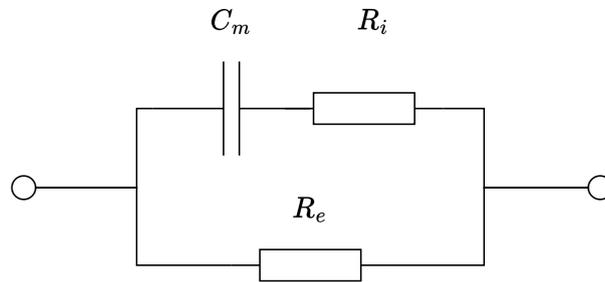
Figure 5: linear Resistive-Capacitive model corresponding to a first hypothesis approximation. R_e stands for the current lines in the extra-cellular medium which is supposed resistive. R_i represents the resistivity of the intra-cellular medium and C_m represents the assembly of membranes per unit of volume that make the tissue capacitive

16. Write formally the equivalent impedance of the tissue.

17. Trace (*by hand and computation*) the form of the plot of the module and phase as a function of frequency. Especially, place transition frequencies and noticeable values.

18. With the measurement that you performed on a raw potato, identify by hand values for R_e, R_i and C_m.

19. Adding code in the script *load_and_plot.py*, superimpose your measurement as well as the impedance you have identified by hand.

20. Comment your result. Is the model correctly fitting the observation and if not, what seems to be the main source of error?

4 A bit of math

You should by now be able to explain with a simple model the measures you are doing. However the process is not really automatic. In the next sections, we will have a look at :

- how to identify a model with a script,
- how to improve the electrical model we are using.

4.1 Automatic model fitting

It is possible to use minimization algorithms to automatically tune a model to fit a measurement. We will not dig into the mathematics properly, just take a look at the picture so you can modify it for your own specifications. Identification is usually performed by minimizing the error between observation and a model. Here we are doing spectral measurements, so that the input of our system is a set of frequencies at which we do the measurement. These values are called the input vector. To a specific input vector, we measure the module and phase of the impedance which are the output vectors. For a linear system the phase is proportional to the derivative of the modulus (Bode actually derived this relation), so we will only use the module for the identification, and compare the phases for validation.

Global error is computed by integrating the quadratic difference between the observation and model. Simple model tuning algorithms include linear or non-linear least square regression or more generally iterative optimization algorithms such as gradient descend. We won't care about this step. The overall process can be synthesized in the following Figure:
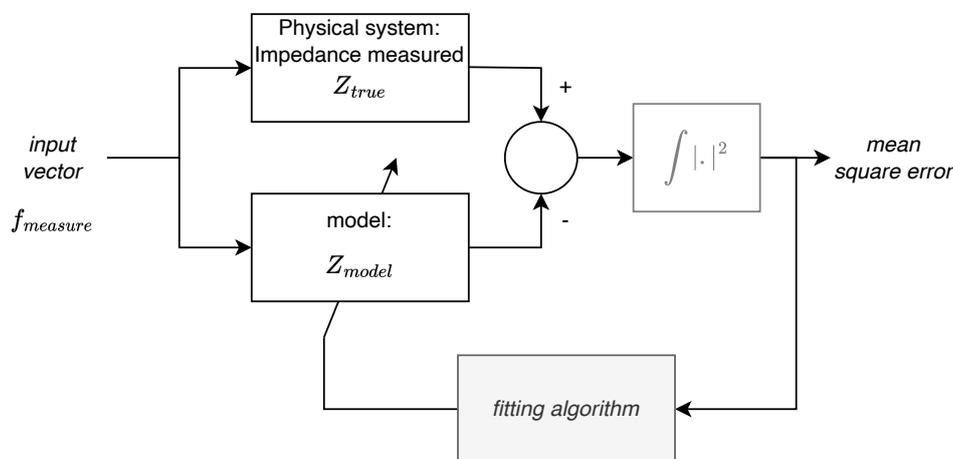
Figure 6: Iterative approach for automated model fitting

21. Open the script *identify_model.py*. Read the code and identify the lines to corresponding blocks in the Figure 6.

22. Write the content of the function *my_model* with the previous model.

23. Complete the *p0* argument of the *curve_fit* function call with the values you obtained on manual identification

24. Run the script, observe and comment results.

4.2 A more complex model - optional depending on your timing

As you can observe, the resistive-capacitive model is poorly fitting the measurement. You can observe that the slope during the so-called capacitive phase is not really one decade of Ohm loss per decade. This is physically due to the irregular repartition of membrena in the tissue. As a consequence the impedance appears to be fractional, and the corresponding passive electrical dipole to model such phenomenon is a constant phase element noted with the following symbol:
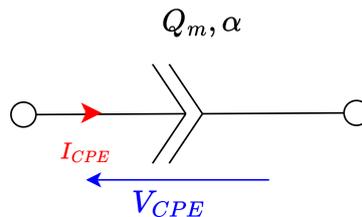
The impedance of this element can be written:

$$Z_{CPE} = \frac{1}{Q_m \left(\jmath \omega \right)^\alpha}$$

where $\jmath^2 = -1$ and $0 \leq \alpha \leq 1$. It can be easily derived (using Euler's formula) that:

$$\begin{cases} |Z_{CPE}| = \frac{1}{Q_m \cdot \omega^\alpha} \\ \angle Z_{CPE} = -\alpha \frac{\pi}{2} \end{cases}$$

Justifying the name of 'Constant Phase'. If $\alpha = 0$, the CPE is purely resistive, if $\alpha = 1$ it is purely capacitive, if not it is a sort 'in-between'. The name of fractional comes from the time domain. It is possible to show with much more computation than the previous one that :

$$i_{CPE} = \frac{1}{Q_m} \frac{\mathrm{d}^\alpha v_{CPE}}{\mathrm{d}t^\alpha}$$

using a *fractional derivative.* This kind of mathematical object do exist and have formal definition that we will not need or use. Only the impedance is enough to modify the model.

We will use a relatively simple model depicted in the next Figure:
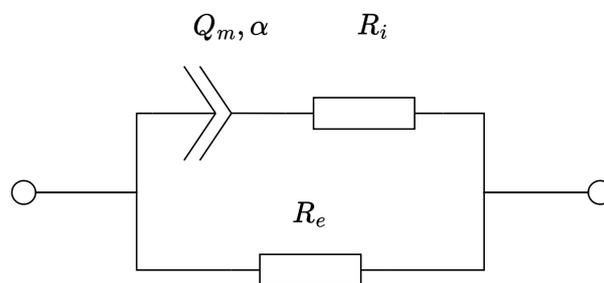
Figure 7: Modification of the RCR model to take into account the fractional behaviour

25. Using the properties of the CPE in the frequencial domain, identify *by hand* a first value for α

26. Write in the *Python* script a model function that takes into account the fractional behavior of the impedance.

27. Run the script, and comment values and results. Is the model more accurate? How, and how far?

28. Feel free to improve the model (we have not taken into account HF parasitics such as cross-talk capacitance or cable inductance for instance).

The mystery potato challenge

29. As final question, here is a small challenge: perform as many test as you want, re-use the code provided during the lab. **Write a programm that measures the impedance of a potato a identifies if the potatos is healthy (*raw*) or injured (*cooked*). Remember that you are not supposed to eat the subjects during experiments...**

I hope you enjoyed your ~~lunch~~ lab