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Determining Mammalian Cells State by Fractal Micromotion

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Objective

Study the evolution of the experimental cell culture impedance signal, in only one frequency, as a fractal geometric structure.

Test if the fractal dimension is a characteristic value of the cellular state by two independent experiments.

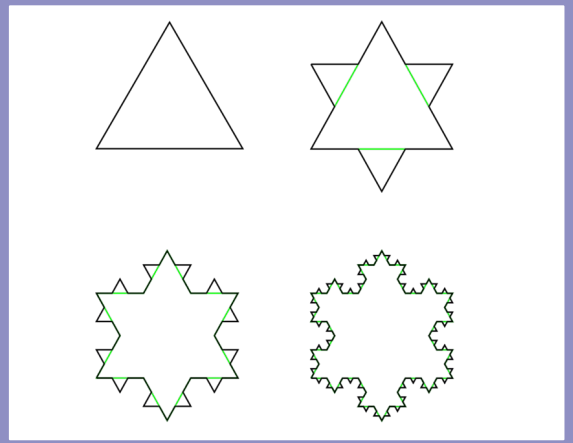
Motivation

To obtain information of the impedance fluctuations associated with the cell micromotion, and see if this is complementary to the culture impedance spectrum.

What is a fractal?

- A structure composed of smaller parts that resemble the whole.
- For these structures the fractal dimension can be calculated.
  - For mathematical fractals can me analytically calculated.
  - For natural fractal structures can be estimated.
- The dimension is a characteristic of the structure.

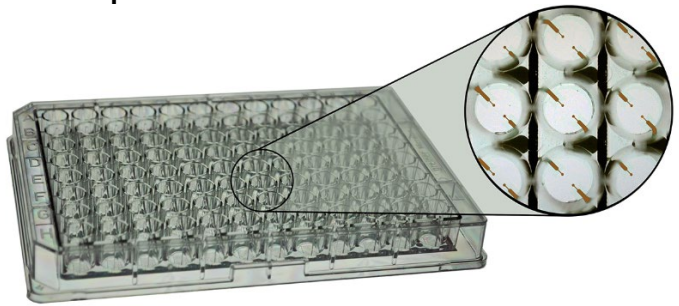
Romanesco broccoli structure (2D natural fractal).



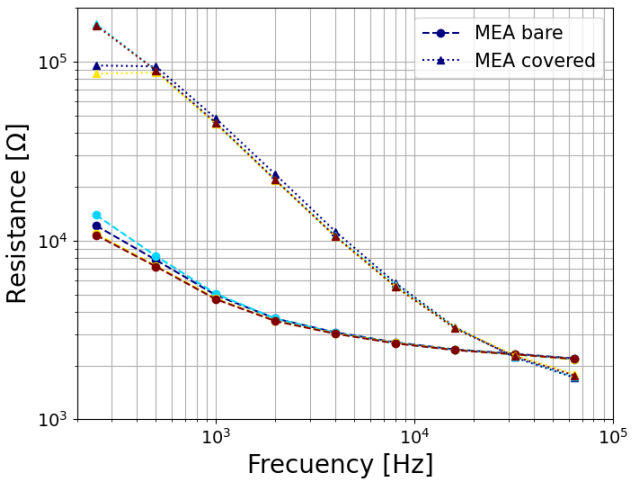
Koch snowflake (1D mathematical fractal).

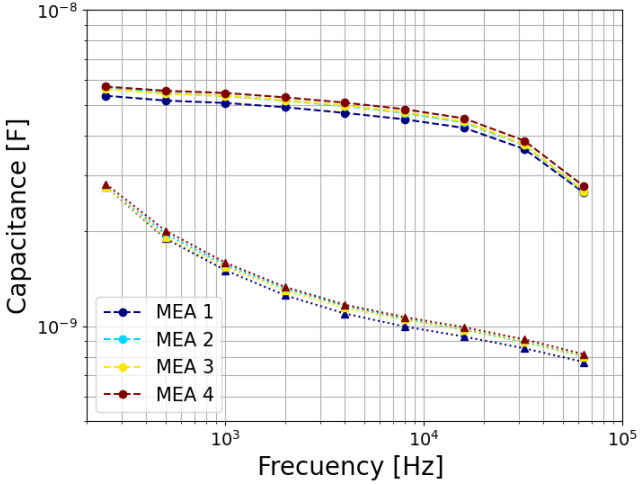
Electrical Cell-Substrate Impedance Sensing technique

Non invasive technique that focus in the study of the spectral impedance of a culture.



- We used normal MDCK type II cells.
- Cultivated over Biophysics 96W1E electrodes.

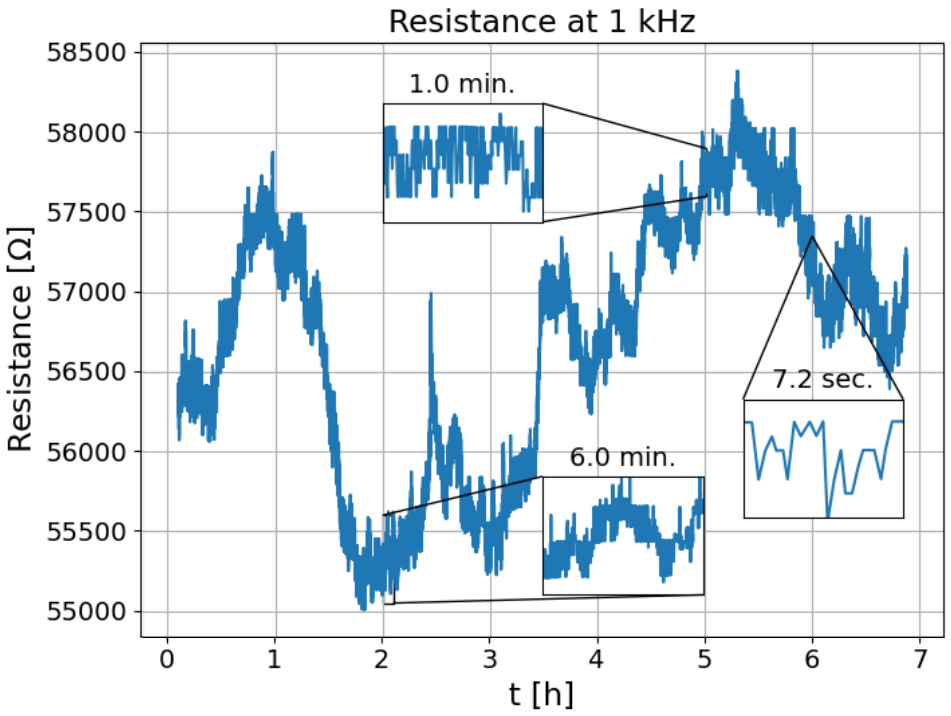




Bare (without cells) and covered electrodes impedance spectrums.

Micromotion

- When sampled rapidly the impedance signal of a confluent culture show fluctuations over time.
- The fluctuations are associated to cell micromotion over the electrode.
- The evolution presents fractal behaviour.
- Then the fractal dimension (FD) can be estimated.
- For signals FD = 1 indicates a flat line and FD = 2 indicates white noise influence.

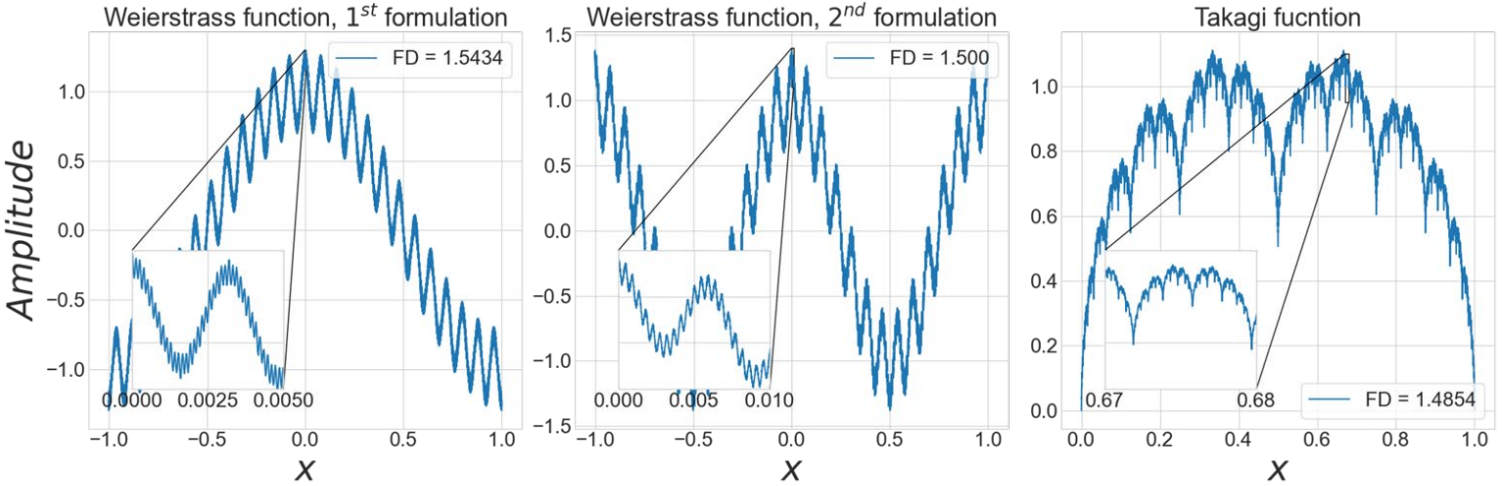


Fractal dimension algorithms

- Higuchi.
- Rescaled Range.
- Multiresolution Box-counting.
- Multiresolution Length-based.

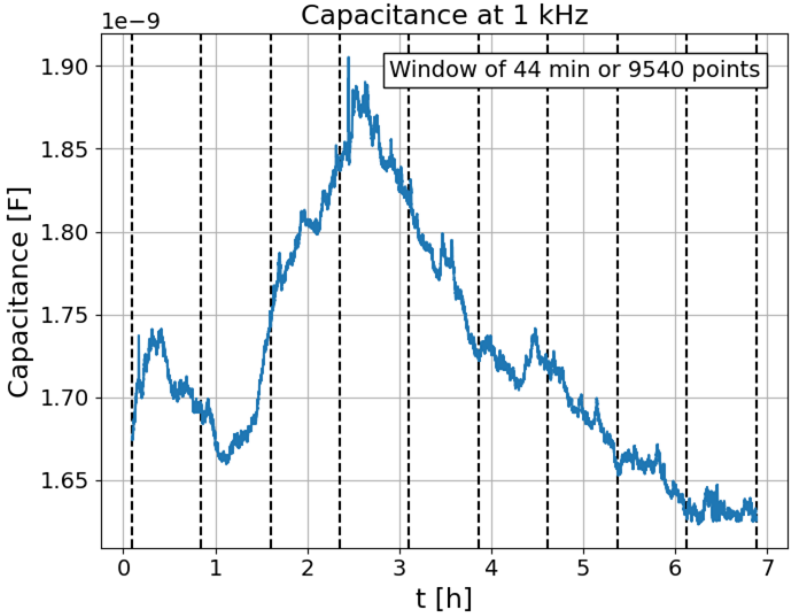
Validation of the algorithms

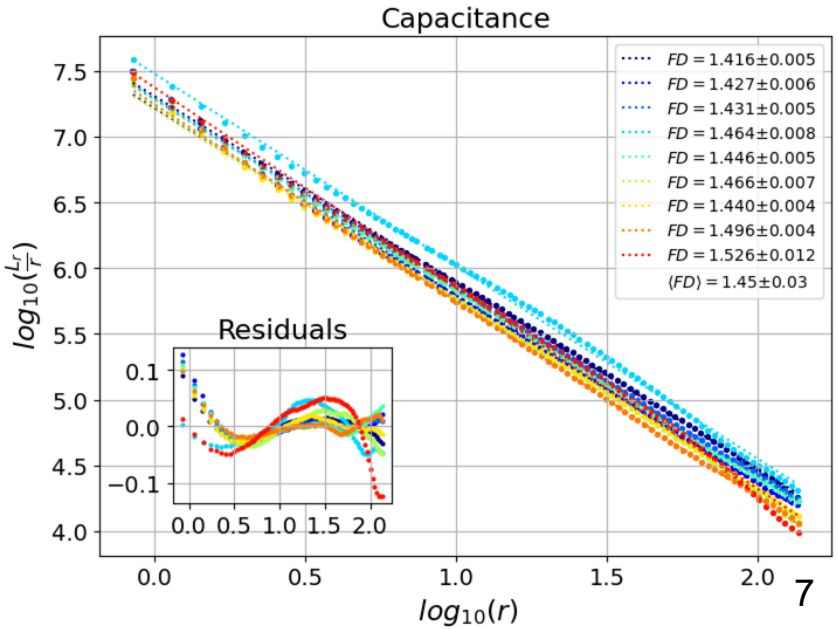
The algorithms were validated using topological functions of FD manipulable.
The conditions for a correct estimations were also studied.



Experimental signal processing

1. The signal is segmented and treated as independent signals.
2. Each segment is rescaled and the FD is calculated.
3. An statistical value of the FD is associated to the cell state.





Measurements

To study the exciting frequency dependency measurements were made at 1 kHz or 64 kHz for different cell states.

Characterization

- Bare electrode.
  - Starting point of culture fractal behaviour characterization.
- Normal culture: Seeding and confluent culture.
  - We used MDCK type II normal cells.

Experiments

- Electrically wounded and healed culture.
  - The wound is generated by applying 5 V_{rms} at 40 kHz for 30” to the culture.
- Experimental oncological drug administration.
  - This drug induced cell death between a 24 hs window.

Results

All the statistical estimations are summarized in this two tables.

You are invited to see and discuss every estimation by the poster.

Table 1: Resistance's over time signal fractal dimension estimation table for different culture states.

Cell status	Freq.	HI.	MR. BC.	MR. LB.	RS.
Bare electrode A	LF	1.997 ± 0.001	2.012 ± 0.002	2.012 ± 0.002	1.72 ± 0.03
Bare electrode A	HF	2.000 ± 0.001	2.009 ± 0.001	2.001 ± 0.001	1.84 ± 0.02
Bare electrode B	LF	1.999 ± 0.001	2.012 ± 0.001	2.012 ± 0.001	1.75 ± 0.03
Seeding process	LF	1.57 ± 0.13	1.67 ± 0.11	1.67 ± 0.11	1.72 ± 0.02
Confluent culture	LF	1.76 ± 0.05	1.77 ± 0.04	1.77 ± 0.04	1.65 ± 0.03
Confluent culture	HF	1.95 ± 0.03	1.97 ± 0.02	1.97 ± 0.02	1.61 ± 0.02
Wounded culture (healing)	LF	1.29 ± 0.12	1.43 ± 0.13	1.43 ± 0.13	1.70 ± 0.04
Healed confluence (Recently)	LF	1.68 ± 0.04	1.72 ± 0.03	1.72 ± 0.03	1.68 ± 0.03
Healed confluence (Post 24hs)	LF	1.48 ± 0.06	1.53 ± 0.06	1.53 ± 0.06	1.56 ± 0.04
Pharmacological assay	LF	1.56 ± 0.05	1.59 ± 0.05	1.59 ± 0.05	1.58 ± 0.04
Pharmacological assay	HF	1.71 ± 0.12	1.81 ± 0.09	1.81 ± 0.09	1.64 ± 0.04

Table 2: Capacitance's over time signal fractal dimension estimation table for different culture states.

Cell status	Freq.	HI.	MR. BC.	MR. LB.	RS.
Bare electrode A	LF	1.987 ± 0.001	2.007 ± 0.001	2.007 ± 0.001	1.79 ± 0.04
Bare electrode A	HF	1.998 ± 0.001	2.008 ± 0.001	2.008 ± 0.001	1.76 ± 0.02
Bare electrode B	LF	1.994 ± 0.001	2.009 ± 0.001	2.009 ± 0.001	1.75 ± 0.02
Seeding process	LF	1.51 ± 0.06	1.64 ± 0.04	1.64 ± 0.04	1.74 ± 0.02
Confluent culture	LF	1.46 ± 0.04	1.44 ± 0.03	1.44 ± 0.03	1.41 ± 0.04
Confluent culture	HF	1.92 ± 0.06	1.95 ± 0.05	1.95 ± 0.05	1.65 ± 0.02
Wounded culture (healing)	LF	1.31 ± 0.12	1.42 ± 0.13	1.42 ± 0.13	1.63 ± 0.08
Healed confluence (Recently)	LF	1.44 ± 0.05	1.40 ± 0.04	1.40 ± 0.04	1.39 ± 0.02
Healed confluence (Post 24hs)	LF	1.38 ± 0.04	1.37 ± 0.04	1.37 ± 0.04	1.39 ± 0.02
Pharmacological assay	LF	1.48 ± 0.04	1.46 ± 0.03	1.46 ± 0.03	1.45 ± 0.02
Pharmacological assay	HF	1.68 ± 0.05	1.80 ± 0.04	1.80 ± 0.04	1.66 ± 0.05

Summary

- Bare electrode and normal culture
  - The bare electrode impedance evolution presented white noise influence, FD ~ 2.
  - The FD discriminates at 1 kHz between a bare electrode and a confluent one, FD ~ 1.7.
  - This did not differ at high excitation frequencies (64 kHz), FD ~ 2.

- Wound-Healing assay
  - A wounded culture fractal behaviour differ from bare electrodes, even with similar spectral impedance.
  - The confluent fractal dimension was re-obtained after cicatrization at 1kHz.

- Drug assay
  - The fractal dimension was reduced after the drug administration, in both high and low excitation frequencies. This change was not detected in the spectral impedance.

Conclusions

- The fractal dimension is a valid value to detect certain process of the cellular culture.
- Not all the algorithms are sensible to the same changes in cell state.
- Most processes were detected estimating the resistance fractal dimension.
- The estimation depends of the used excitation frequency.

Line of work continuation

- Simulate the culture influence in the bare electrodes signal, as a low pass filter.
- Try other signal processing tools to detect culture changes (using the same dataset).
- Study if other drugs administration are reflected in the fractal dimension.

Thanks for listening