Tracking electrochemically active biofilms using an electrochemical quartz crystal microbalance (eQCM)

GAMRY INSTRUMENTS

Perspective and goal

- Perspective:
  - Biofilm researcher looking for a system with low barrier to entry into eQCM
- Goal:
  - Provide a practical framework to run eQCM experiments for biofilm research with minimal investment and instead focusing on your research questions.

Common tools to biofilm research

- Autoclave for steam sterilization
- Biosafety cabinet
- Environmental chamber for anaerobic microbes
- Basic PPE and protocols for disinfecting surfaces
- Microscopes (such as SEM)

Hardware topics

- QCM
- Potentiostat (not covered here)
- eQCM cell and sensor holder

Applications

- Growing electrochemically active biofilms (EAB)
- Basic biofilm electrochemistry
- Tracking *Geobacter sulfurreducens* biofilm growth with eQCM

What is QCM?

- QCM is a device that consists of a mass sensor and the electronics that control the mass sensor
- We care about the mass sensor only
- The mass sensor is selective—only detects <u>mass</u> that "strongly" <u>attaches</u> to the sensor
- The mass sensor is sensitive, resolving ng-level mass changes

QCM is a device used to study film formation and surface-related physicochemical phenomena

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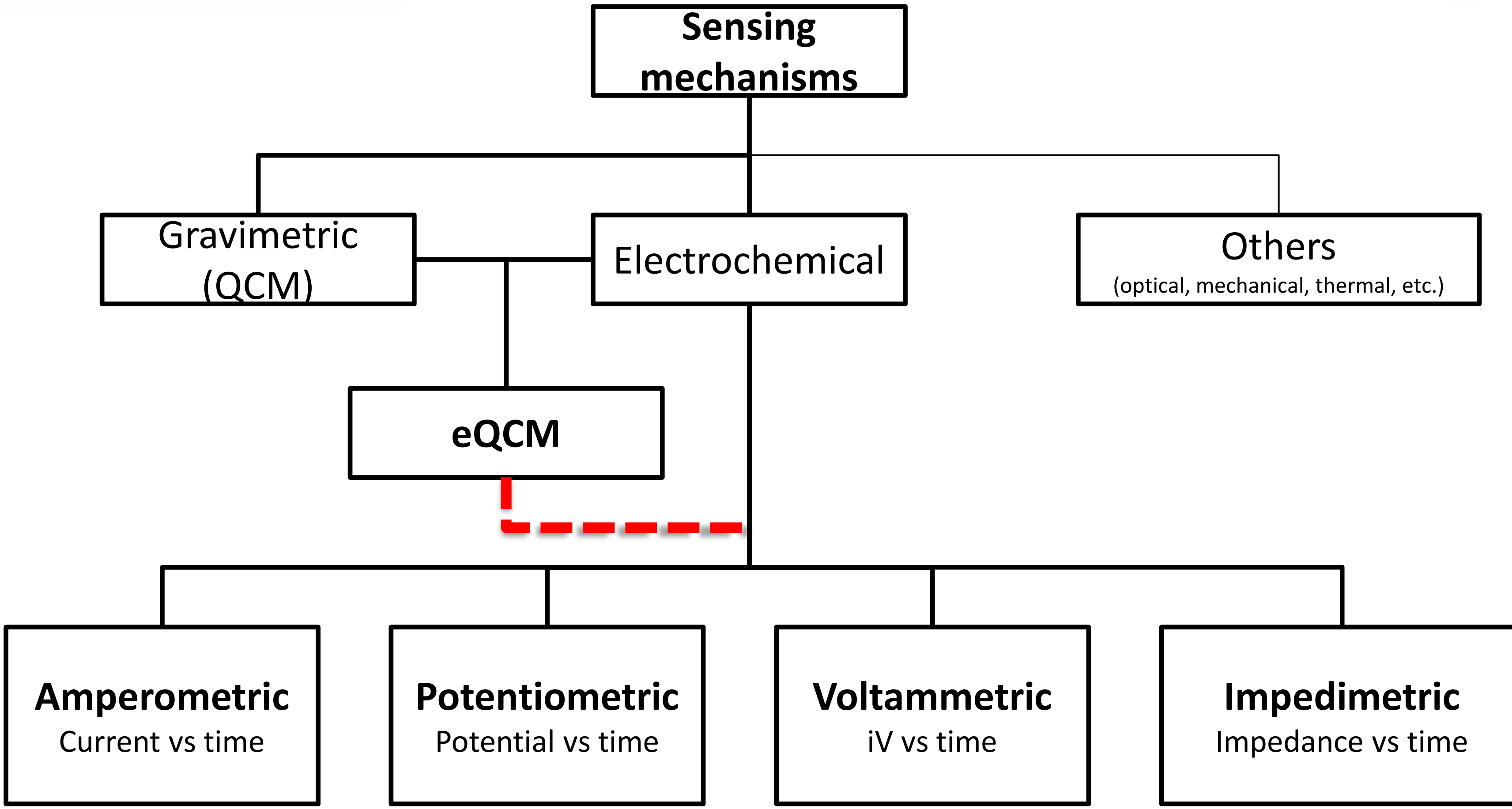
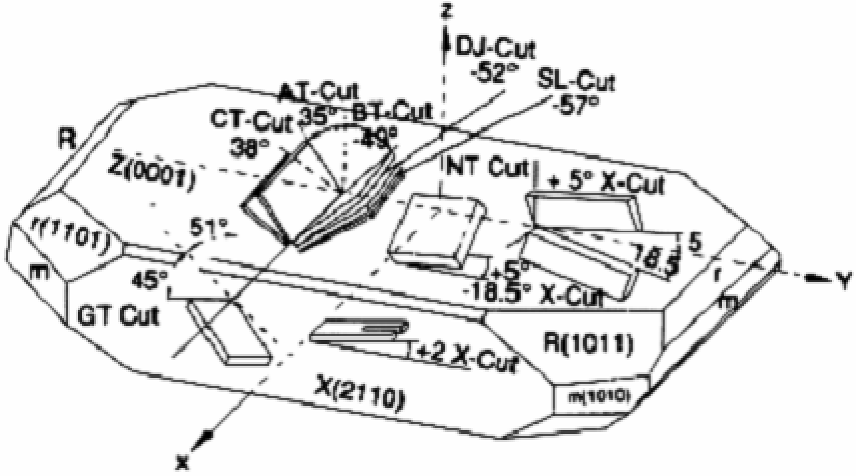
Microsensors and Microscale Gradients in Biofilms

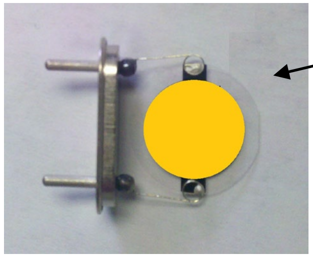
Haluk Beyenal and Jerome Babauta



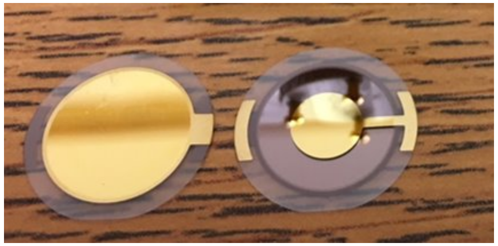
What is the mass sensor?

- Quartz crystal with a particular cut
  - AT-cut is the most common
- Quartz is sandwiched between two electrodes
- Utilizes quartz piezoelectric behavior to make it oscillate
  - Electrodes apply voltage





One face on each side of crystal.

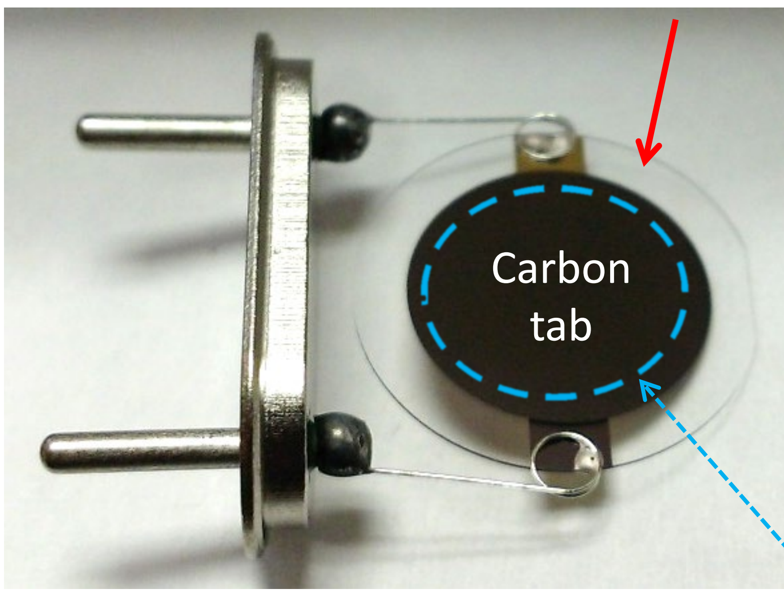


Quartz crystal components

Important crystal parameters:

1) area of overlap
2) electrode surface area
3) fundamental frequency
4) Q factor

quartz



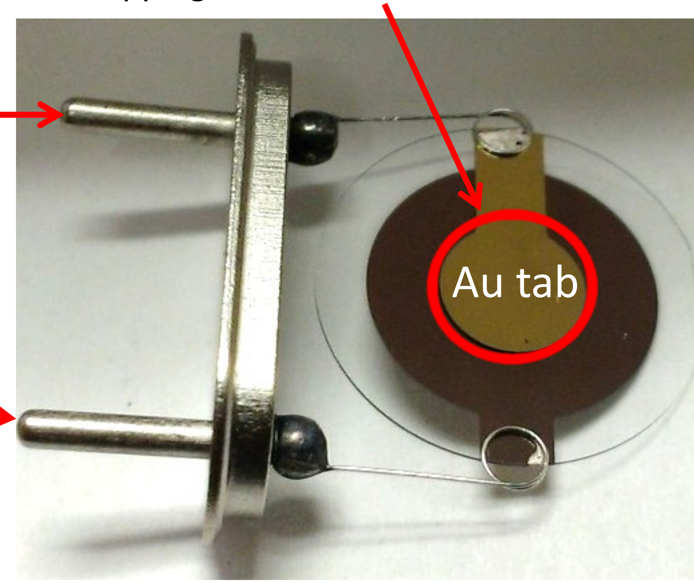
Carbon face-up

When clamped in a cell, only this area is exposed to solution

Oscillation of the quartz is restricted to the overlapping areas between C-tab and Au-tab

Au-contact

C-contact

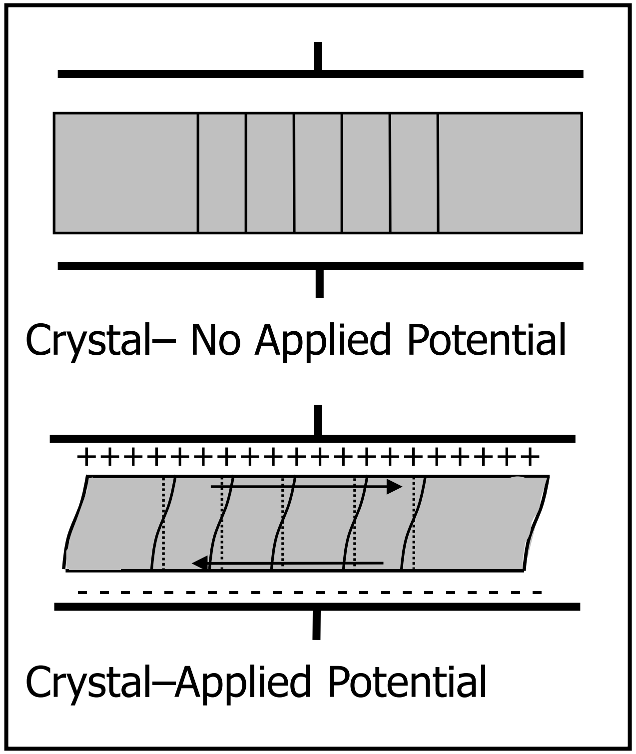


Carbon face-down

What is a QCM?

- Upon application of an alternating potential to the crystal faces, the quartz crystal resonates. At a particular frequency the crystal oscillates with very little energy lost. This is the fundamental frequency (f_0) determined by the density (ρ_q), thickness (t_q), and shear modulus (μ_q) of the quartz.

$$f_0 = \sqrt{\frac{\mu_q}{\rho_q}} / 2t_q$$



QCM is an electromechanical system

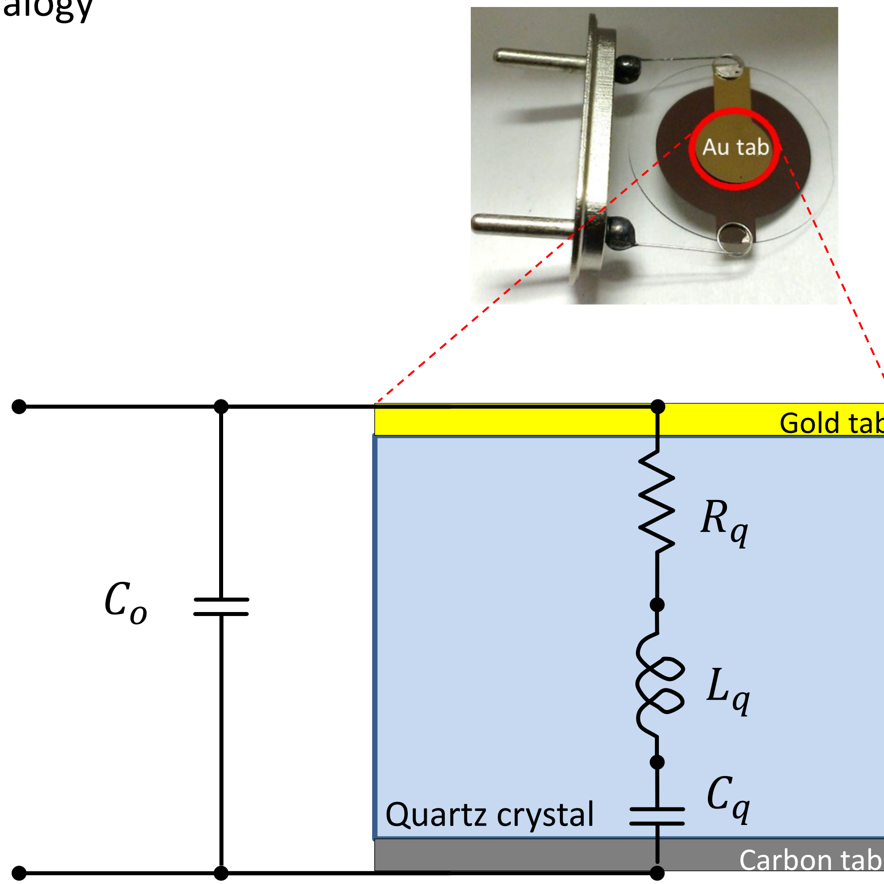
https://en.wikipedia.org/wiki/Impedance_analogy

Elements[30]

Mechanical property	Analogous electrical property
Damping	Resistance
Mass	Inductance
Compliance	Capacitance
Mechanical impedance	Electrical impedance

- Most commercial QCMs will extract frequency information from a change in electrical impedance (i.e. response) of the quartz crystal.

- BvD model describes electrical characteristics of the quartz crystal close to mechanical resonance (f_o).



Butterworth – van Dyke (BvD) model

(Wudy et al., Electrochimica Acta 53 (2008) 6568-6574)

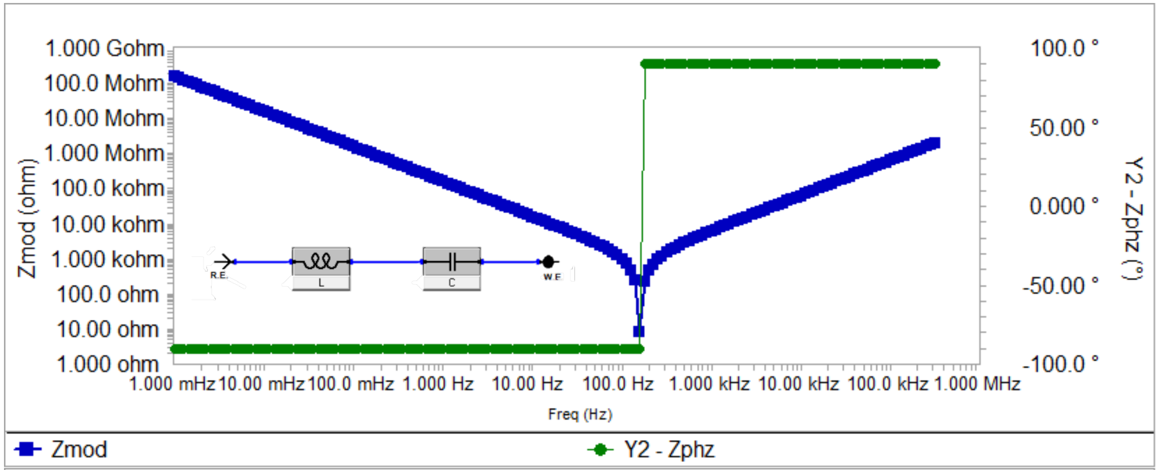
Simplified electrical analogy – LC circuit

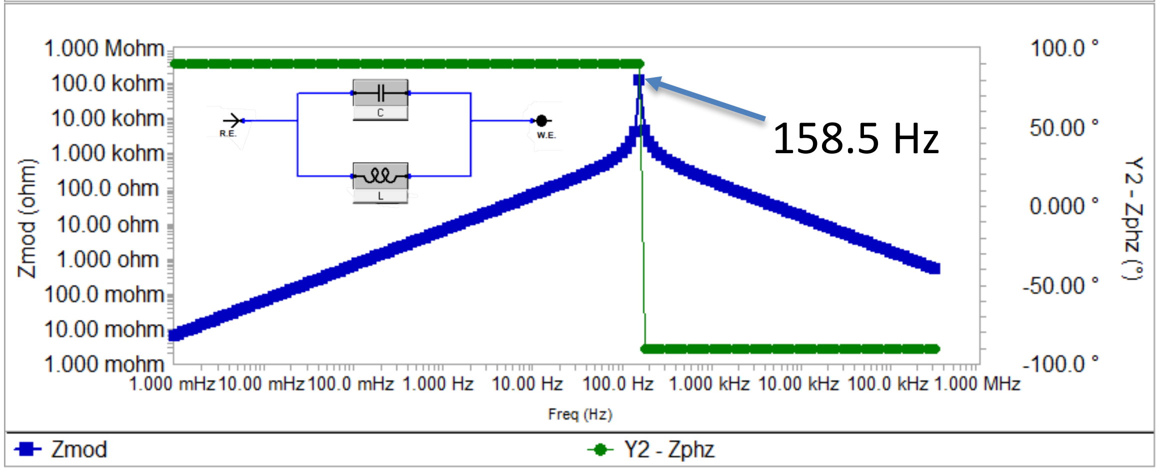
$$f_{resonance} = \frac{1}{2\pi\sqrt{LC}}$$

$$f_{resonance} = 159\ Hz$$

C: 1 μF

L: 1 H





QCM is an electromechanical system

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Mechanical property	Analogous electrical property
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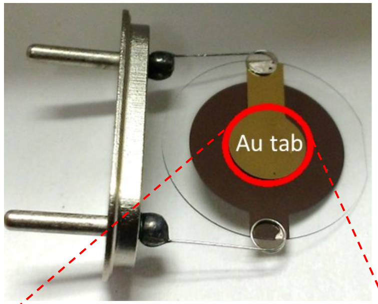
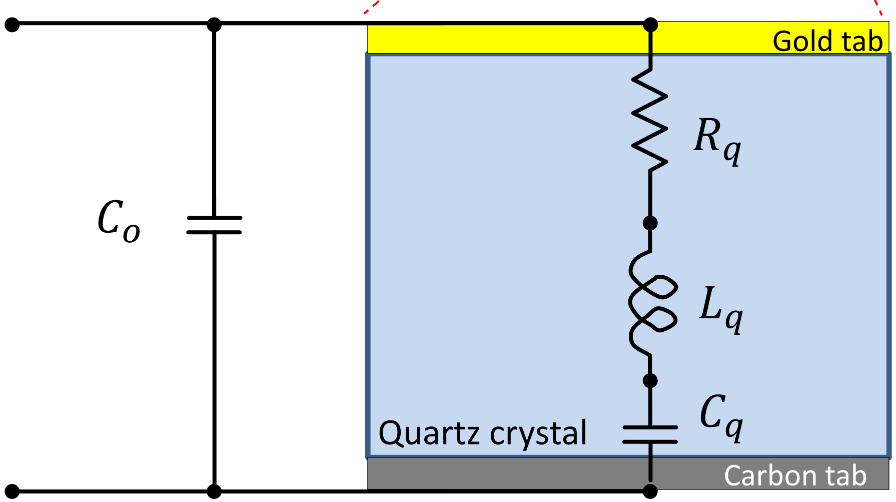
Mechanical impedance	Electrical impedance

- Series resonant frequency, Fs, related to the series LC
- Parallel resonant frequency, Fp, related to the parallel LC





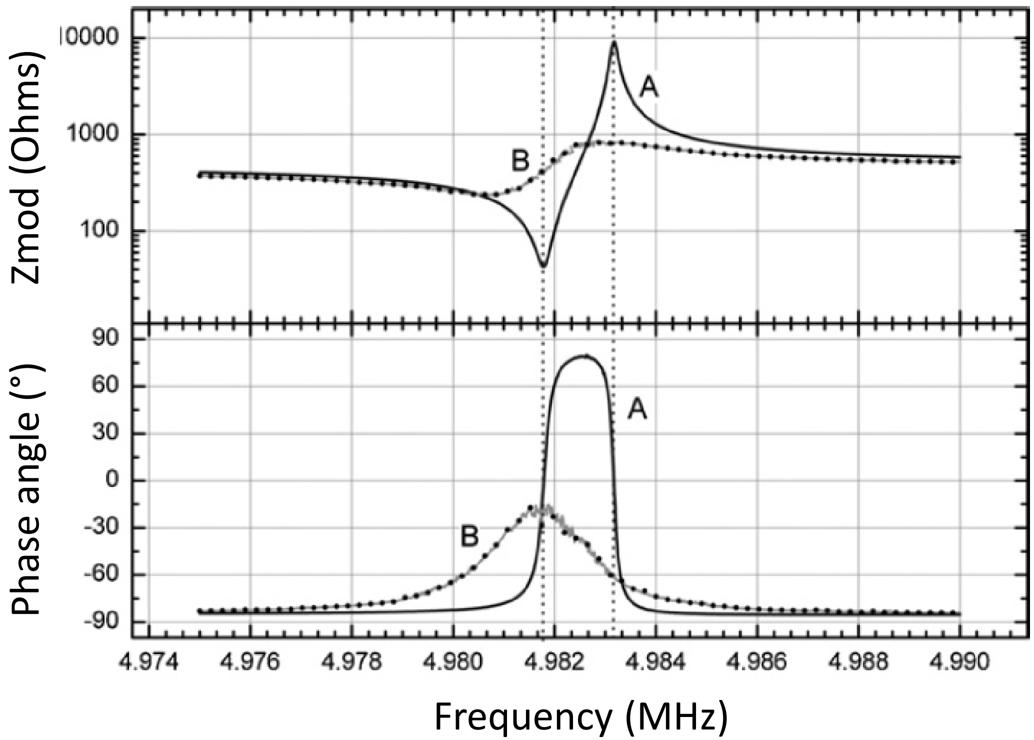
Butterworth – van Dyke (BvD) model

(Wudy et al., Electrochimica Acta 53 (2008) 6568-6574)

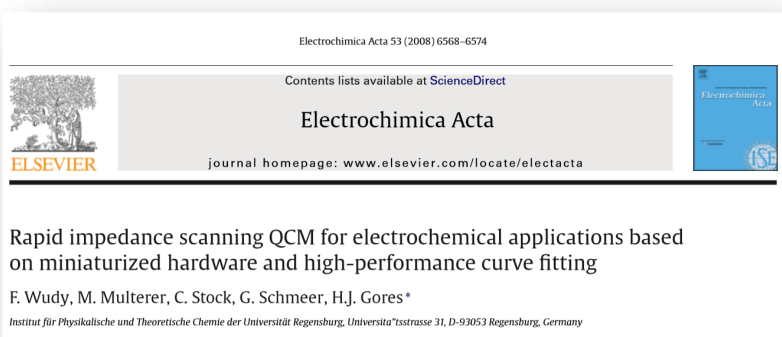
BvD model importance

BvD model predicts two important frequencies in the crystal oscillation behavior:

1) Series frequency shift, Fs
2) Parallel frequency shift, Fp



- Bode plot of an oscillating quartz crystal sensor
  A: in air
  B: in liquid



Electrochimica Acta 53 (2008) 6568–6574

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Rapid impedance scanning QCM for electrochemical applications based on miniaturized hardware and high-performance curve fitting

F. Wudy, M. Multerer, C. Stock, G. Schmeer, H.J. Gores*

Institut für Physikalische und Theoretische Chemie der Universität Regensburg, Universita"tsstrasse 31, D-93053 Regensburg, Germany

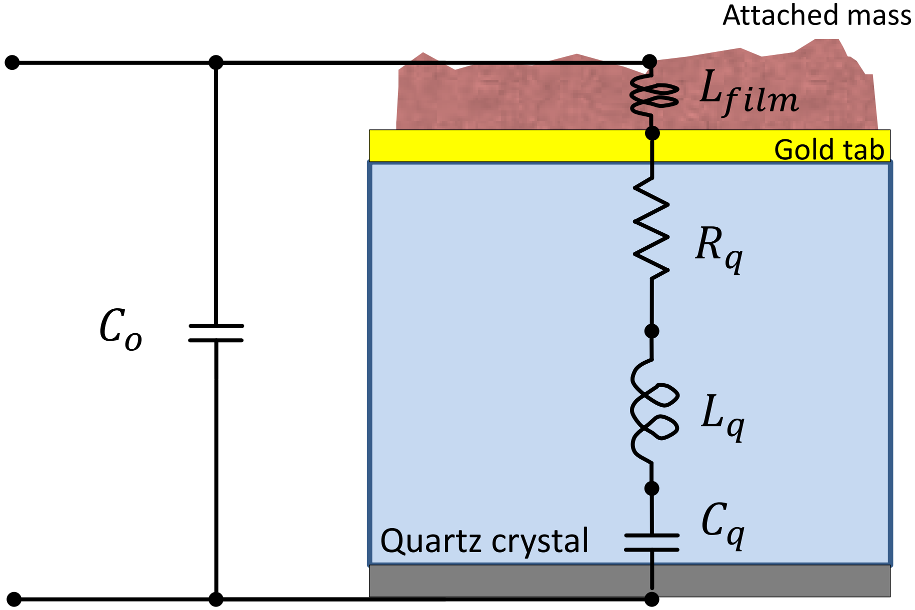
What happens when mass is attached?

https://en.wikipedia.org/wiki/Impedance_analogy

Elements[30]

Mechanical property	Analogous electrical property
Damping	Resistance
Mass	Inductance
Compliance	Capacitance
Mechanical impedance	Electrical impedance

1. Treat the attached mass as a rigid extension of the quartz
2. The mass is evenly distributed across the electrode
3. Mass attached is << mass of the oscillating crystal

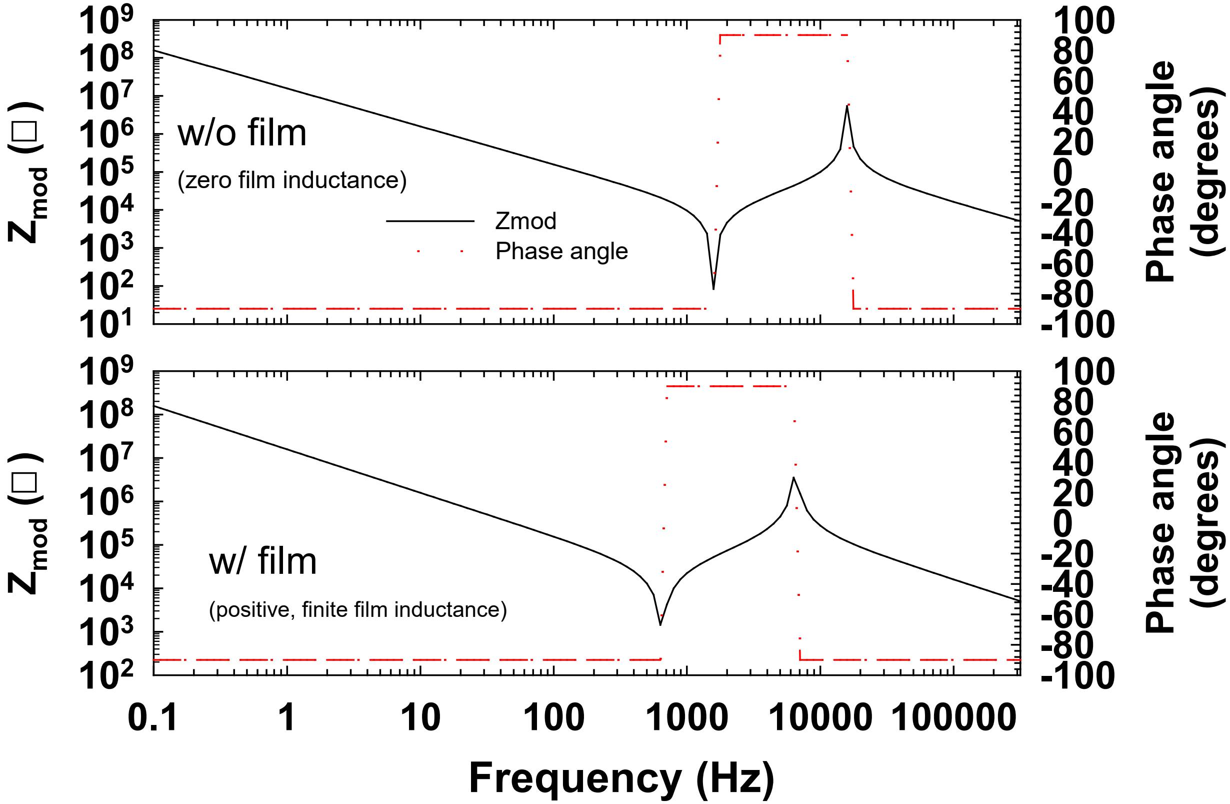


$$f_{s,baseline} = \frac{1}{2\pi\sqrt{L_q C_q}} \Rightarrow f_{s,film} = \frac{1}{2\pi\sqrt{(L_q + L_{film}) C_q}}$$

When film is “loaded” onto the electrode…

- Increase in mass is modeled as increase in inductance
- BvD model predicts that both series (f_s) and parallel (f_p) resonant frequencies to shift to the left

$$\frac{f_p}{f_s} = \left(1 + \frac{C_q}{C_o}\right)^{\frac{1}{2}}$$

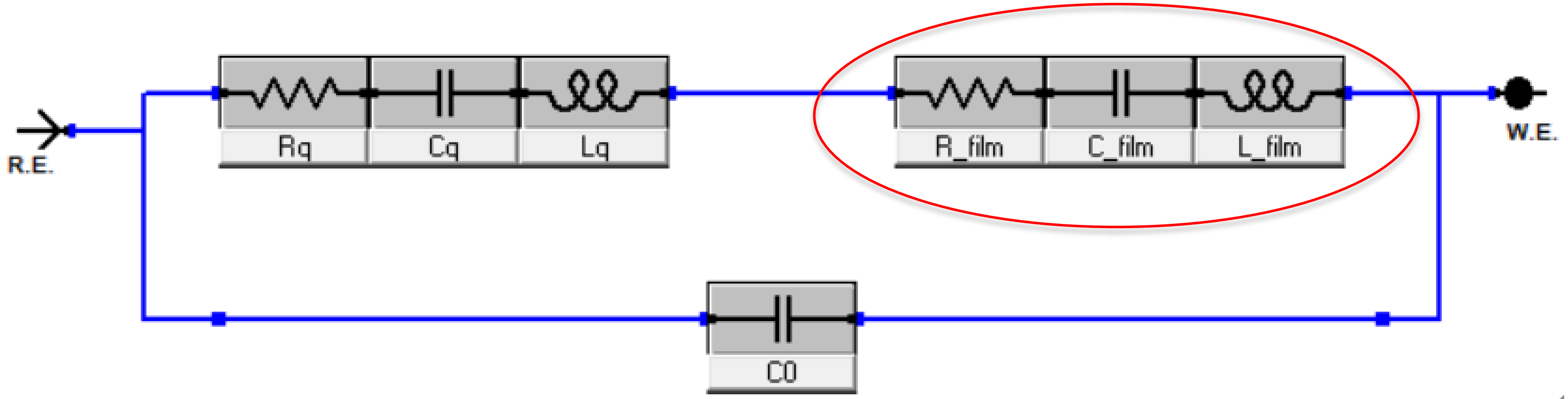


QCM tracks changes in f_s and f_p

- L adds in series (net increase)
- C adds in parallel (net decrease)
- f_s and f_p insensitive to small changes in R

$$f_s = \frac{1}{2\pi\sqrt{L_q C_q}}$$

$$\frac{f_p}{f_s} = \left(1 + \frac{C_q}{C_o}\right)^{\frac{1}{2}}$$



Sauerbrey equation

- Variation in f_s (Δf_s)can be correlated to mass attaching to the electrode.
- For most films, this is not a true mass and is instead an apparent mass.
- Often called "Sauerbrey mass"

$$\Delta f_s = -\frac{2\Delta m n f_0^2}{(\mu\rho)^{1/2}}$$

Δm = mass change

μ = shear modulus of quartz crystal (cut dependent)

ρ = density of quartz

f_o = fundamental frequency

n = harmonic (n=1 for 5 MHz crystal resonating at 5 MHz)

Reduce above to:

$$\Delta f_s = -C_f \Delta m$$

C_f = 56.6 Hz cm^2/ug for 5 MHz AT-cut

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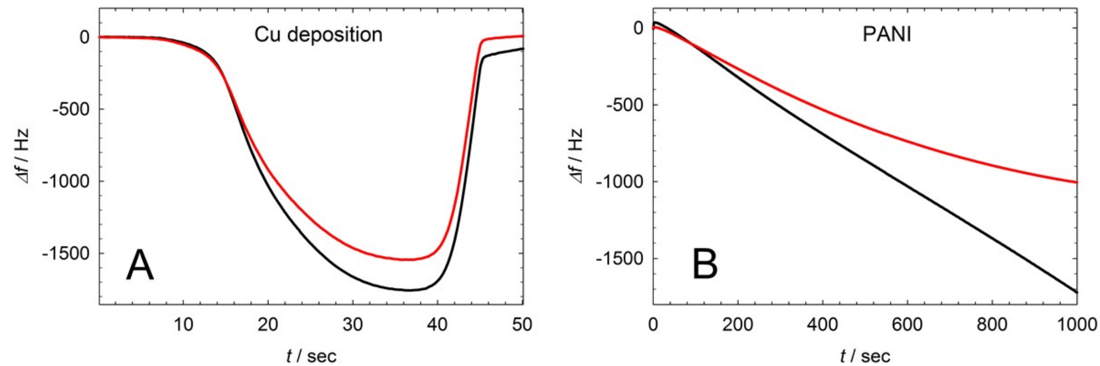
Investigation of Electron Transfer by *Geobacter sulfurreducens* Biofilms by using an Electrochemical Quartz Crystal Microbalance

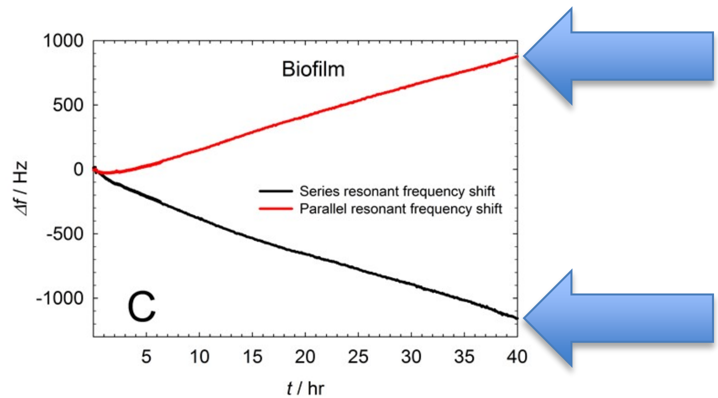
Jerome T. Babauta,[a] Christopher A. Beasley,[b] and Haluk Beyenal*[a]

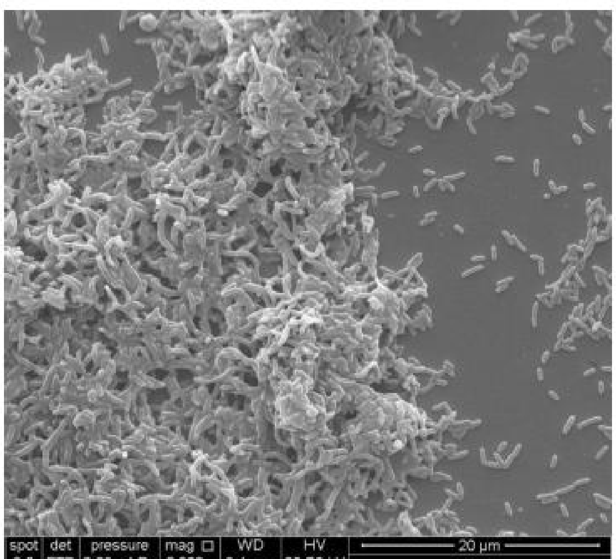
Experimental data

$$\frac{f_p}{f_s} = \left(1+\frac{C_q}{C_o}\right)^{\frac{1}{2}}$$

A. Copper deposition
B. Polyaniline deposition
C. Bacterial biofilm deposition







G. Sulfurreducens biofilm on Au-coated quartz crystal.

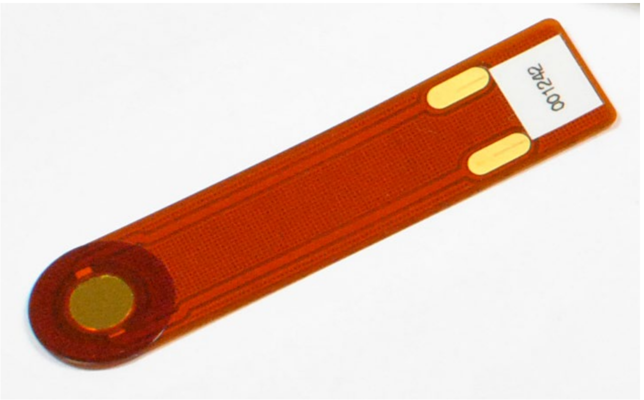
What is an eQCM?

- Electrochemical Quartz Crystal Microbalance – a QCM that has been interfaced to a potentiostat or galvanostat.
  - The potentiostat or galvanostat is used to induce adsorption, desorption or changes in a film deposited on an electrode.
  - Can correlate electrochemical change to gravimetric change.

eQCM cell for biofilm growth

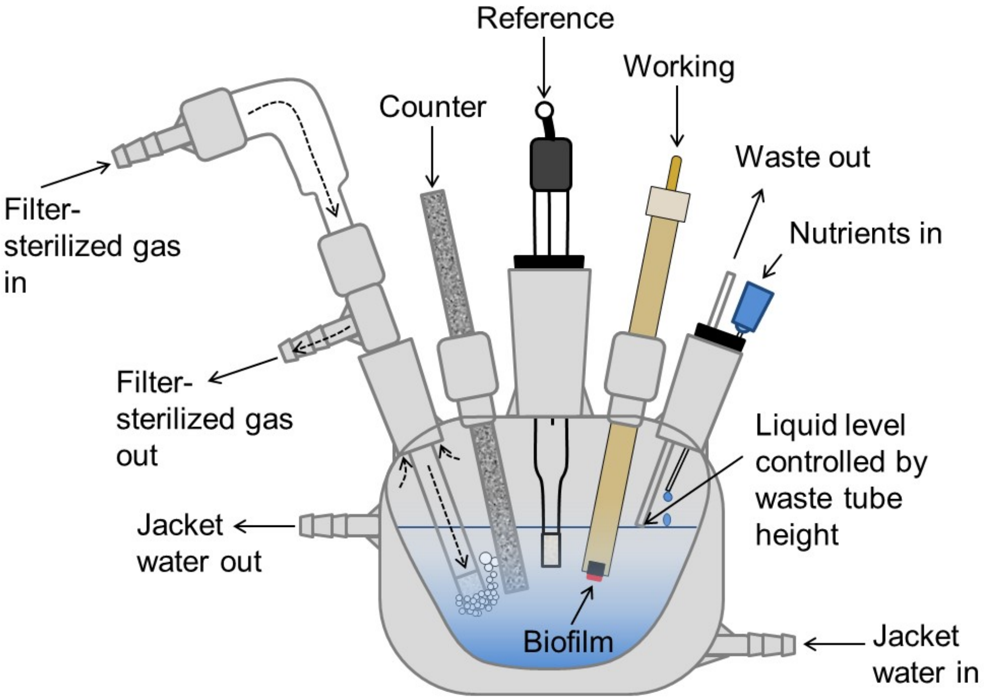
- Easy to sterilize
- Quartz crystal easily removable for further biofilm analysis
- Standard electrode geometry and electrode parts
- Built-in gas purge assembly for anaerobic biofilm
- Replaceable parts

eSorption probe



Convert jacketed Eurocell / RDE cell kit

- 24/40 ports will work with a #4 or #5 rubber stopper
- 14/20 ports will work with a #1 or #0 rubber stopper
- Use a borosilicate glass capillary tube as the waste tube that controls the liquid level
- Sterile hypodermic needle inserted after sterilization



Electrochemically active biofilm

- Electrochemically active bacteria (EAB):
  - biofilm that can transfer electrons to and from a solid electrode
  - biofilm that generate a product that is electro-active

- Biofilm electrochemistry:
  - The study of electrochemically active biofilm using electrochemical theory
  - Electrode behavior typically represents the overall response of cells respiring in the biofilm (causality)

Cathodic EAB vs. Anodic EAB

- Cathodic EAB
  - Grow in an oxidized environment such as the cathodes of microbial fuel cells
  - Accept electrons from the electrode and force the electrode potential towards more positive voltages
  - Ex: Metal-oxidizing bacteria such as *Leptothrix discophora* SP-6

- Anodic EAB
  - Grow in a reduced environment such as the anodes of microbial fuel cells
  - Donate electrons to the electrode and force the electrode potential towards more negative voltages
  - Metal-reducing bacteria such as *Shewanella oneidensis* MR-1

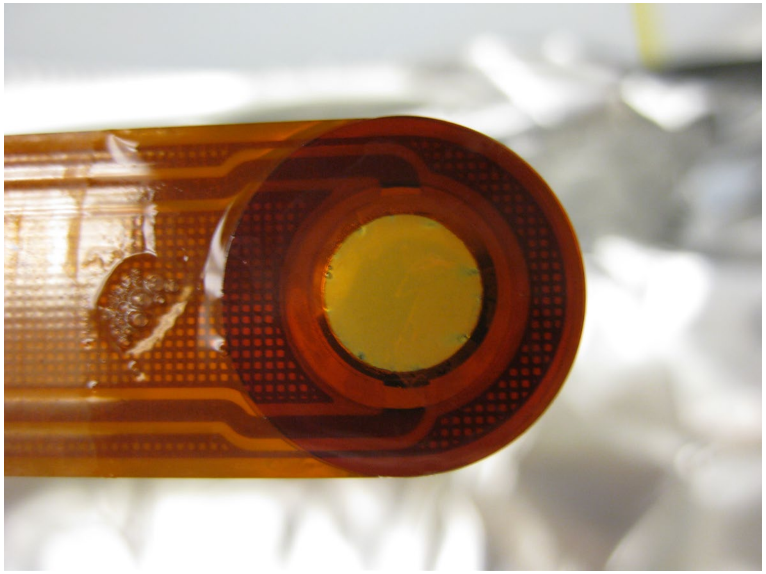
Geobacter sulfurreducens biofilm on electrodes

- *G. sulfurreducens*, an oxygen-intolerant species of bacteria able to grow as biofilms on electrodes[1].
- Biofilm metabolizes acetate (a source of organic carbon).

$$\text{Acetate} \xrightarrow{\text{biofilm}} CO_2 + \text{protons} + \text{electrons}$$

- Biofilm can be grown on the Au-coated crystal.
- Biofilm is only found on Au because the electrode is the electron sink for the electrons generated from respiration

When studying EABs, the electrochemical system is designed so that the only electron sink is the electrode.

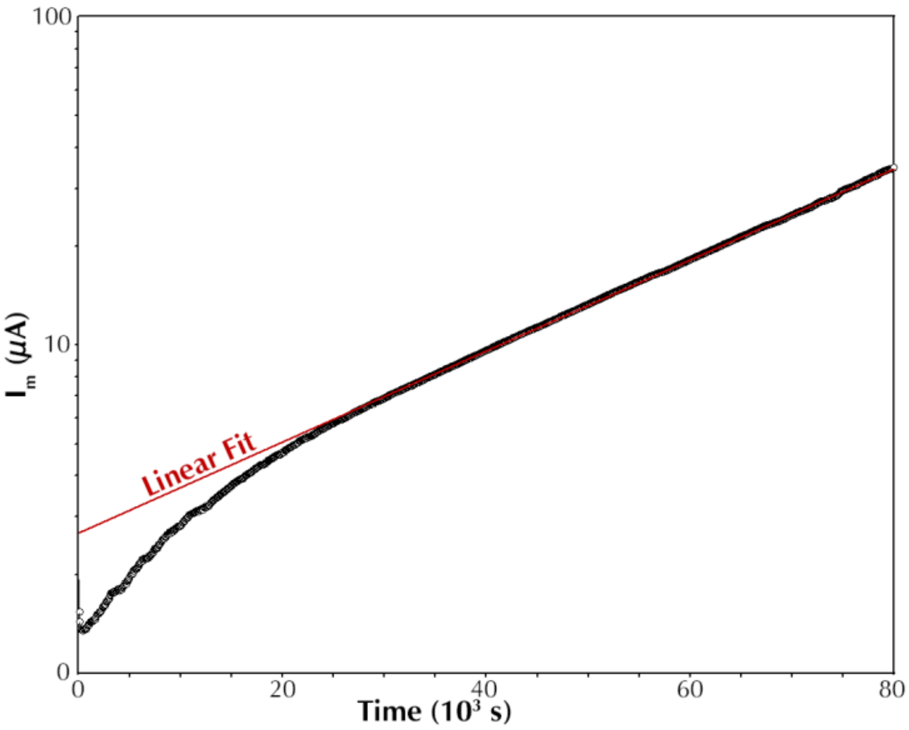


Photograph of biofilm grown on the 10 MHz Au-coated crystal for the eSorptionProbe

[1]Bond, D.R. and Lovley, D.R., *Appl. Environ. Microb.* 2003, **69**(3), 1548−1555.

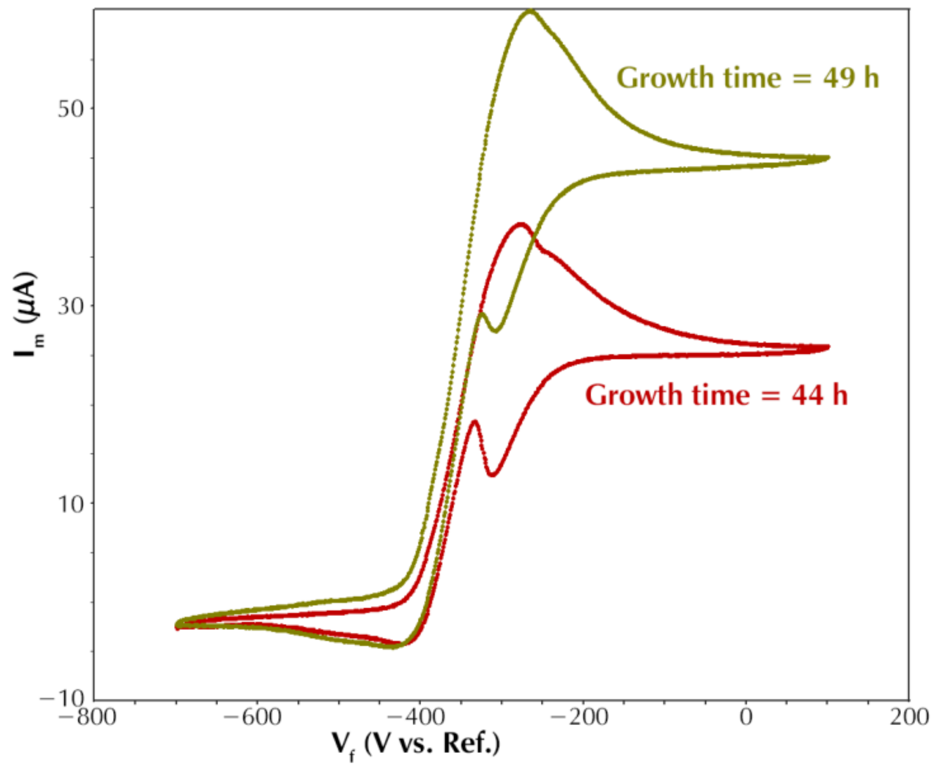
Current is a proxy for biofilm respiration rate

- For *G. sulfurreducens* biofilm, the acetate half-reaction is activated above −0.4 $V_{Ag/AgCl}$.
- The electrode potential needs to be polarized above this to allow biofilm growth.
- *G. sulfurreducens* cells are added to the eQCM cell.
- Cells that attach to the electrode form the initial biofilm.
- The initial biofilm metabolizes acetate and produces electrons at an increasing rate.

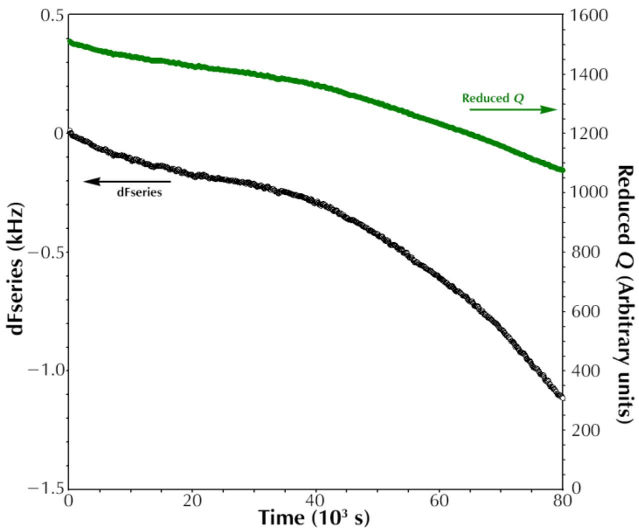


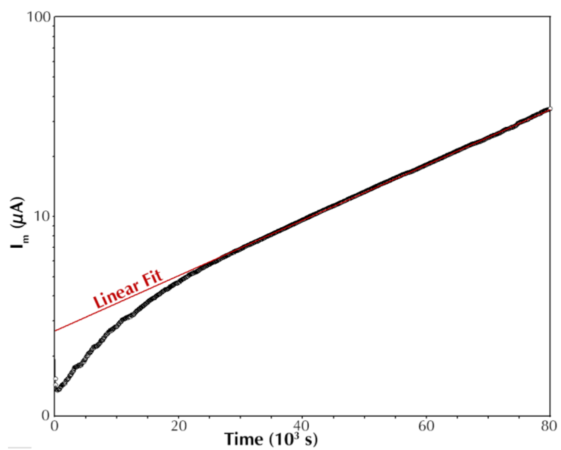
Cyclic voltammetry (CV)

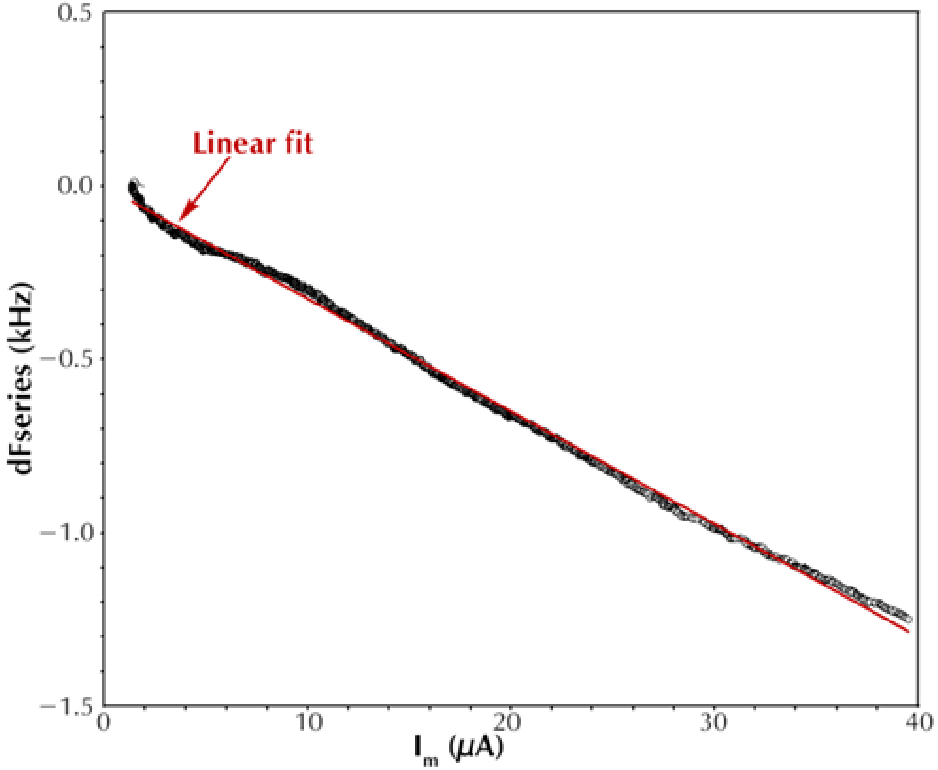
- During biofilm growth, the chronoamperometry script can be stopped without damaging the biofilm.
- Run cyclic voltammetry script (scan rate of 30 mV/s)
- A catalytic wave is observed with several redox peaks superimposed



Frequency shift vs. current







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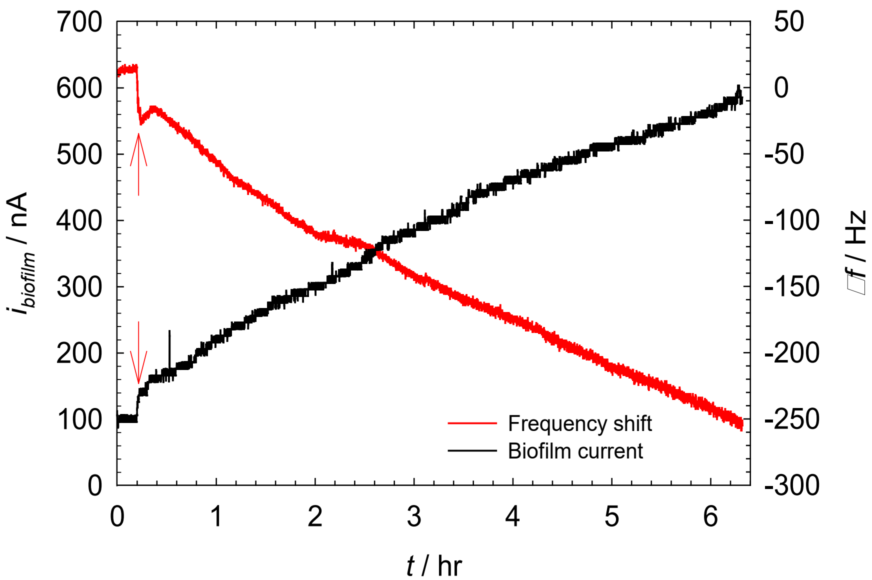
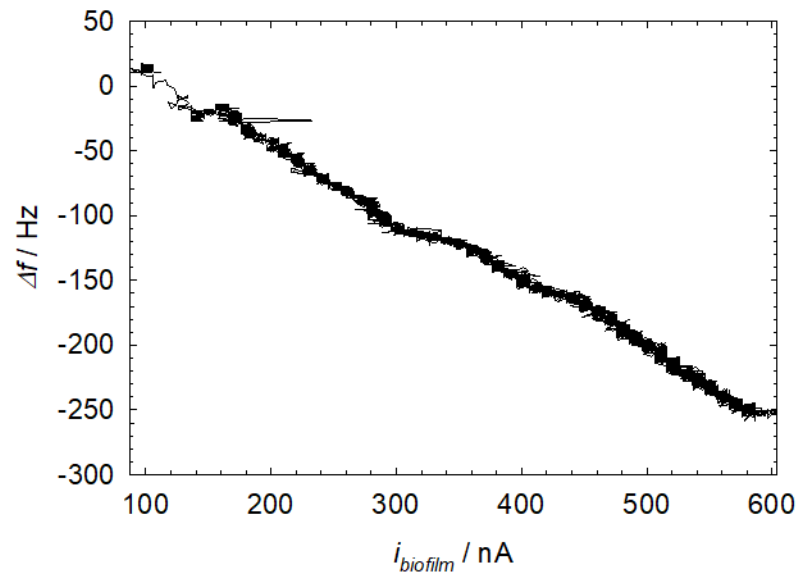
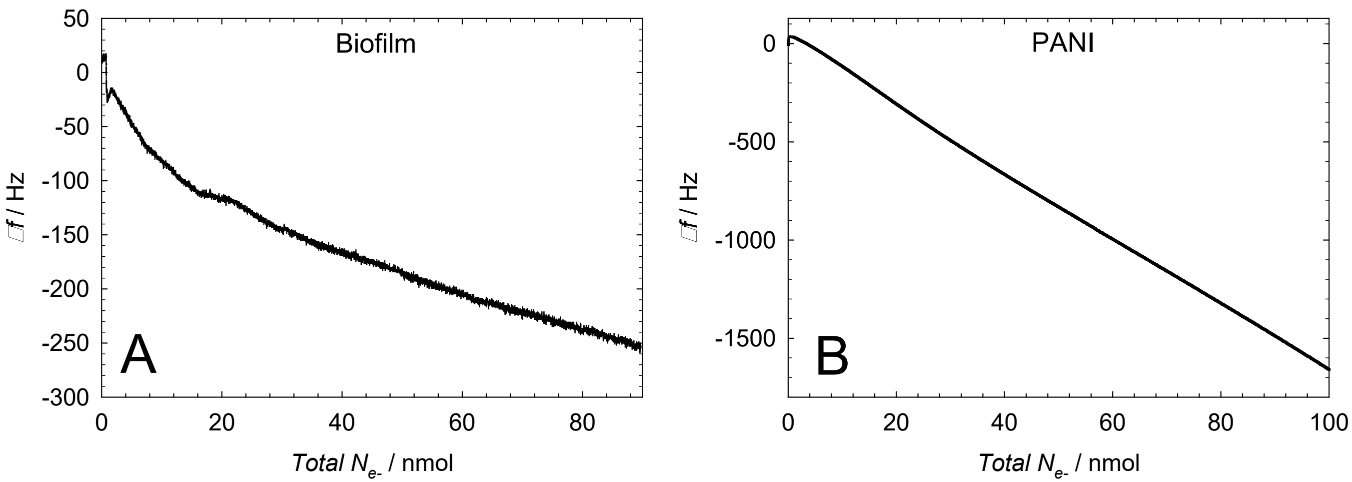
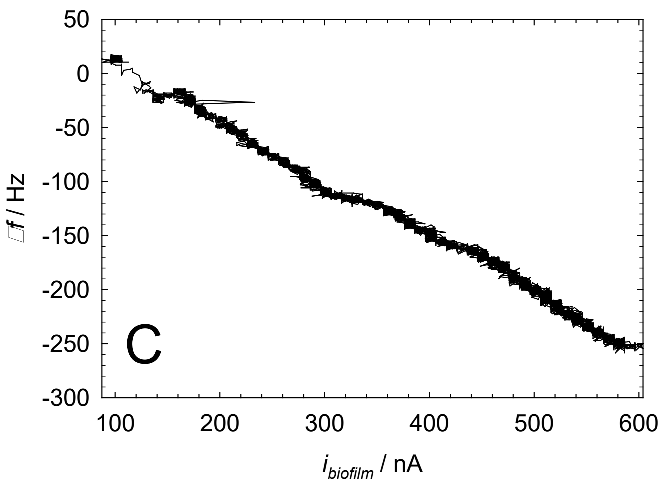
Closer look at initial attachment





Comparison between biofilm and a conductive polymer, polyaniline (PANI)

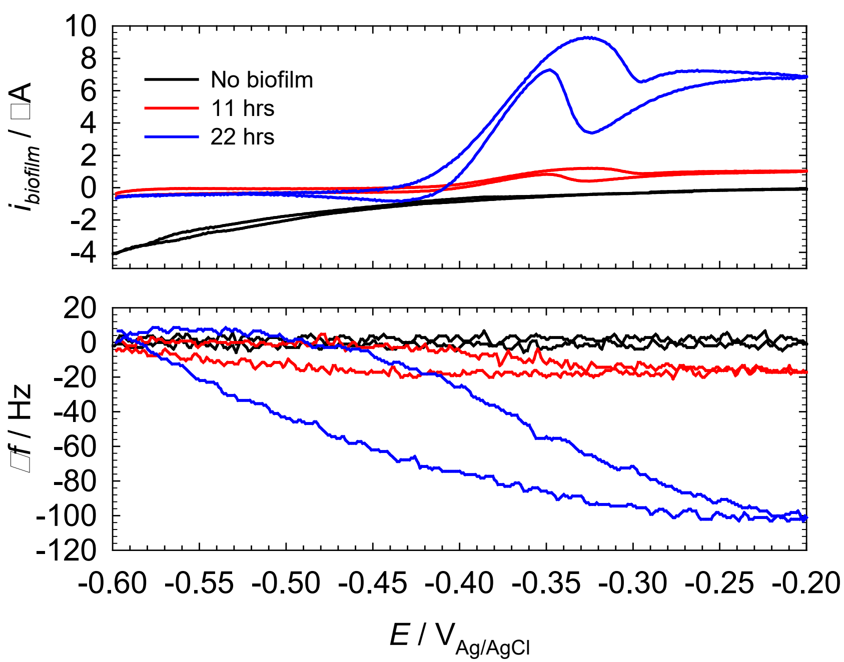




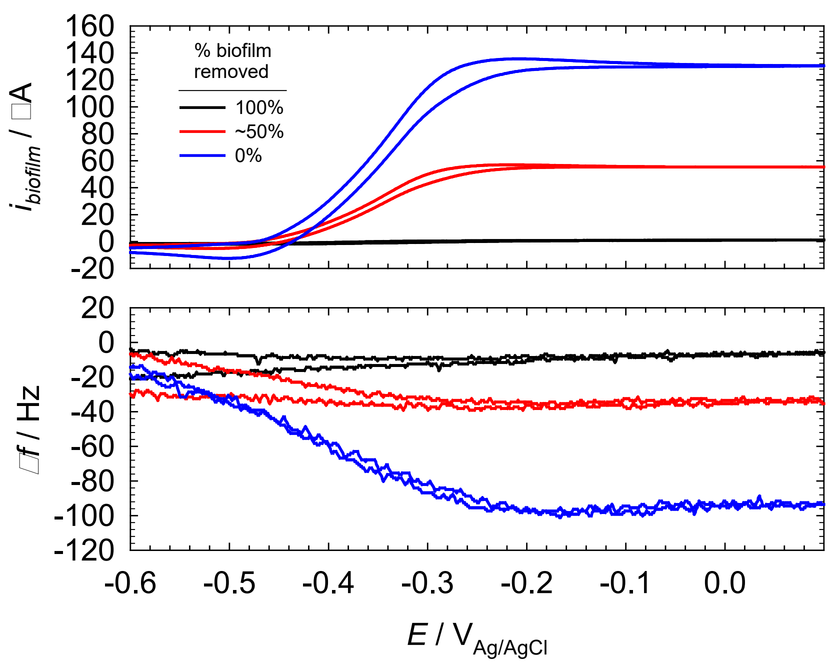
- PANI propagation requires stoichiometric amounts of electrons to be deposited
- Biofilm (and individual cells within it) require a certain amount of electron flux

Transient frequency shift during CV

Young biofilm

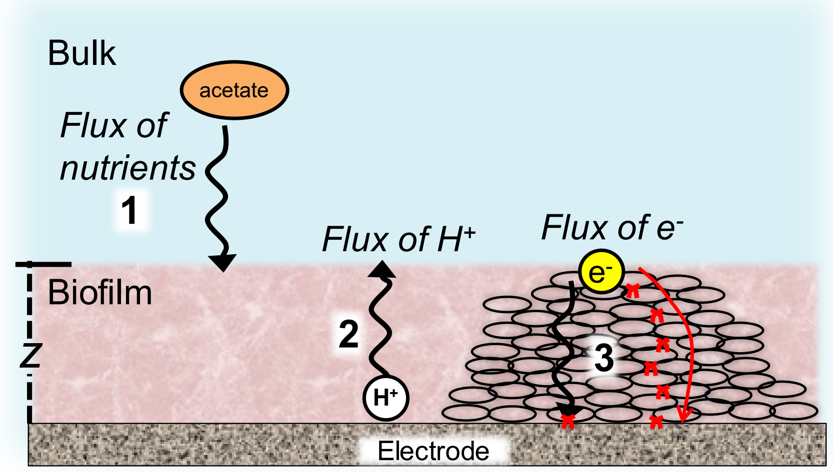


Mature biofilm



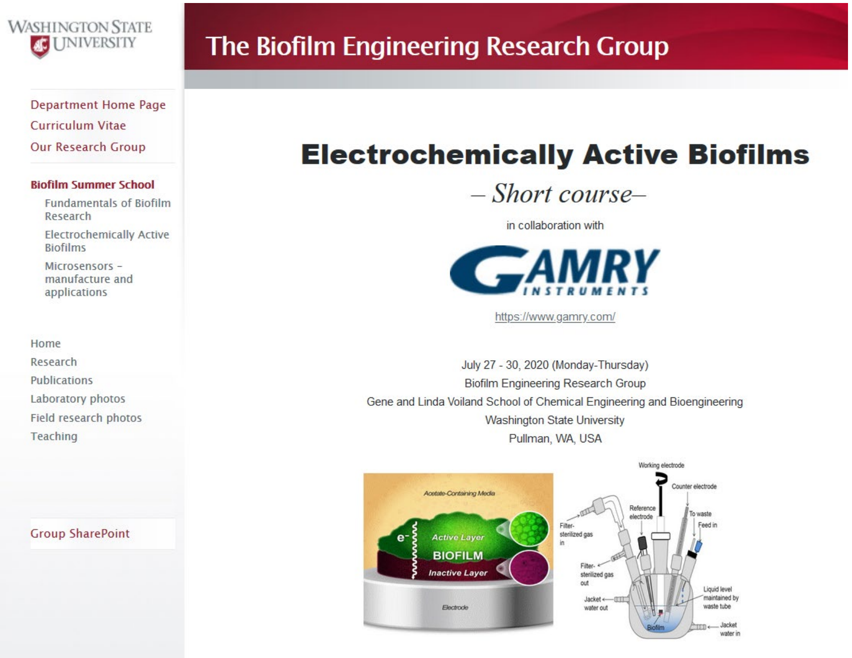
Conclusion

- eQCM provided an unprecedented amount of information about the native state of electrode-respiring *G. sulfurreducens* biofilms in relation to their initial attachment, growth, and oxidation state.

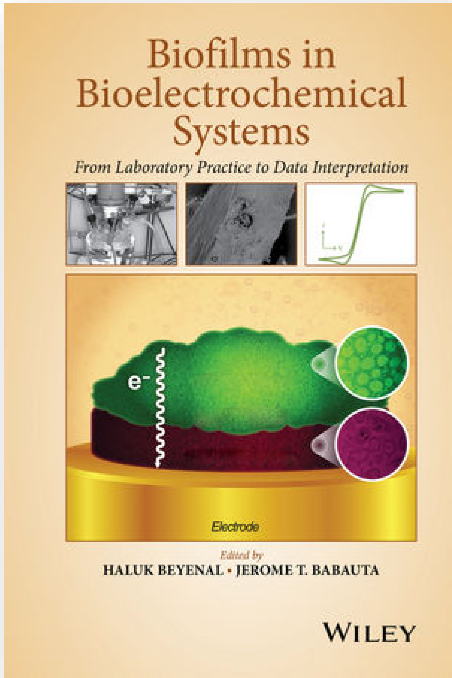


Additional resources

Annual short course



Practical guide to BES



(CH5 covers biofilm electrochemistry)

QUESTIONS?