

Application Note

Your First QCMD Experiment

Summary

A guide to conducting one's first QCMD experiment is presented. Diagnostics of common problems is discussed. Basics of quantitative data analysis are introduced.

Introduction

You now have an AWSensors advanced QCMD instrument in your laboratory and are on your way to making exciting discoveries. The question that commonly arises is, where does one start? This *Application Note* is intended to help you make your initial steps with QCMD.

AWSensors supplies single-channel (X1) and four-channel (X4) advanced QCMDs with integrated fluidic control (Figure 1). Both instruments provide frequency and dissipation measurements on multiple overtones, from $n = 1$ to $n = 13$ for 5 MHz sensors, for $n = 1$ and $n = 3$ for 50 MHz sensors, and for the fundamental for the 100 MHz sensors. Further information can be found in the corresponding *AWSensors QCMD Technology Note*¹ and product catalogue.

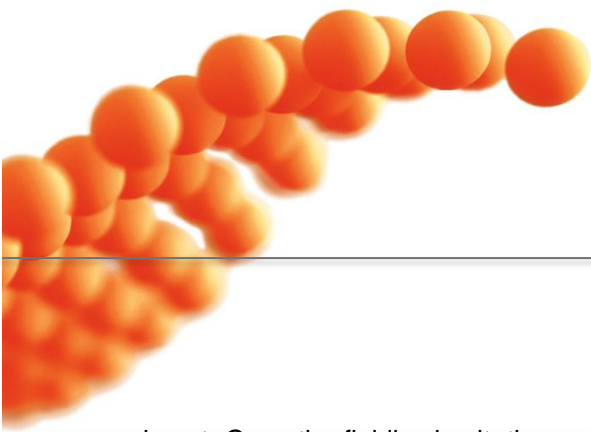
Since its original publication, supported lipid bilayer (SLB) formation from liposome solutions on SiO₂-coated sensors remains the benchmark experiment for evaluating QCMD instrument performance and operator skill.^{2,3} However, SLB formation experiments require expertise in the areas of liposome and surface preparation. Indeed, the very sensitivity that makes them an ideal benchmark also makes them difficult for beginners to perform. Therefore, here we chose a simple and accessible system: readily available coffee cream on gold-coated, polished 5 MHz QCMD sensors.

Experimental set-up

For performing the experiment described in this *Application Note*, you should be familiar with the basics of setting up the instrument and operating the AWS Suite software, such as wiring and cleaning the fluidics and the measurement cells, mounting sensors, and starting the experiment. This information is normally provided during training and is available in the manuals and *AWSensors Cleaning Protocol*.⁴ Cleaning the fluidics and the sensor is critical for the success of any



Figure 1: AWSensors single-channel (X1) and 4-channel (X4) advanced QCMD instruments with Flow Control Units (FCUs).



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experiment. Once the fluidic circuit, the measurement cell, and the sensor are clean and dry, the sensor is installed in the measurement cell, and the cell is mounted in the instrument, you are ready to start the experiment.

Sensor Diagnostics

In AWS Suite, one begins by setting up and running a *Tracking Mode* experiment in air. AWS Suite will perform an automatic setup, the results of which can be used to check the health of the sensor (Figure 2). A good-quality sensor exhibits the following features: all resonance curves (circles) have the same origin, conductance G_{\max} at $n = 1$ is between ~ 50 and ~ 100 mS, and the quality factor Q for $n = 3$ to $n = 13$ decreases monotonously with n . When evaluating the dependence of the quality factor on the overtone order, the fundamental is ignored: firstly, in quartzes where energy trapping is optimized for overtones, the fundamental does not perform well, and secondly, its performance is affected by the mounting O-ring due to the large amplitude distribution.⁹

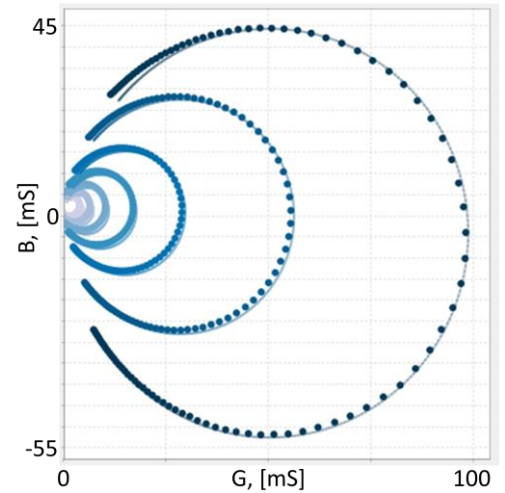
Varying points of origin for the resonance curves for the different overtones, non-monotonous behavior of Q as a function of n , or conductance that is below ~ 40 mS for $n = 1$, indicate problems with the sensor. Try unmounting the sensor and drying it again. If that fails, use a different sensor.

The next step is to record an air-to-buffer (or air-to-water) transition. To this end, let the sensor equilibrate in air for ~ 15 min, then start the flow of the buffer at a flow rate of $25 \mu\text{L}/\text{min}$. The buffer needs to be freshly filtered through 200 nm pore diameter filters and temperature equilibrated.

In AWS Suite, set up the plot such that $\Delta F/n$ (frequency shifts) and $\Delta\Gamma/n$ (bandwidth shifts) are plotted on the primary and secondary y-axis for $n = 3$ and up (Figure 3). The plots should appear symmetrical, as in Figure 4, as per the Kanazawa-Gordon relation: $\Delta f + i\Delta\Gamma =$

$$\frac{\sqrt{nf_0^3}}{\sqrt{\pi G_q \rho_q}} (i\sqrt{\rho_l \eta_l} - \sqrt{\rho_l \eta_l}),$$

where f_0 is the resonance frequency, G_q and ρ_q are the shear elastic modulus and density of

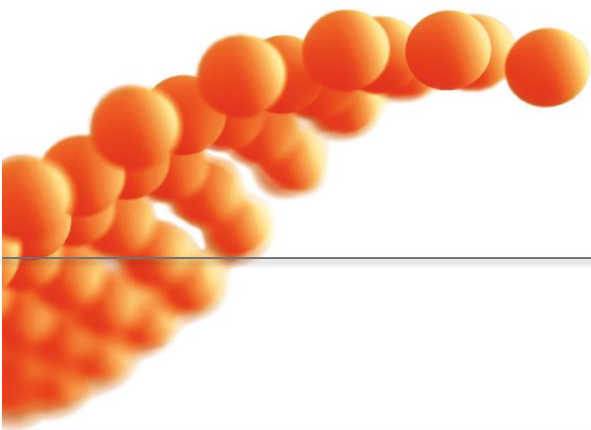


Harmonic	Frequency (Hz)	Gmax (S)	Q factor
1	4,956,318.73	9.69E-02	Ignore n = 1
3	14,852,653.37	5.56E-02	3.05E+05
5	24,751,271.96	2.93E-02	2.89E+05
7	34,648,492.79	1.75E-02	2.56E+05
9	44,546,376.68	1.10E-02	2.09E+05
11	54,444,007.67	7.38E-03	1.63E+05
13	64,342,222.84	4.87E-03	1.24E+05

Figure 2: Sensor Diagnostics: Automatic Setup Results.

Top: resonance curves in the susceptance vs. conductance (B vs. G) plane appear as circles. For a sensor in air the resonance curves for the different overtones should all originate at or close to (0,0).

Bottom: a table with the results of the fit of the resonance curves to the phase-shifted Lorentzian model. Note the maximum conductance, G_{\max} , ($\sim 96 \text{ mS}$) for $n = 1$, and the monotonous decrease of the quality factor for $n = 3 \dots 13$. Both the image and the table are accessible in the AWS Suite software.



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quartz; ρ_l and η_l are liquid density and viscosity.⁵ The shifts, $\Delta f/n$, and $\Delta\Gamma/n$, should be $\sim \pm 400$ Hz for $n = 3$ for water or PBS buffer. The absolute values of the frequency and bandwidth shifts will depend on the kind of liquid used. The absolute values of the frequency and bandwidth shifts will depend on the kind of liquid used, but if the values of $-\Delta f/n$ and $\Delta\Gamma/n$ deviate from each other by more than $\sim 10\%$, there is a problem: most commonly, bubbles, contamination, or a damaged sensor.

The sensor that passes these diagnostic criteria can be used in an experiment.

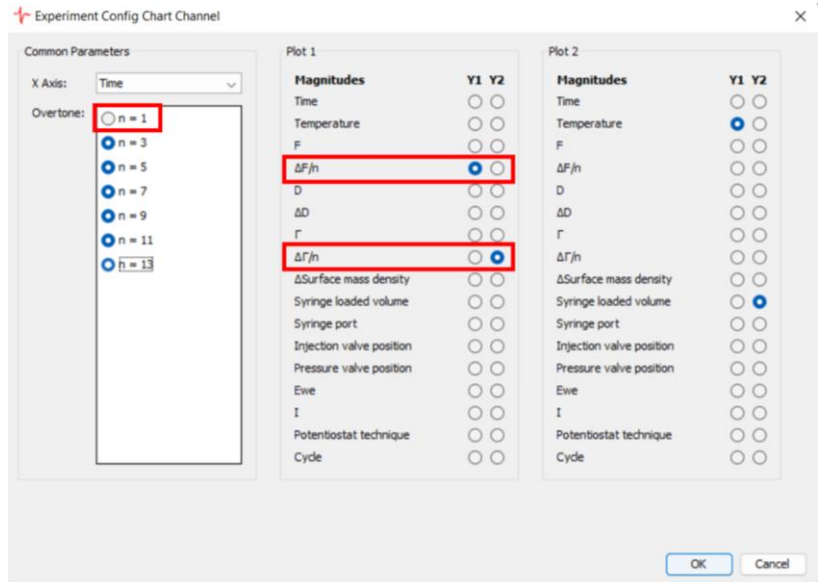


Figure 3: AWS Suite plot setup for the air-to-buffer transition measurement.

Data Acquisition

A QCMD experiment entails acquiring a drift-free baseline in the liquid of choice, injecting a certain volume of the sample, and acquiring signals post-injection in the same liquid as the baseline. As a general rule, only differences between frequency and dissipation signals acquired in the same liquid should be used to characterize samples, because of the possible contribution of the liquid density and viscosity to the frequency and dissipation shifts. The results of such an experiment are shown in Figure 5A, where 50 μl of coffee cream diluted 10 : 1 in PBS buffer was injected into the flow cell at time $t = 0$ s.

Examining the time traces of the frequency and distribution signals shown in Figure 5A, one can see that the injection of coffee cream leads to a decrease in the frequency and an increase in the dissipation. The frequency shifts stabilize after ~ 300 s at a set of values that are different for the different overtones (different colors). The dissipation shifts go

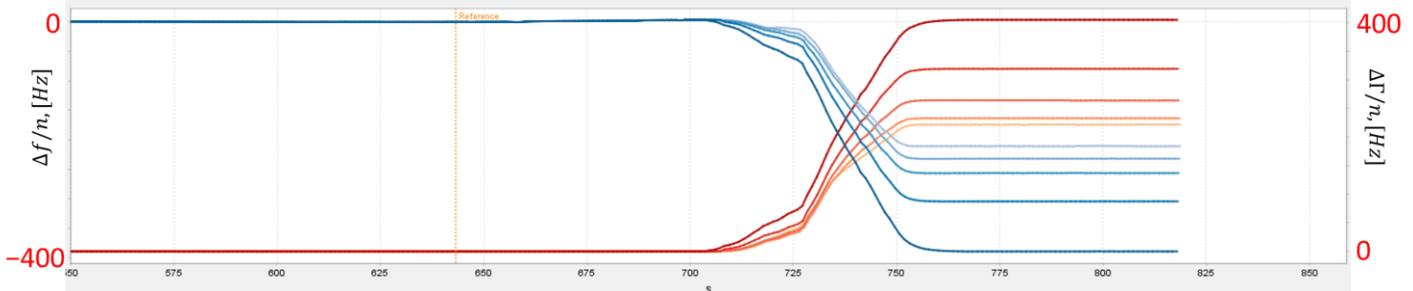
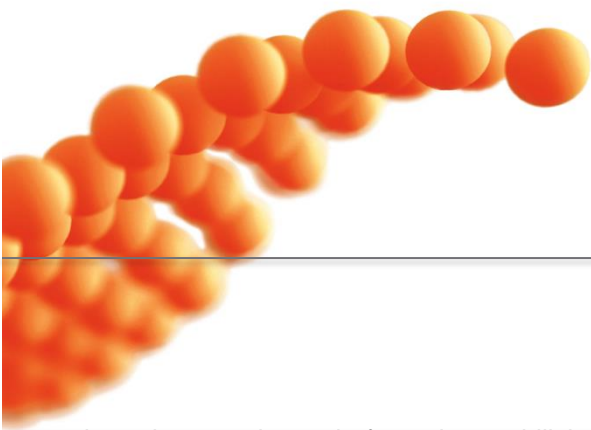


Figure 4: Sensor Diagnostics: Air-Buffer shifts.
Note the symmetrical features of the plot in terms of frequency and bandwidth shifts.



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through a maximum before also stabilizing at a set of values, different for each overtone. These values are referred to as “asymptotic shifts”.

Data Analysis Basics

The first step in the analysis of QCMD data is to calculate baseline-corrected asymptotic frequency and dissipation shifts for each overtone. To this end, the data are exported from AWS Suite and imported into Excel, or another suitable software. Time averages of the frequency and dissipation shifts in the baseline region and the asymptotic shifts region are calculated and subtracted from each other, correcting for drift if necessary. The results are shown in Figure 5B. Subsequent data analysis steps depend on the goal.

The results shown in Figure 5 indicate that the Sauerbrey relationship between the frequency shift and the adsorbed mass, $\Delta f_n = -\frac{2nf_0^2}{\sqrt{G_q\rho_q}}\Delta m$,⁶ does not apply. For the Sauerbrey relationship to be valid, the data should not exhibit overtone dispersion and the ratio of the dissipation shifts to frequency shifts should be \ll than $\sim 4E-7$ [1/Hz]; in this case, it is $\sim 2E-7$ [1/Hz] and is too high for the Sauerbrey mass to be reliable.⁷ Therefore, if the goal is to determine the amount of material adsorbed to the surface, one needs to fit the data to the viscoelastic model. The model entails five parameters: adsorbed mass and the four parameters describing the frequency-dependent complex compliance $\tilde{J} \sim J'_{cen} \left(\frac{f}{f_{cen}}\right)^{\beta'} - iJ''_{cen} \left(\frac{f}{f_{cen}}\right)^{\beta''}$: the values J'_{cen} and J''_{cen} at an arbitrary central frequency, f_{cen} (typically, 30 MHz), and the two power law exponents, β' and β'' , that approximate the frequency dependence.

The fitting was performed in *PyQTM*.⁸ Fit results are shown in Table 1. In particular, the adsorbed mass determined from the fit

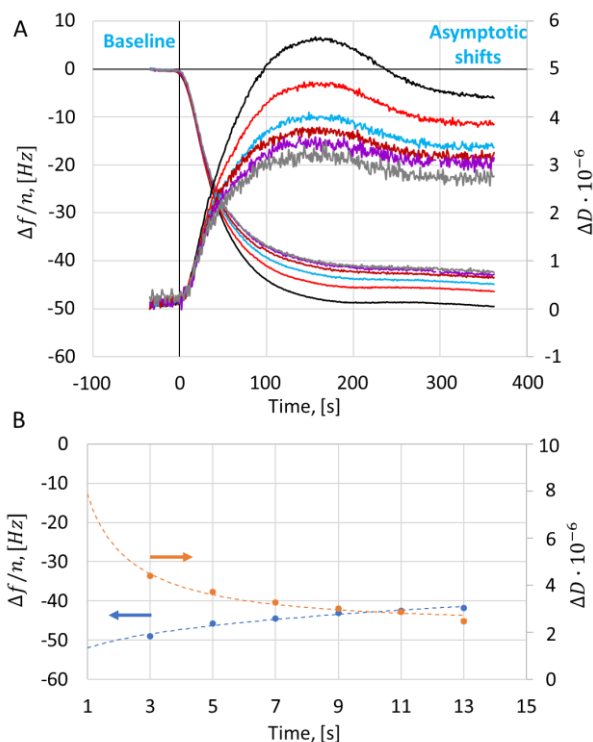
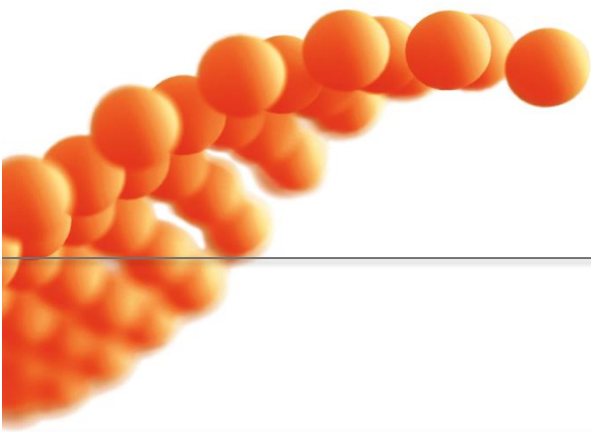


Figure 5: Typical QCMD data (A) and analysis (B).

A: Frequency and dissipation shifts as a function of time on multiple overtones. Coffee cream injected at time $t = 0$ s. **B:** baseline-subtracted frequency and dissipation shifts as a function of the overtone order. Dashed line are fits to viscoelastic model (Table 1).

Table 1: Viscoelastic model fit parameters.

Mass, ng/cm ²	1100 ± 400
Thickness, [nm]	11 ± 4
J'_{cen} , [MPa ⁻¹]	0.6 ± 0.4
β'	-1.6 ± 0.2
J''_{cen} , [MPa ⁻¹]	2 ± 1
β''	-0.8 ± 0.3



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is 1100 ± 400 [ng/cm²], while the Sauerbrey mass calculated from the average over the frequency shifts on the overtones $n = 3 \dots n = 13$ yields a value of 790 ± 50 [ng/cm²]; a significant underestimation. Detailed discussion of the rest of the model parameters is beyond the scope of this *Application Note*; the reader is referred to refs. 9 and 10.

If the adsorbed mass is not of primary interest, one might pick one of the overtones and plot the value of the frequency shift as a function of some experimental parameter, such as dilution. Typically, the 7th overtone is used in such cases because there, one finds a balance between edge- and compressional wave effects that plague the lower overtones and the noise that becomes a problem on the higher overtones. Such a plot is shown in Figure 6, revealing a rather complex adsorption behavior of the coffee cream components to the gold surface.

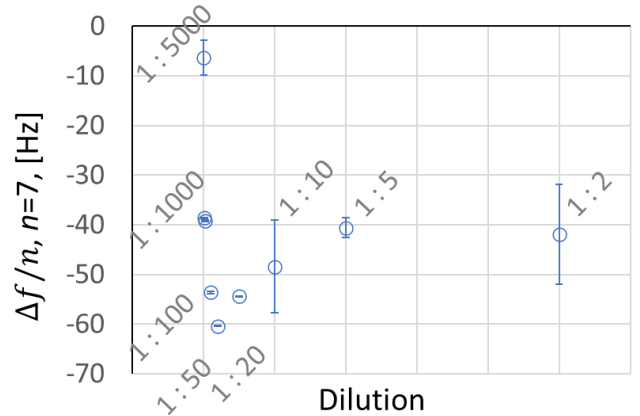
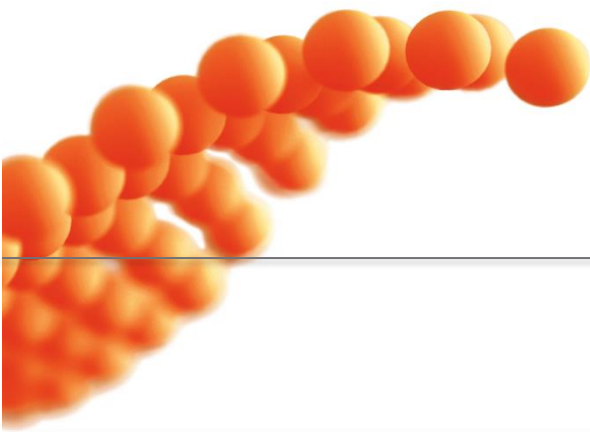


Figure 6: Effect of coffee cream dilution on the frequency shift.



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References

- ¹ AWSensors QCMD *Technology Notes* can be found on the web at awsensors.com/tech-and-app-notes/
 - ² Keller, C. A.; Kasemo, B. *Surface Specific Kinetics of Lipid Vesicle Adsorption Measured with a Quartz Crystal Microbalance*. *Biophysical Journal* 1998, 75 (3), 1397–1402. [https://doi.org/10.1016/S0006-3495\(98\)74057-3](https://doi.org/10.1016/S0006-3495(98)74057-3).
 - ³ AWSensors QCMD Application Note *Supported Lipid Bilayer formation followed at low- and high-fundamental frequencies* can be found on the web at awsensors.com/tech-and-app-notes/
 - ⁴ AWSensors cleaning protocol can be found at AWSensors' Customer Area on the web (customers.awsensors.com).
 - ⁵ Kanazawa, K. Keiji.; Gordon, J. G. *Frequency of a Quartz Microbalance in Contact with Liquid*. *Anal. Chem.* 1985, 57 (8), 1770–1771. <https://doi.org/10.1021/ac00285a062>.
 - ⁶ Sauerbrey, G. *Verwendung von Schwingquarzen zur Wägung dünner Schichten und zur Mikrowägung*. *Z. Physik* 1959, 155 (2), 206–222. <https://doi.org/10.1007/BF01337937>.
 - ⁷ Reviakine I, Johannsmann D, Richter RP. *Hearing what you cannot see and visualizing what you hear: interpreting quartz crystal microbalance data from solvated interfaces*. *Anal Chem.* 2011;83(23):8838-48. doi: 10.1021/ac201778h.
 - ⁸ PyQTM is a free, open-source software available for download from www.pc.tu-clausthal.de/en/research/qcm-modelling/
 - ⁹ Johannsmann, D. *The Quartz Crystal Microbalance in Soft Matter Research*; Springer Berlin Heidelberg: New York, NY, 2014.
 - ¹⁰ Johannsmann, D.; Langhoff, A.; Leppin, C.; Reviakine, I.; Maan, A. M. C. *Effect of Noise on Determining Ultrathin-Film Parameters from QCM-D Data with the Viscoelastic Model*. *Sensors* 2023, 23 (3), 1348. <https://doi.org/10.3390/s23031348>.
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- Eisele, N. B.; Andersson, F. I.; Frey, S.; Richter, R. P. *Viscoelasticity of Thin Biomolecular Films: A Case Study on Nucleoporin Phenylalanine-Glycine Repeats Grafted to a Histidine-Tag Capturing QCM-D Sensor*. *Biomacromolecules* 2012, 13 (8), 2322–2332. <https://doi.org/10.1021/bm300577s>.