Simultaneous AFM and QCM measurements: application to the adsorption of proteins on metallic surfaces

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Linz, February 1-4 2002

Presentation overview

- Aim
- Detection methods
- Interaction of the two techniques
- Interaction analysis
- Experimental setup
- Results (AFM)
- Results (QCM)
- Conclusion and perspectives

Aim

Combine QCM and AFM:

- complementary scales (AFM: nm^2, QCM: cm^2)
- complementary techniques (AFM: topography/stiffness, QCM: adsorbed mass)

Test QCM model hypothesis: uniform material layer strongly binded to the sensing (grounded) electrode $\Rightarrow \Delta f \propto \Delta m$

Biological application: identify protein adsorption method

Detection methods

AFM: sharp tip at the end of a flexible cantilever vibrates over the sample. Interaction forces fluctuations $\rightarrow$ topography/stiffness of the sample High lateral resolution (tip convolution $\Rightarrow$ 10 nm objects) adequat for vizualising large biomolecules (Molecular Imaging, AZ, USA).

QCM: piezoelectric resonator disturbed by mass addition to its electrode. Resonance frequency and Q factor tracking (Q-Sense AB, Göteborg, Sweden)
High mass sensitivity BUT sensitive to external parameters (hydrostatic pressure, temperature)

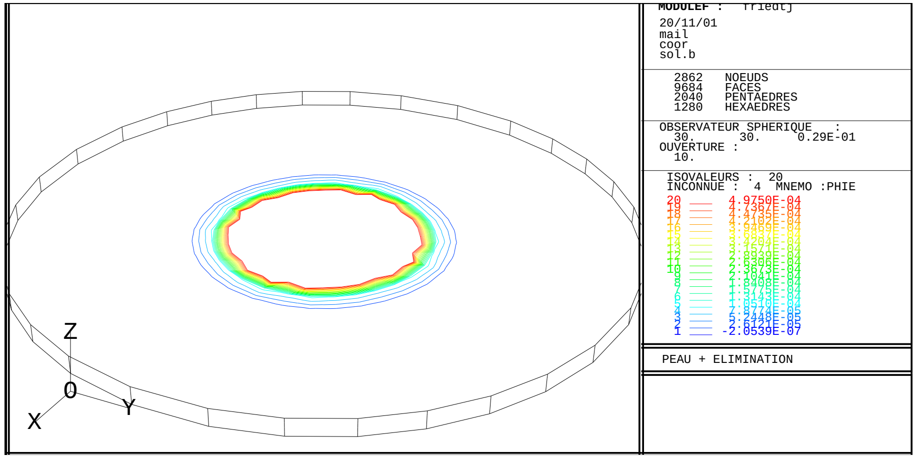
Interactions of the two techniques ?

- QCM $\rightarrow$ AFM: resolution loss ? Surface flatness ?

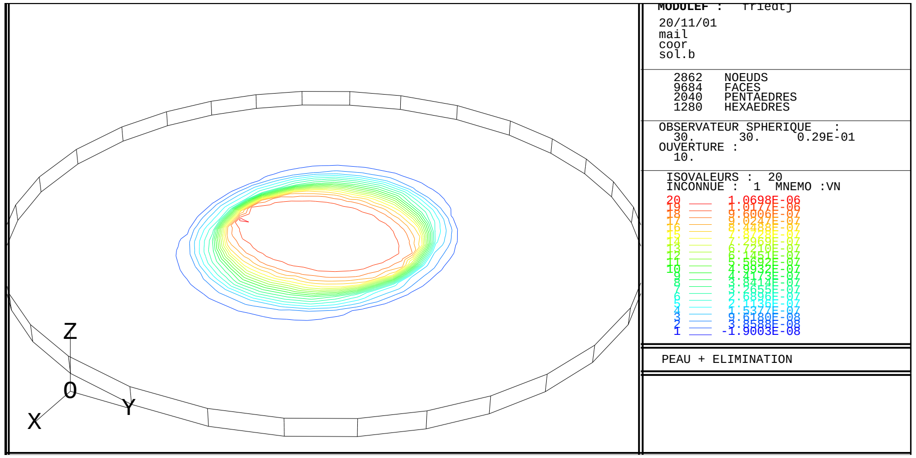
- AFM $\rightarrow$ QCM: frequency stability ?

Interactions analysis

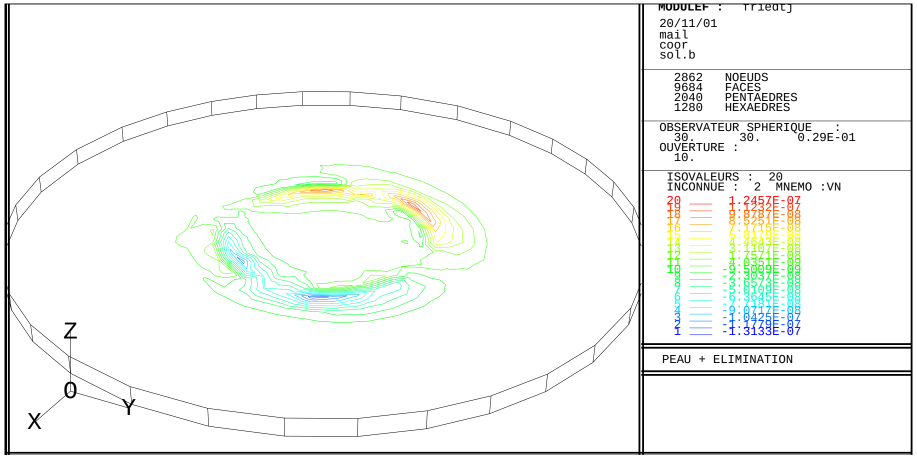
Finite Element Analysis of *static* displacement of QCM (AT cut quartz) (Modulef, INRIA, France)



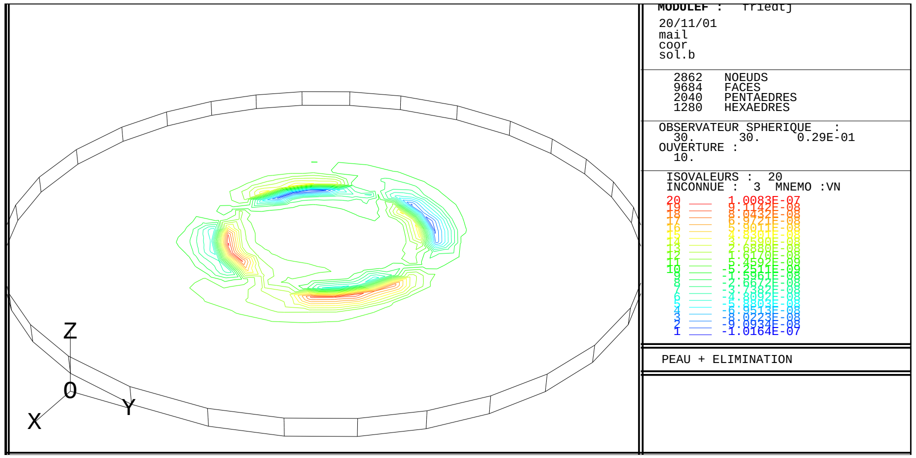
DC potential (0.5 V)



In-plane displacement (1 pm)



In-plane displacement (0.1 pm)



Out-of-plane displacements (0.1 pm)

Extension to the dynamic case by multiplying displacement by Q factor

$\rightarrow$ in-plane displacement smaller than AFM pixel size ($Q \simeq 3000 \Rightarrow 0.3$ nm)
$\rightarrow$ surprisingly high out-of-plane displacement (1/10 in-plane displacement)

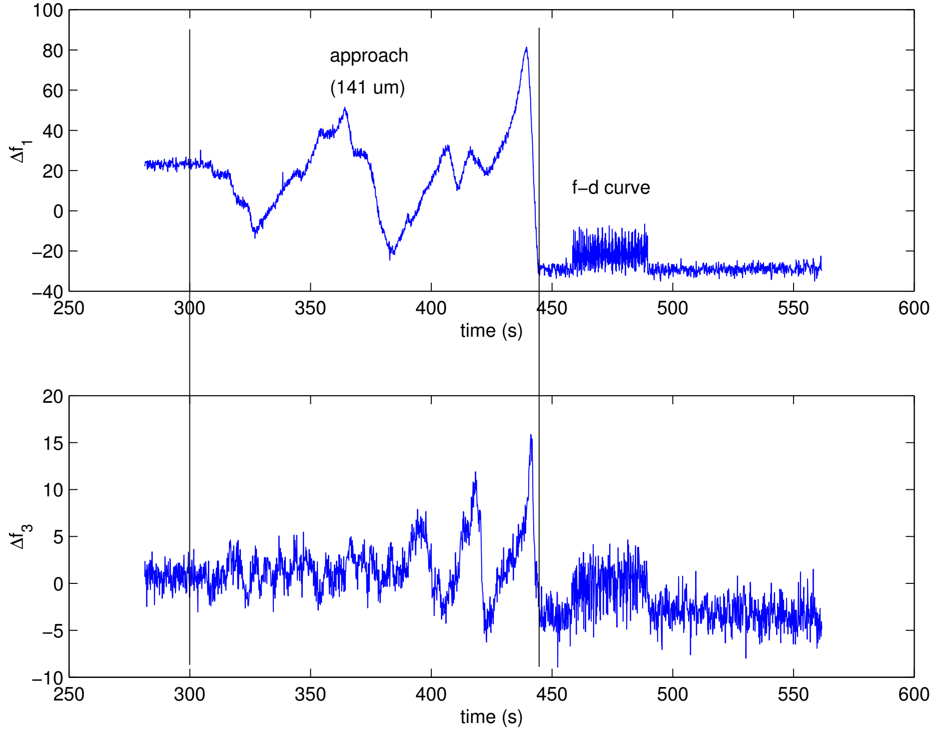
The QCM does not affect the AFM imaging
The AFM cantilever motion can affect the QCM stability

Interactions analysis (2)

Large out-of-plane displacement is due to the finite size of the counter electrode (should be 0 for an infinite electrode)

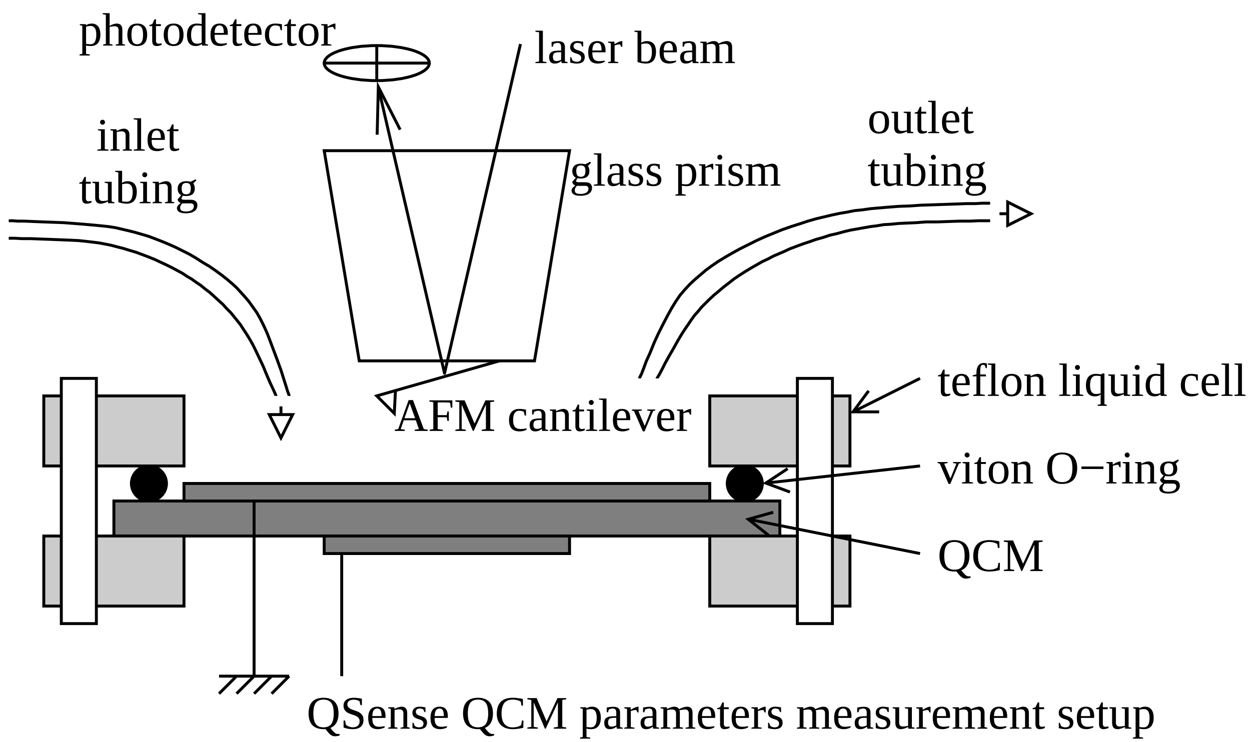
Interpretation: standing wave pattern between the QCM and the flat cantilever holder.
Depending on the node/anti-node position, QCM parameters fluctuate
$\Rightarrow$ stability loss $\Rightarrow$ sensitivity loss

This effect should **NOT** happen with STM (no tip holder)



Schematic of the experiment

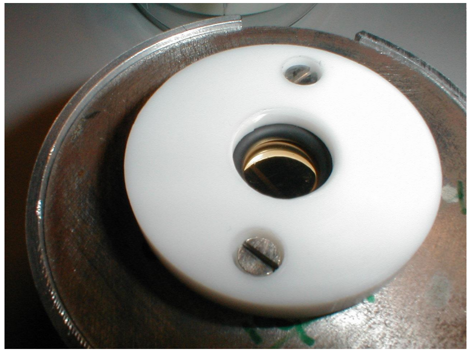
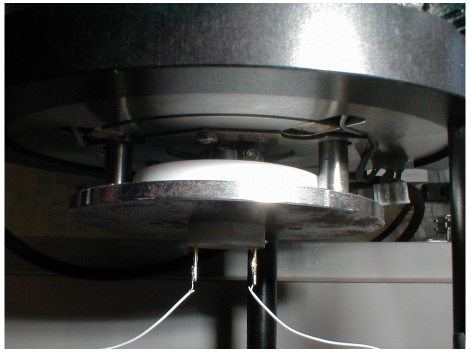
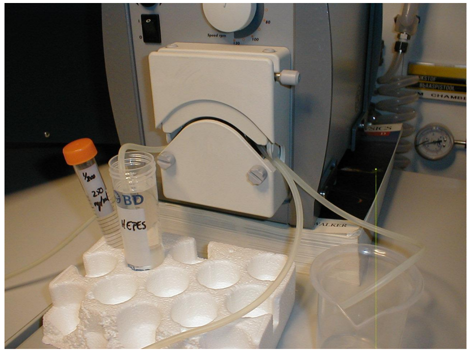
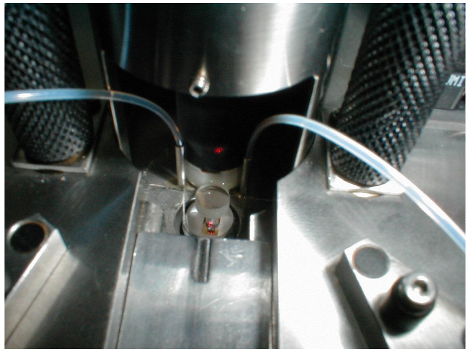
Flow produced by peristatic pump (pushing and sucking liquid): required for solution exchanges.
Teflon liquid cell. Both electrical contacts with the QCM are on the dry side.
$\rightarrow$ replace passive Au substrate by active Au surface



Experimental setup

QCM is a potentially flat (2 nm$_{pp}$ roughness of the quartz wafer) Ti/Au coated surface.

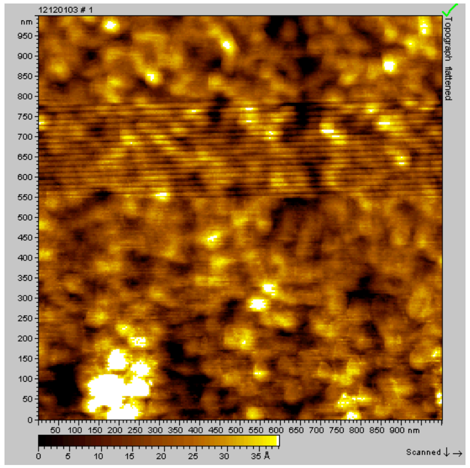
Open-bottom SPM: a lot of space to introduce additional hardware.

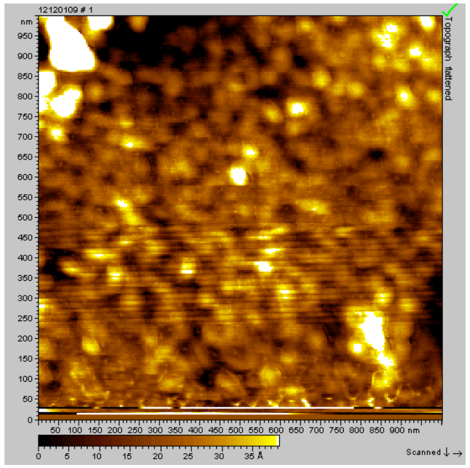

Results (AFM)

Human Plasma Fibrinogen adsorption pattern on the QCM surface: proteins are visible on the $1 \times 1\ \mu m^2$ image as dots and lines between Au grains

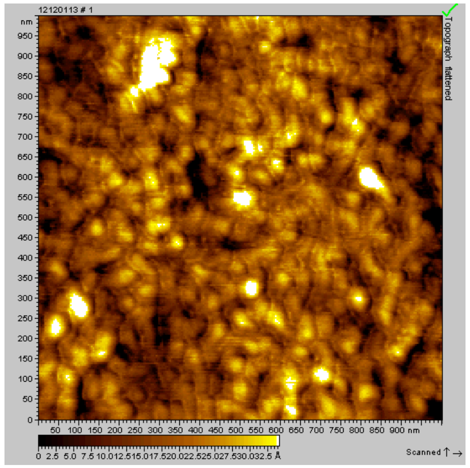
Horizontal stripes: pump noise degrades AFM image quality (only during flow for solution exchange)



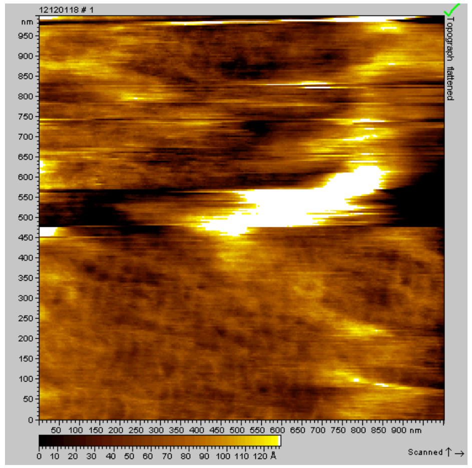
$\phi \rightarrow$ 250 ng/ml ($\downarrow$)



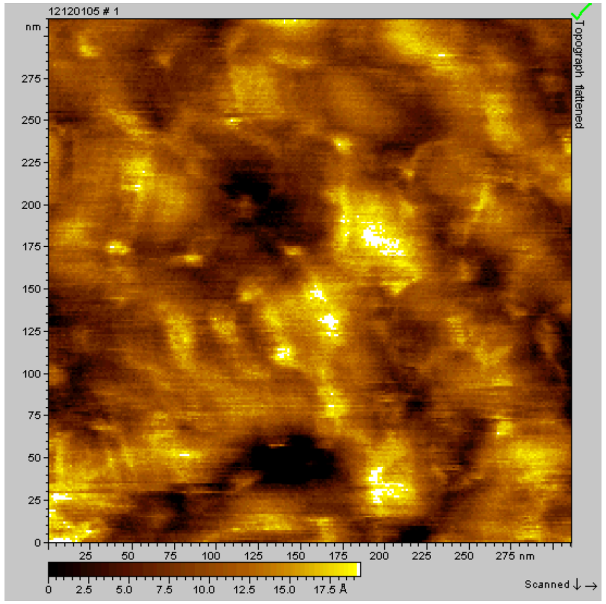
250 ng/ml $\rightarrow$ 2.5 μg/ml ($\downarrow$)

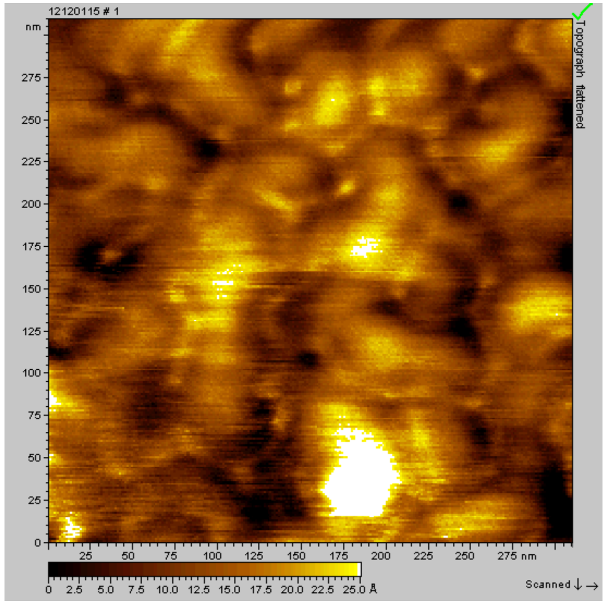


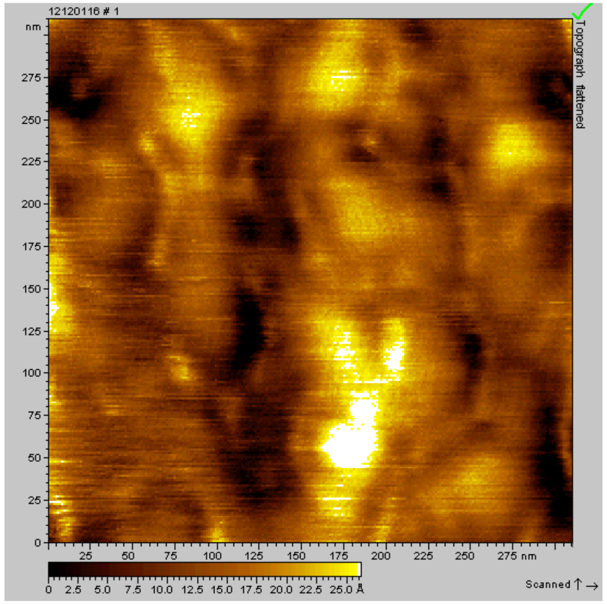
25 μg/ml



100 μg/ml

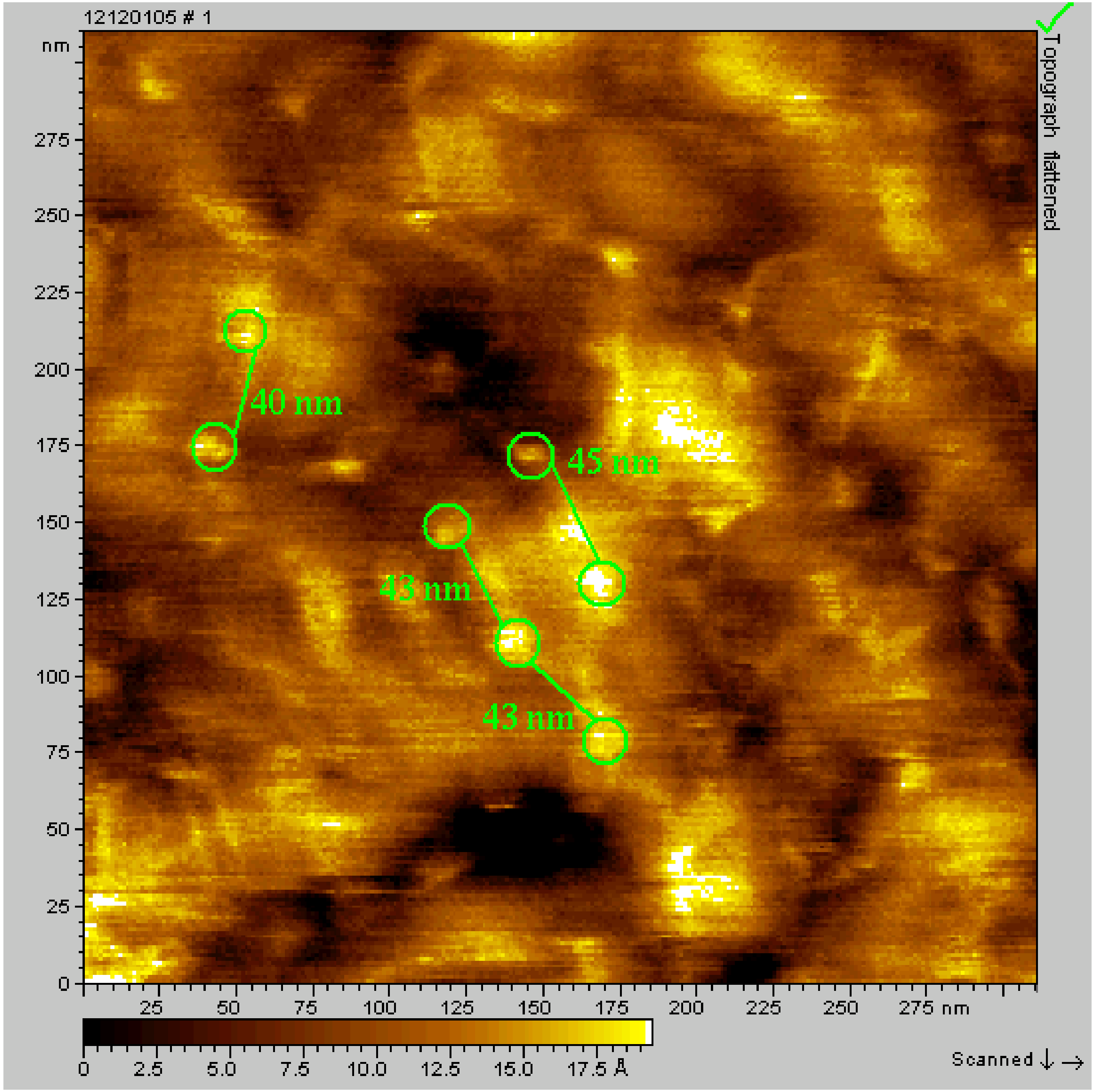






250 ng/ml | 25 μg/ml | 25 μg/ml

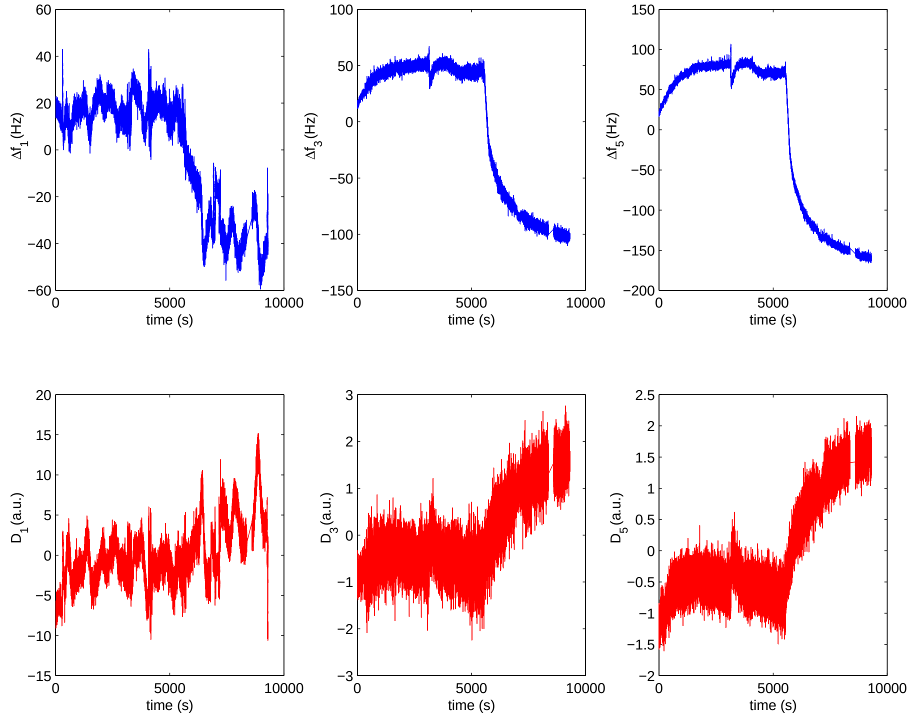
Zoom in (300×300 nm^2 image): sharper shapes compatible with HPF shape ($\simeq$10 nm beads separated by 40 to 60 nm, connected by thin rods.)



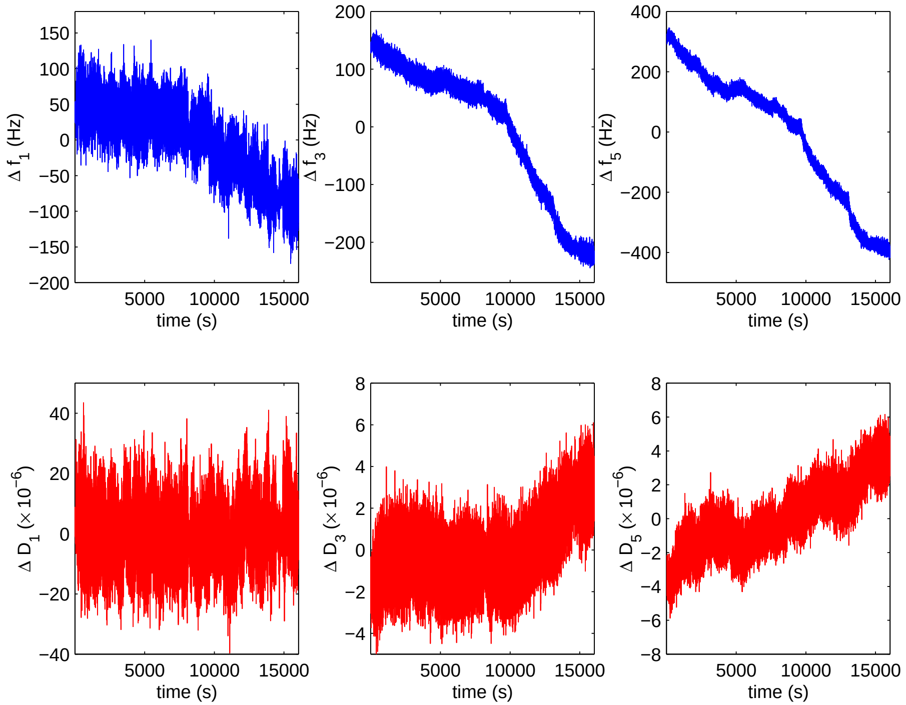
12120105 # 1
Topograph flattened
nm
275
250
225
200
175
150
125
100
75
50
25
0
25
50
75
100
125
150
175
200
225
250
275 nm
40 nm
45 nm
43 nm
43 nm
0
2.5
5.0
7.5
10.0
12.5
15.0
17.5 Å
Scanned

Results (QCM)

Simultaneous QCM resonance frequency shift and damping ($D \doteq Q^{-1}$) monitoring (3 modes, 1-3-5 around 5-15-25 MHz respectively)
Fundamental mode (5 MHz) too sensitive to environmental changes to be useful



HPF in HEPES buffer, Q-Sense QCM



HPF in PBS buffer, Ti/Au QCM

Conclusion

Development of a potentially interesting characterization tool

Demonstration of the ability of the tool to study protein adsorption

Test QCM model hypothesis: the adorption in not uniform. Compare Δf_{QCM} with the mass estimated from AFM images ...

Envisionned applications: electrochemisty, biology ... new acoustic wave modes ...