Pesticide detection using a surface stress micro cantilever system

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Keywords: Pesticide detection, cantilever, surface stress, characterization

Abstract
A 2,6 dichlorbenzamide (BAM) pesticide residue assay has been performed using a surface stress induced cantilever based detection system. The stress induced is measured using the CantiLab4© system from cantion with 4 gold coated cantilevers and piezo resistive readout. The detection mechanism is in principle label free, but fluorescent marked antibodies have been used to subsequently verify the binding on the cantilever surface. The profile and vibration mode of each cantilever has also been investigated using a light interferometer and resonance vibrometer device. The system has been analyzed during repeated measurements to verify the BAM assay and characterize the CantiLab4© system.

Introduction
During the last 10 years an increasing number of water wells in Denmark have been polluted by pesticides or its break down products. Pesticide analysis of drinking water is currently being done by manual sampling and laboratory analysis. This means weeks in between sampling and the analysis result. An in-line sensor based on a competitive immunological reaction for the detection of BAM will therefore vastly improve water quality monitoring. The BAM molecules in the water sample compete with BAM attached to a cantilever surface for the binding to anti-BAM monoclonal antibodies. [1][2].The binding of anti-BAM antibodies to the surface of the cantilever will change the surface stress, causing bending of the cantilever. The bending is then detected by a change in resistance of the imbedded piezoelectric layer in the cantilever (fig 1).[2][3]

Experimental
On an inspected, tested and clean CantiChip4®, a mixture of BAM-ovalbumine conjugate is spotted on cantilever B and C. Cantilever A and D is used as reference and spotted with ovalbumine alone. After overnight incubation the chip is placed in the CantiLab4© and a series of stabilization test are conducted. When the system is stable 100µl of 0.1 mg/ml unspecific Cy5 labeled mouse IgG mix is added to the chip, to test for any unspecific signal. This is followed by the adding of 100µl of 0.1 mg/ml Cy3 labeled BAM antibody in 1x PBS 0,05% Tween20 at a flow rate of 20µl/min. The signal is recorded and the data treated. The visible light and fluorescent pictures are taken of the chip, before and after spotting and hybridization (fig 2). The bending profile and vibration mode of each cantilever is recorded using a light interferometer, and a laser based vibrometer with a piezo actuator, before and after spotting and antibody hybridization (fig 3 and 4).

Results and Discussion
The BAM assay has given repeated positive measurements on the cantilever system; however a signal from the unspecific IgG antibody has also been obtained. The fluorescent labelling of the antibodies has verified the attachment of antibodies on the cantilever surface. A clear mass increase has been detected after BAM pesticide has been spotted on the cantilever surface; however, the addition of antibodies has not given a consistent decrease in resonance frequency using the vibrometer device (fig. 3). The bending profile and mass/stiffness values of each cantilever have been a useful tool for the interpretations of the results obtained with the pesticide assay.

References

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Figures

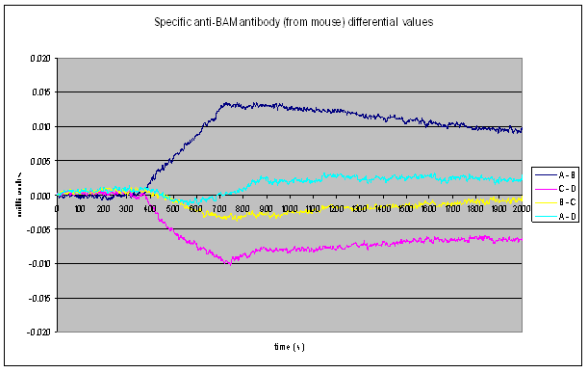

Figure 1. A typical differential signal obtained during the hybridization of anti-BAM to a BAM coated cantilevers..

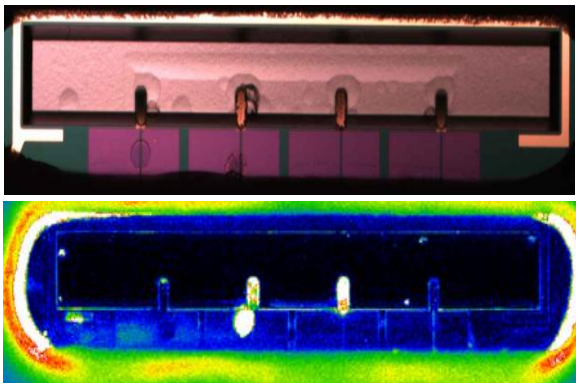

Figure 2. A set of images of a Cantion chip after an experiment, notice the bottom picture shows a clear fluorescent Cy3 signal on cantilever B and C, no signal was obtained from the unspecific IgG antibody

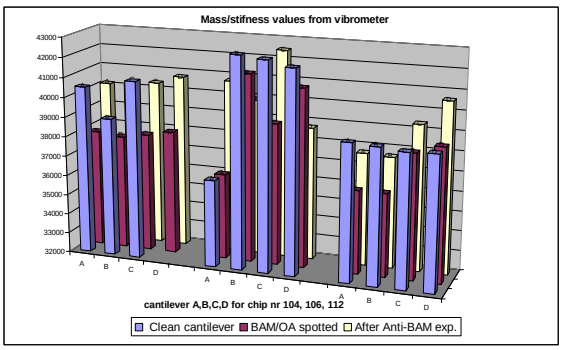

Figure 3. A graph showing 3 clusters of results of vibration frequency of each cantilever from 3 different chips (z axis series: clean, after spotting, after exp). Notice the large variation in between chips and after spotting.

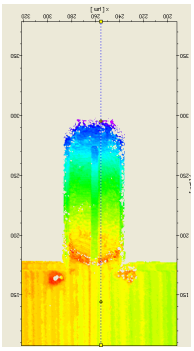

Figure 4. An example of a light interferometer profile image of one of the cantilevers after a pesticide has been spotted onto the surface