Characterization of a cantilever system for a BAM assay

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DTU Nanotech

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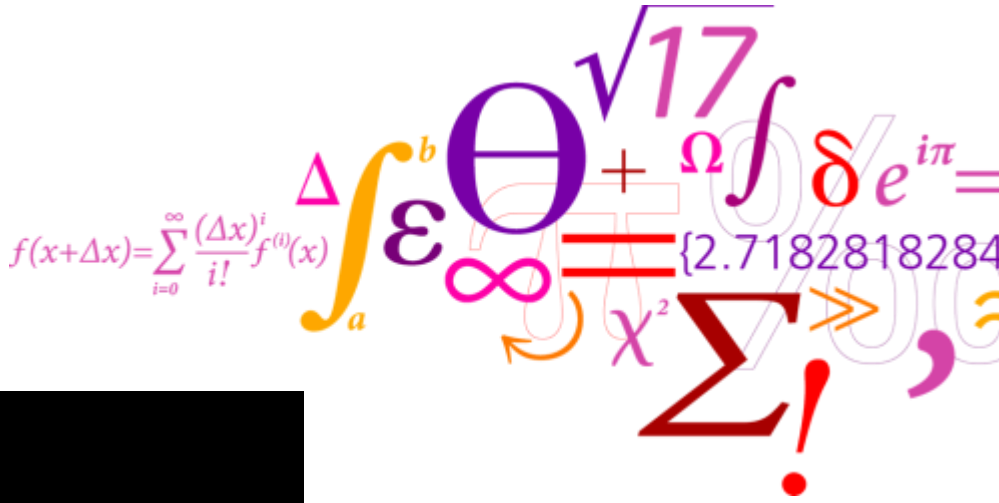
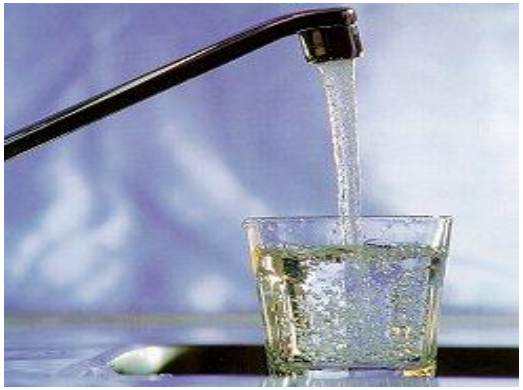


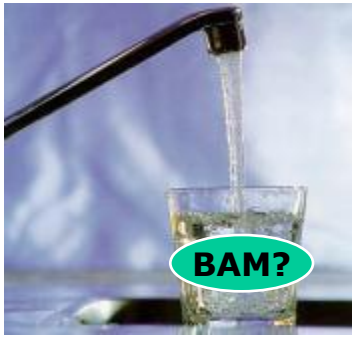
Table of contents

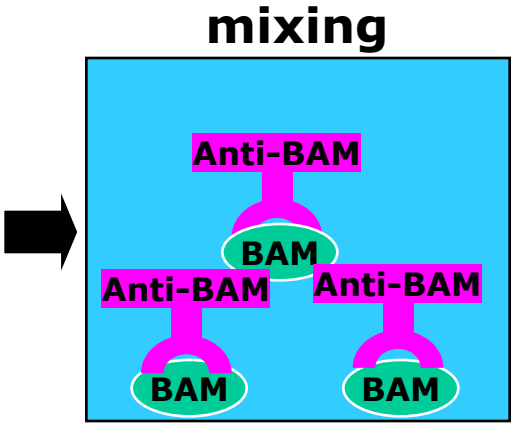
Purpose

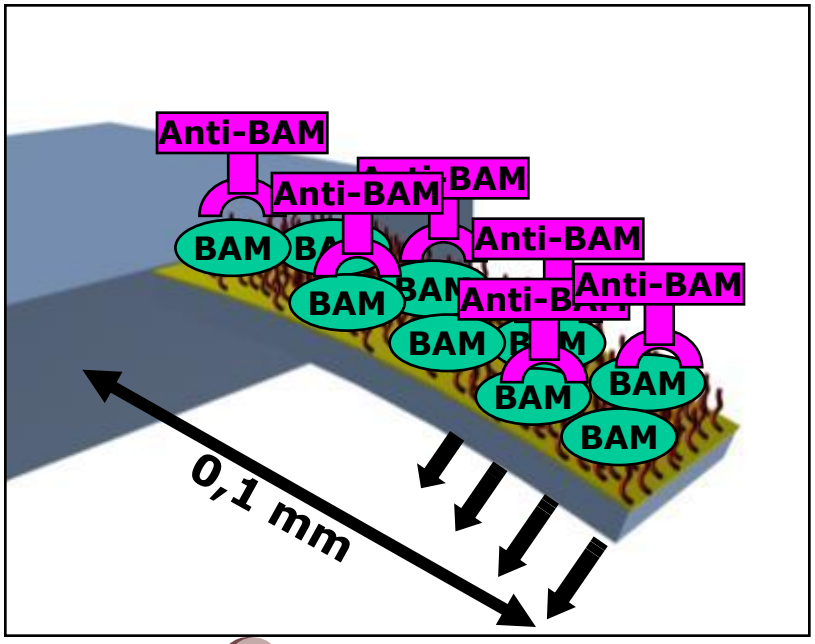
- In-line pesticide sensor for drinking water
- Measuring a breakdown product of dichlorbenil pesticide called 2,4 Dichlorbenzamide (BAM)
- Now done by ELISA (days), goal is 2-3 measurements per day
- Needs to be rugged and unserviceable for 3 months minimum and be placed on selected ground water wells in DK as seen below
- SENSOWAQ = Sensors for monitoring and control of water quality



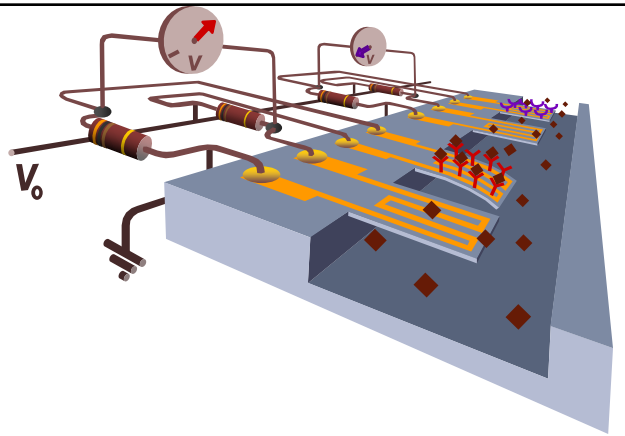
BAM competitive assay concept



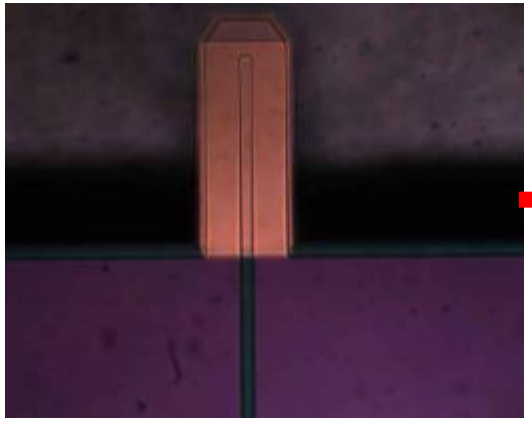


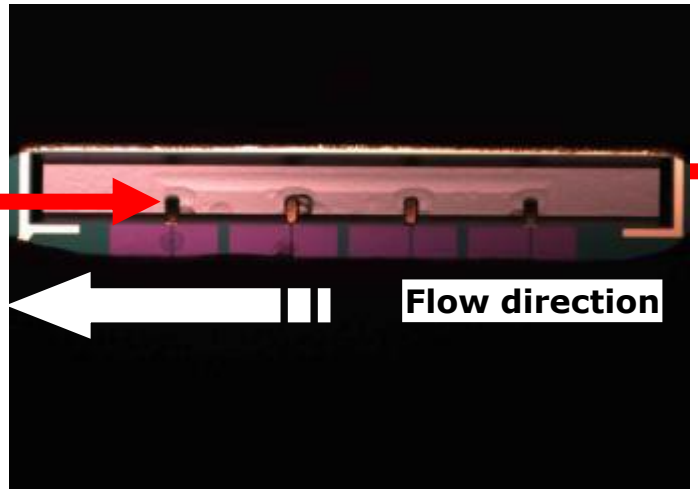


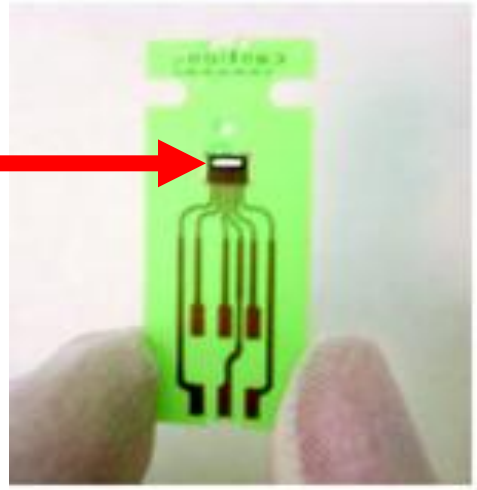
- A built in piezo element changes resistance under stress
- Readout is a change of voltage in a wheatstone bridge setup
- No signal = BAM**;** Signal = no BAM

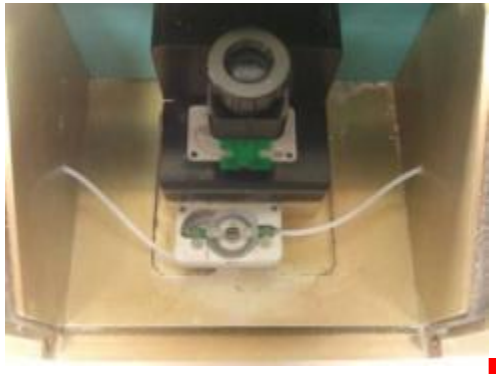


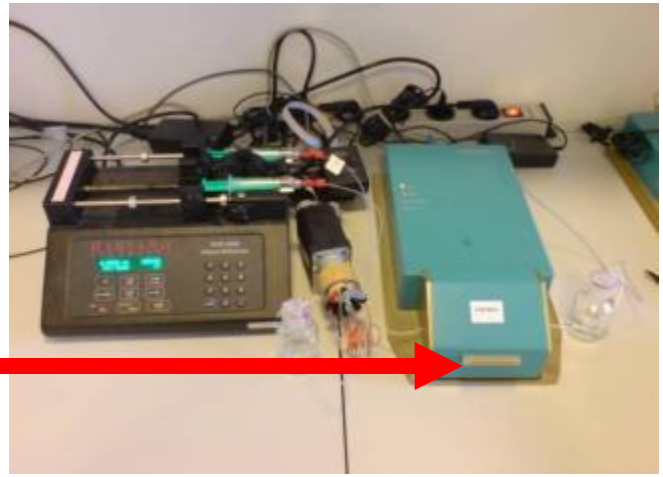
Canti4 Cantilever piezo resistive sensor

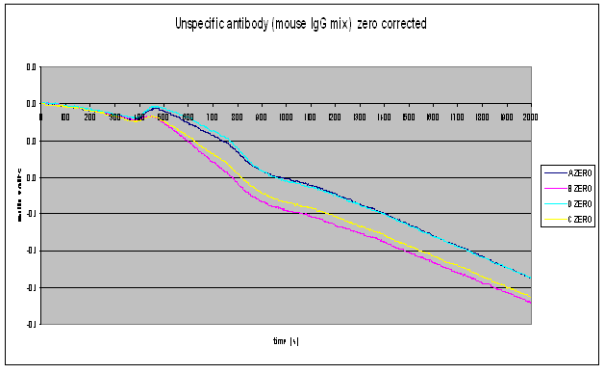




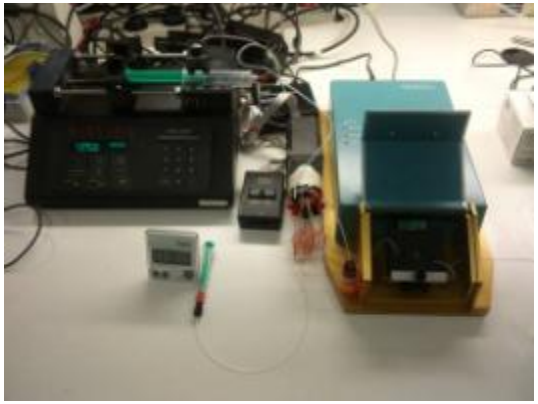




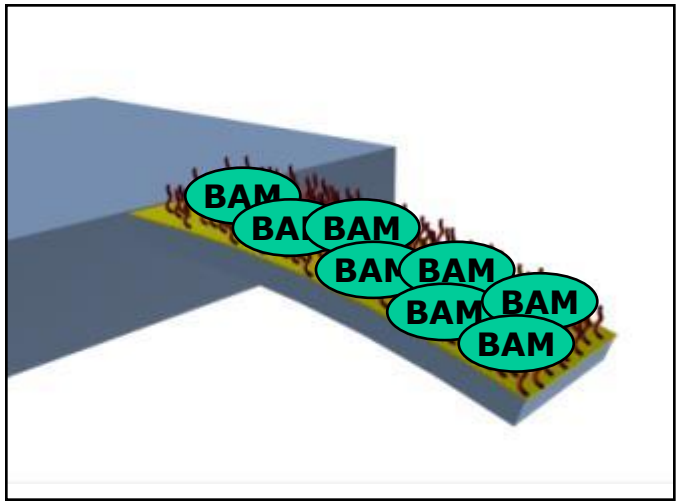


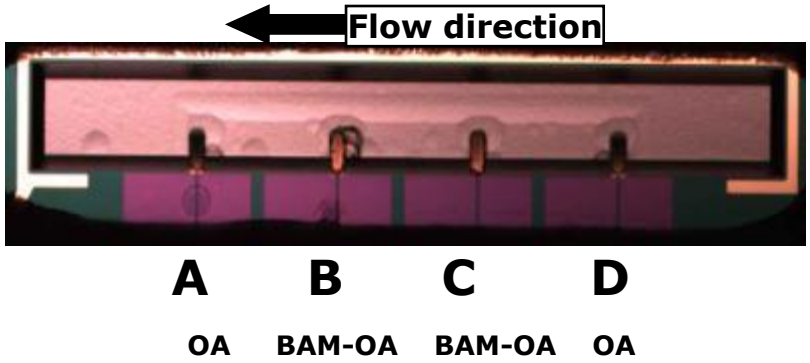


Canti4 fluidics setup

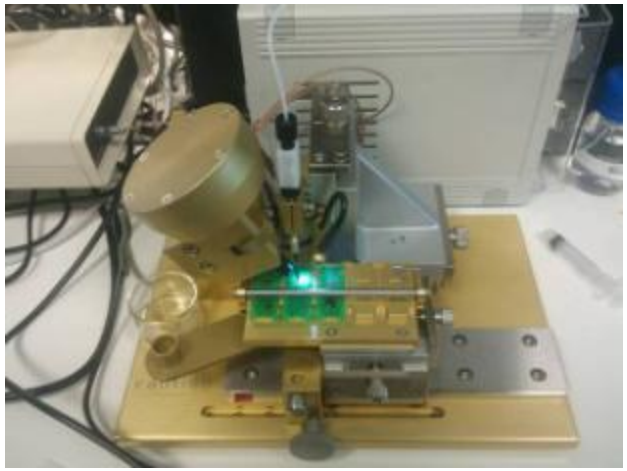


Cantilever spotting setup





- Mix of BAM and ovalbumine is spotted on cantilever B+C
- Ovalbumine is spotted on A+D as reference
- Spotting is done using a cantion microspotter with a camera and pico-liter needle



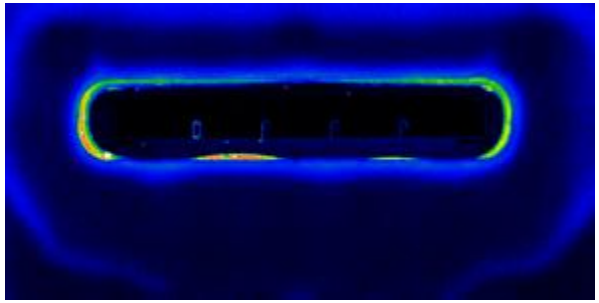
Canti4 BAM assay procedure

1. Chip surface and signal quality check
2. Spotting of BAM-ovalbumine (0.75mg/ml) (B+C) and ovalbumine (1 mg/ml) (A+D) + min 12 hour incubation
3. Chip inserted in cantibox and run until stable in 1xPBS 0,05% Tween20 at 20 µl/min.
4. Switch test from loop 1 to loop 2
5. Buffer induced as sample
6. Unspecific antibody Cy5 IgG from mouse (0,1 mg/ml)
7. Specific mouse antibody Cy3 anti-BAM (0,1 mg/ml)
8. Fluorescent pictures of Cy3 and Cy5

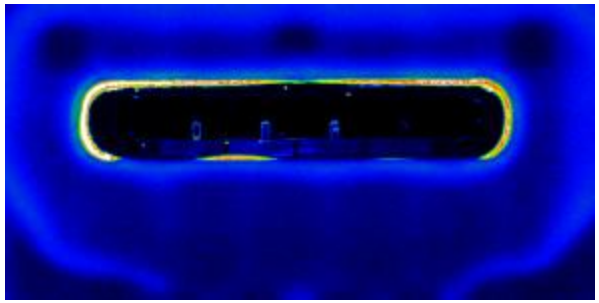
Canti4 Fluorescent pictures

Chip 106

Unspecific Cy5 IgG

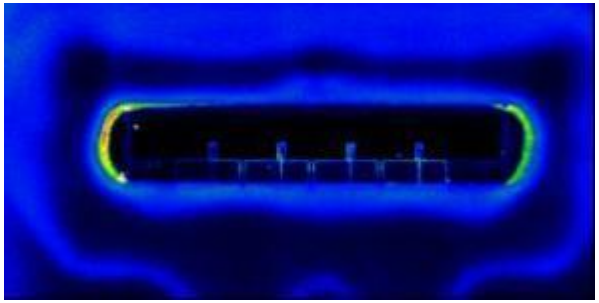


Specific Cy3 Anti-BAM

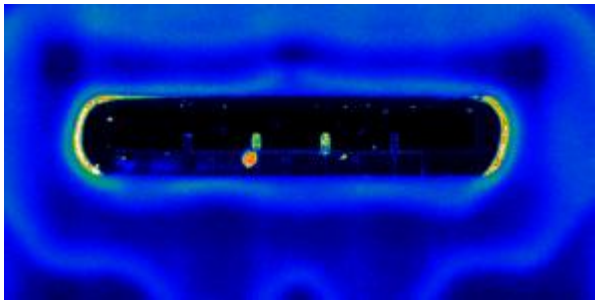


Chip 112

Unspecific Cy5 IgG

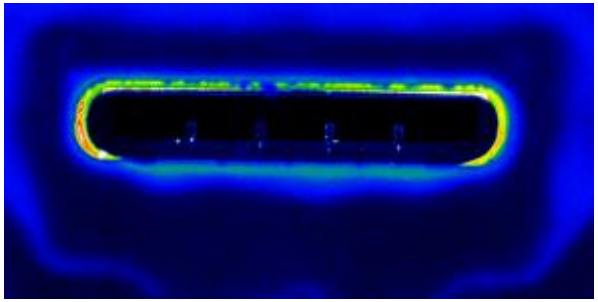


Specific Cy3 Anti-BAM

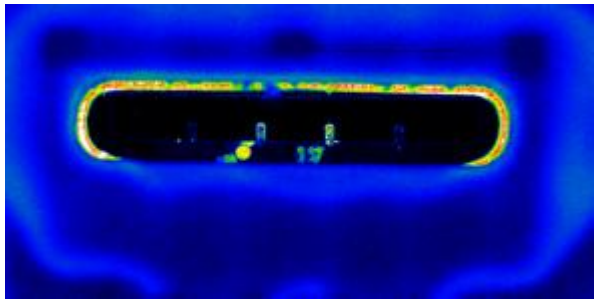


Chip 113

Unspecific Cy5 IgG

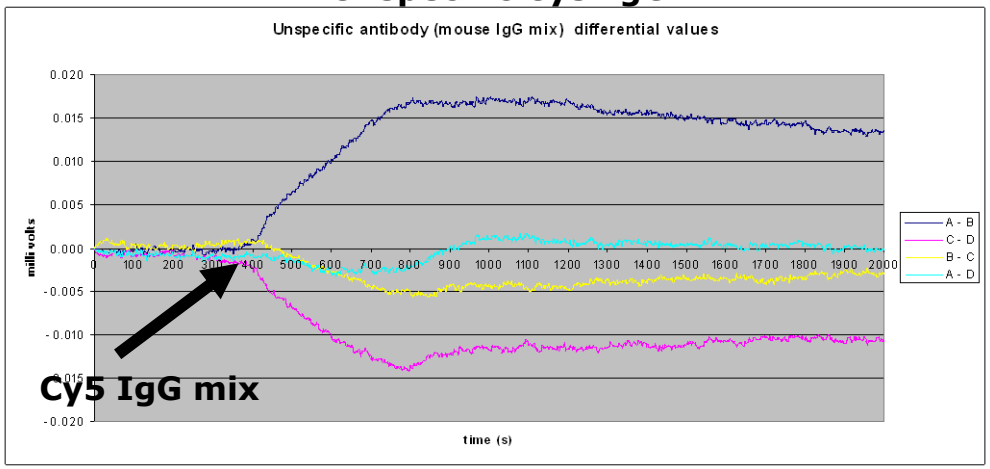


Specific Cy3 Anti-BAM

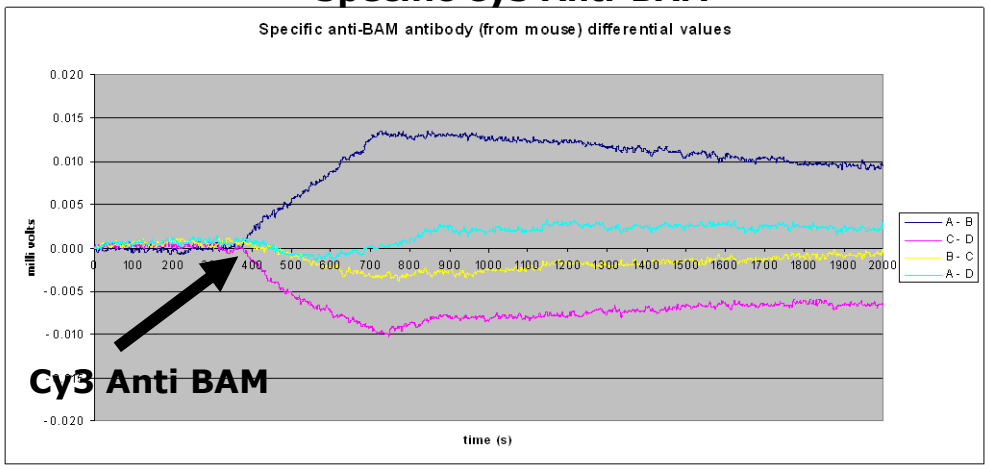


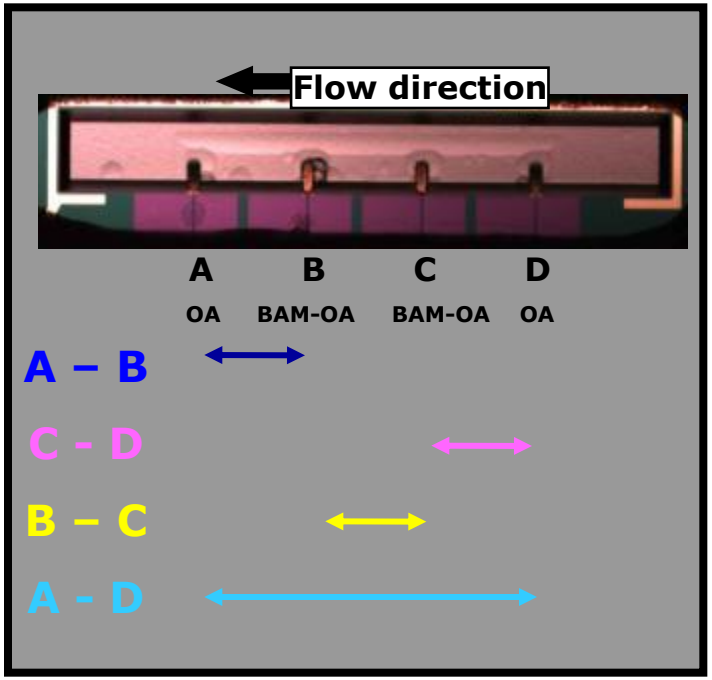
Chip 106 Canti4 data

Unspecific Cy5 IgG



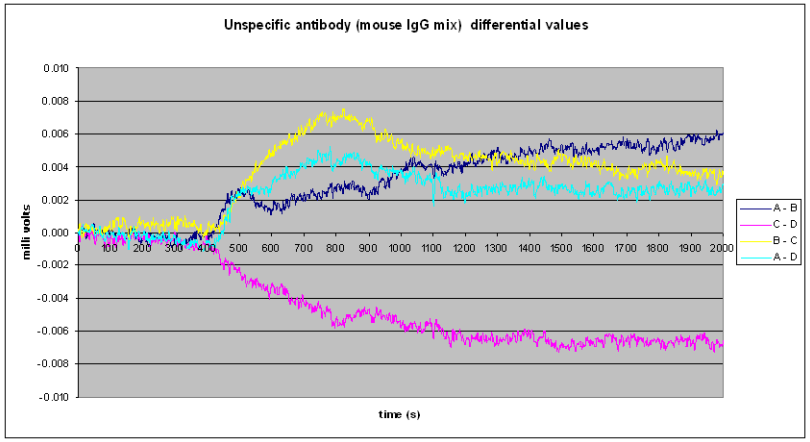
Specific Cy3 Anti-BAM

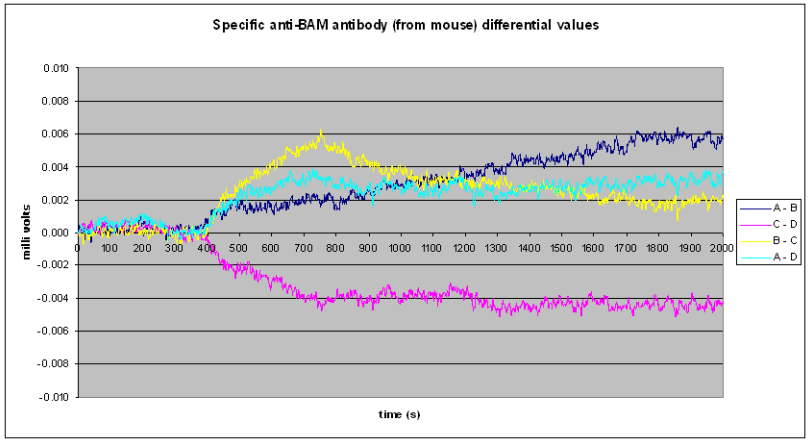




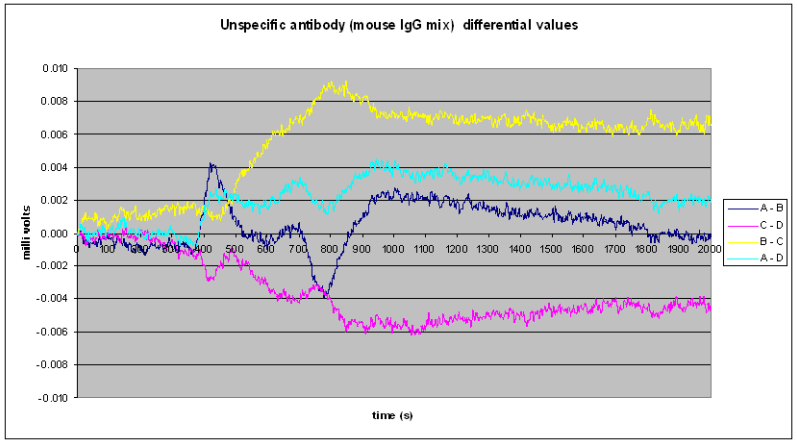
Canti4 data

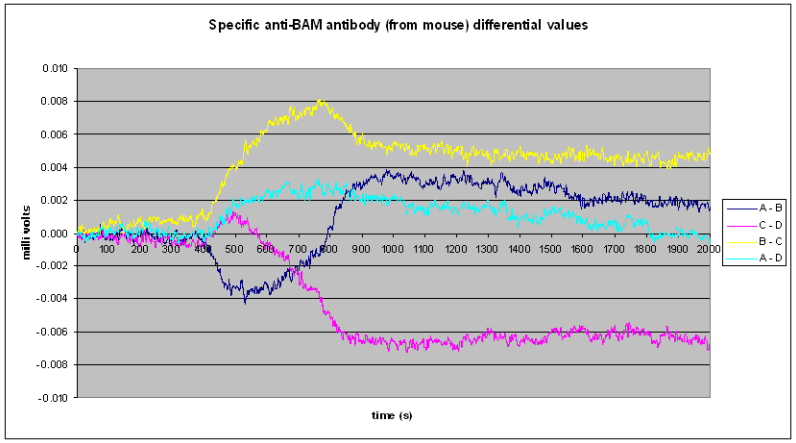
Chip 104





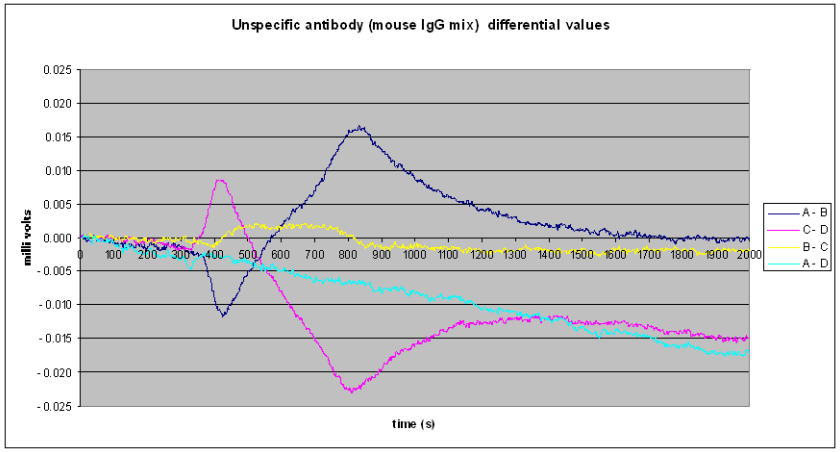
Chip 105

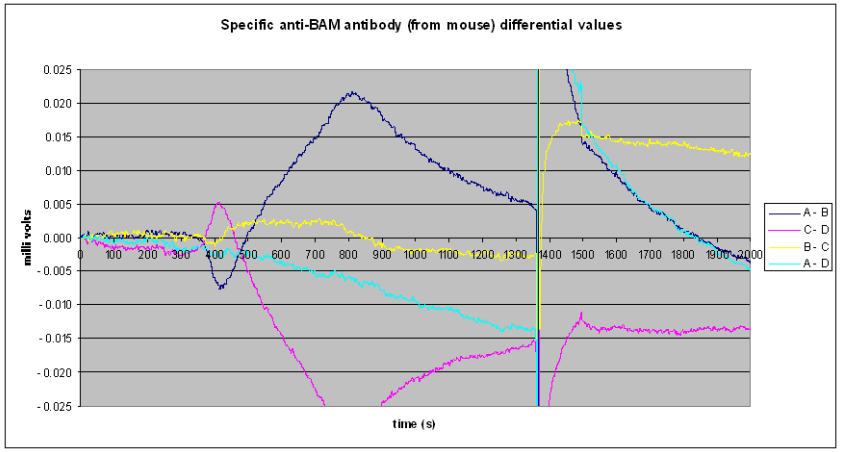




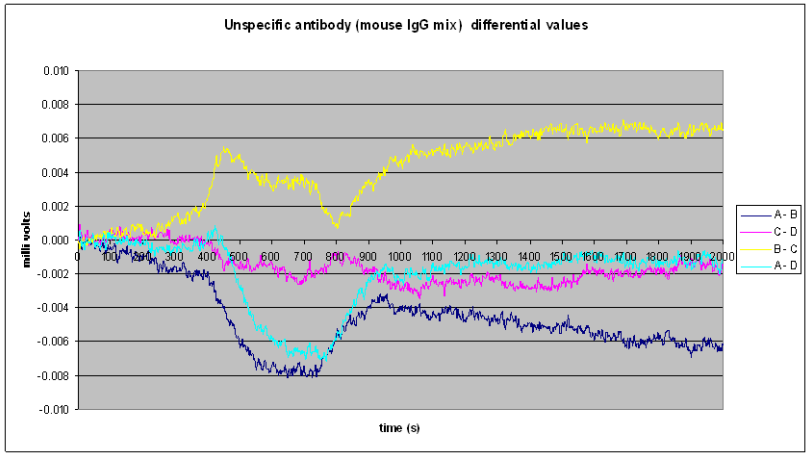
Canti4 data

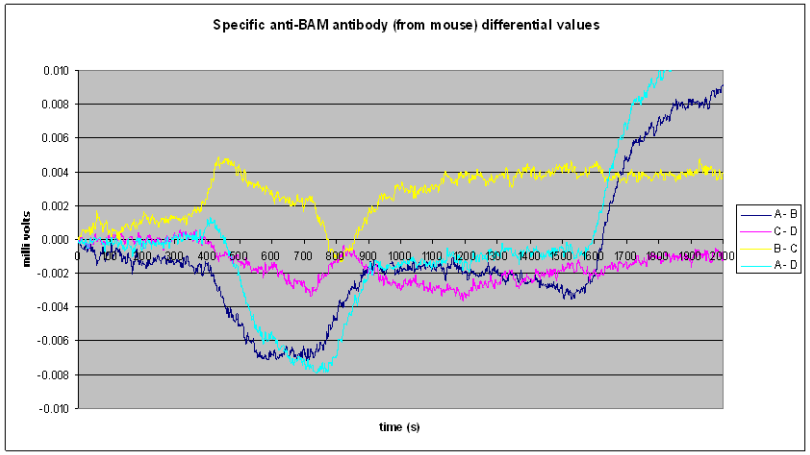
Chip 112





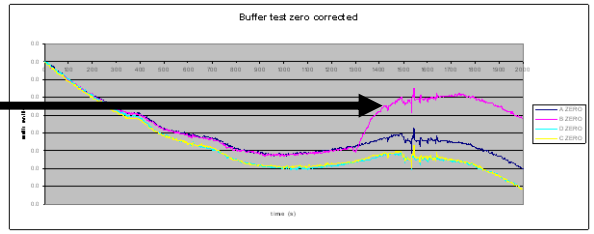
Chip 113



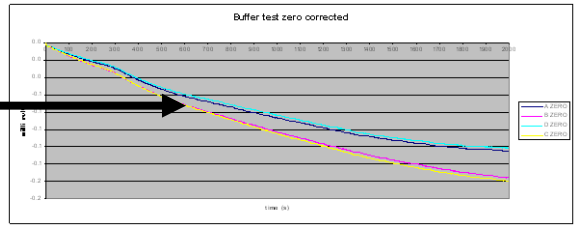


Effects and observations

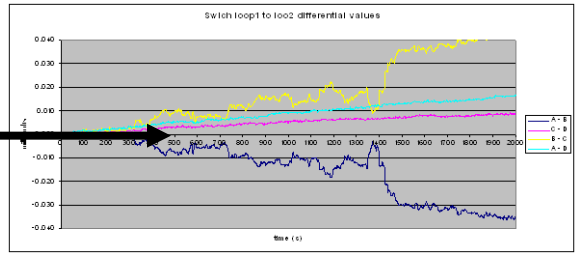
- **Battery effect**: Salinity and pH difference in fluidics acts as a battery, an air bubble blocks the current



- **Signal slope effect**: fixed resistor in the wheatstone bridge does not have a fixed resistance after all.



- **Loop switch effect**: loop switch will likely induce a small change of signal (4-6 µV), probably due to minute changes in the sample buffer, the effect disappears after one loop switch.

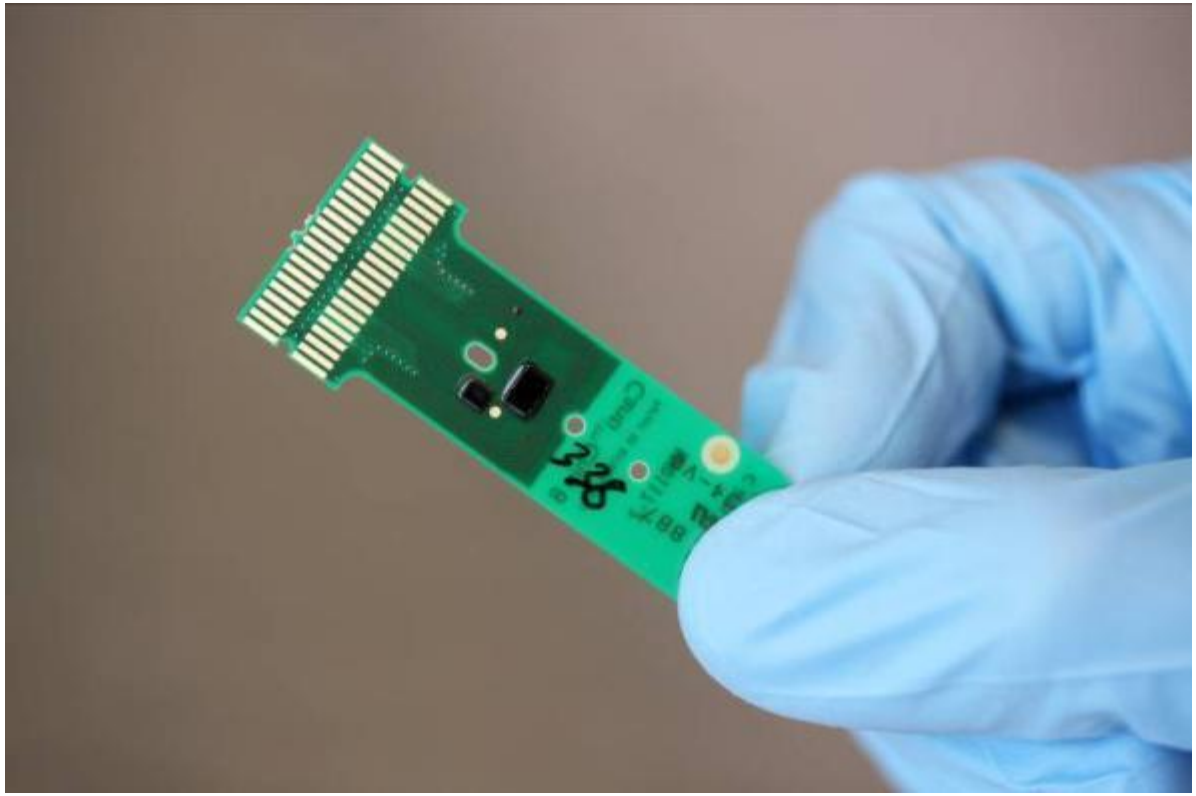


- **Electronic heating effect**: first 30 min of use has a distinct signal profile.

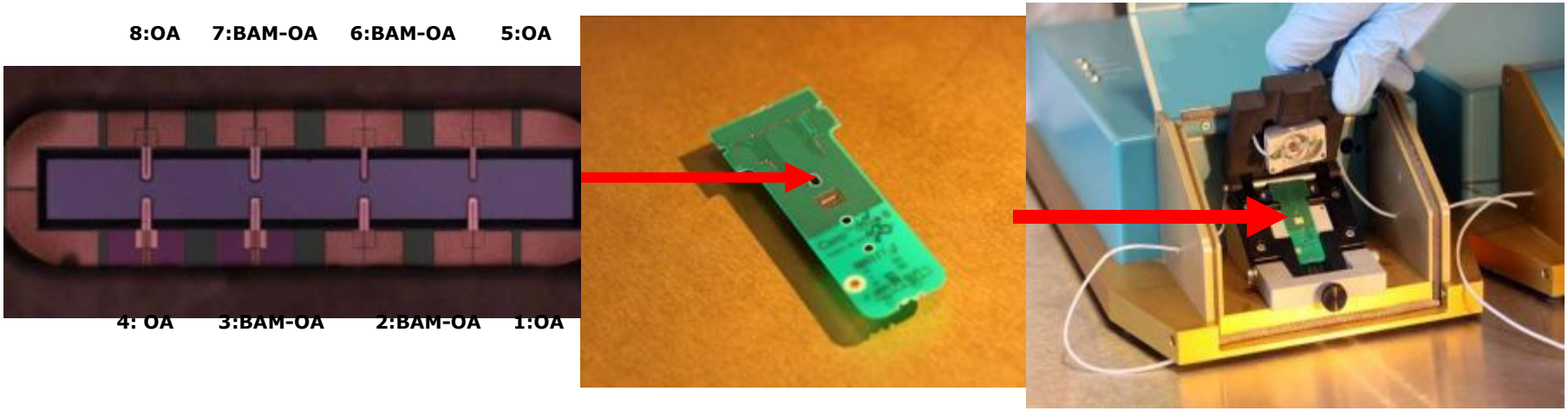
Canti4 characterization status

- Signal Fluorescent pictures show clear Anti BAM bonding to surface and low unspecific bonding
- 5 exp. Show a signal, but also unspecific signal, The concentration of antibodies might be too high (0,1 mg/ml)
- Large signal profile variation from chip to chip
- System is very sensitive to minute changes and impurities

NanoNord Canti8 chip

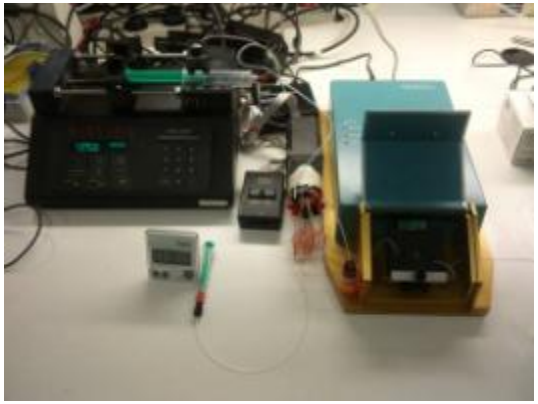


NanoNord Cantichip 8



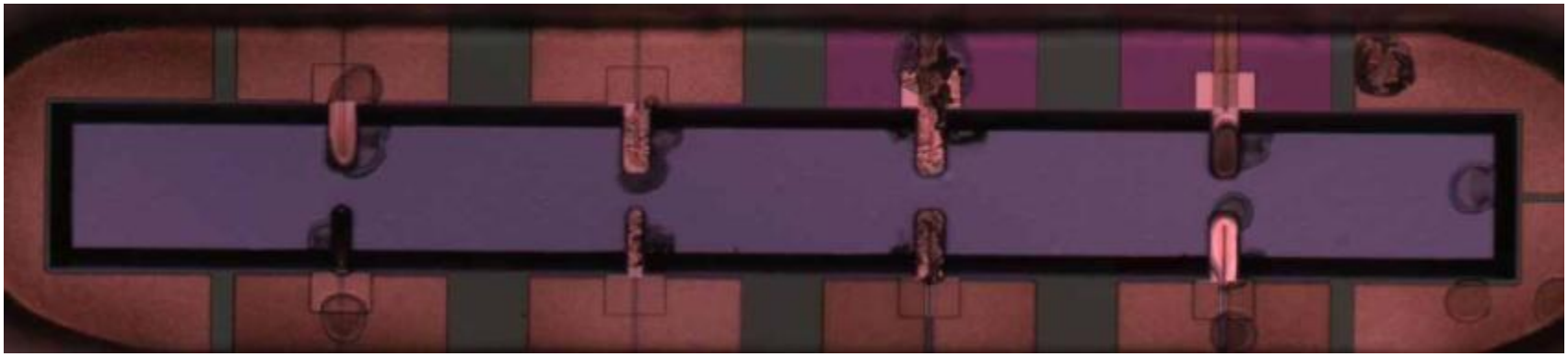
- Built by Nanonord, Århus
- 8 piezo resistive cantilevers
- Better control (Electric field, dynamic mode, voltage…)
- Same outer box but better electronics
- Larger data set

Cantilab 3D fluidic setup

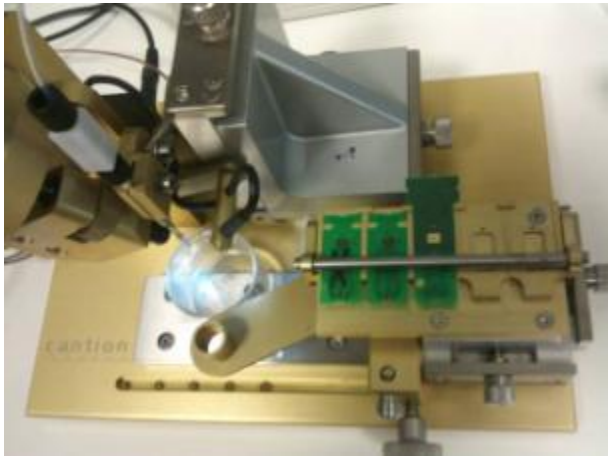


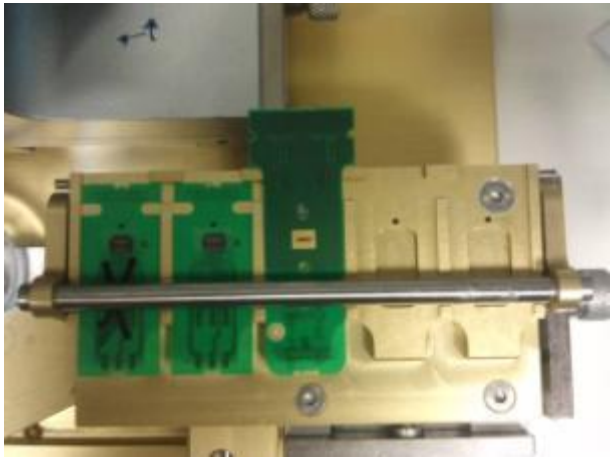
Spotting on Cantichip8

8:OA **7:BAM-OA** **6:BAM-OA** **5:OA**



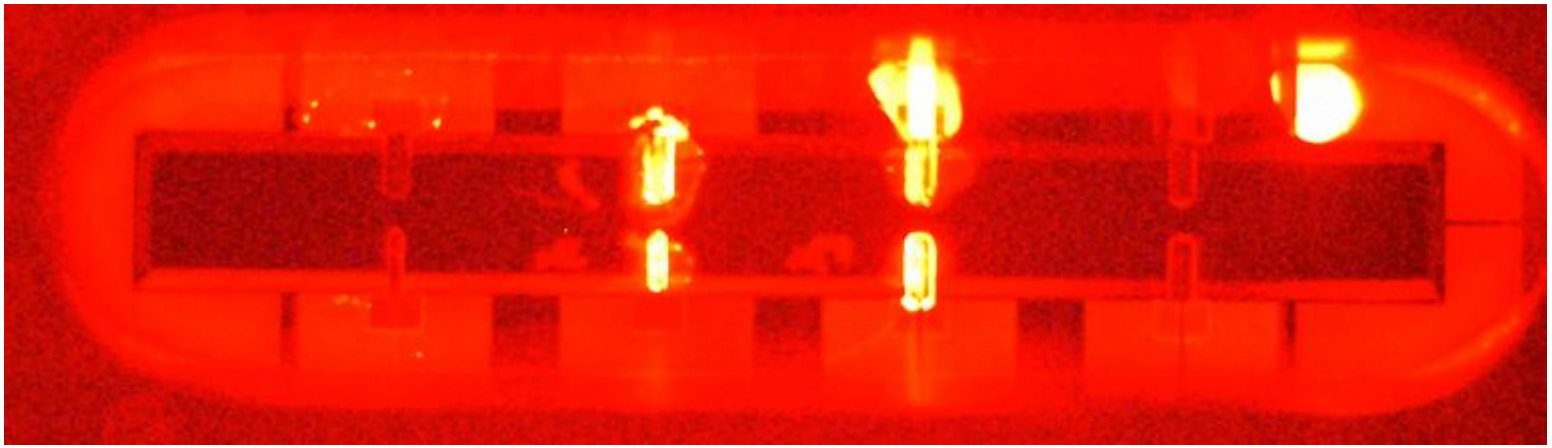
4: OA **3:BAM-OA** **2:BAM-OA** **1:OA**





Cantichip8 Fluorescent picture (Cy3AntiBAM)

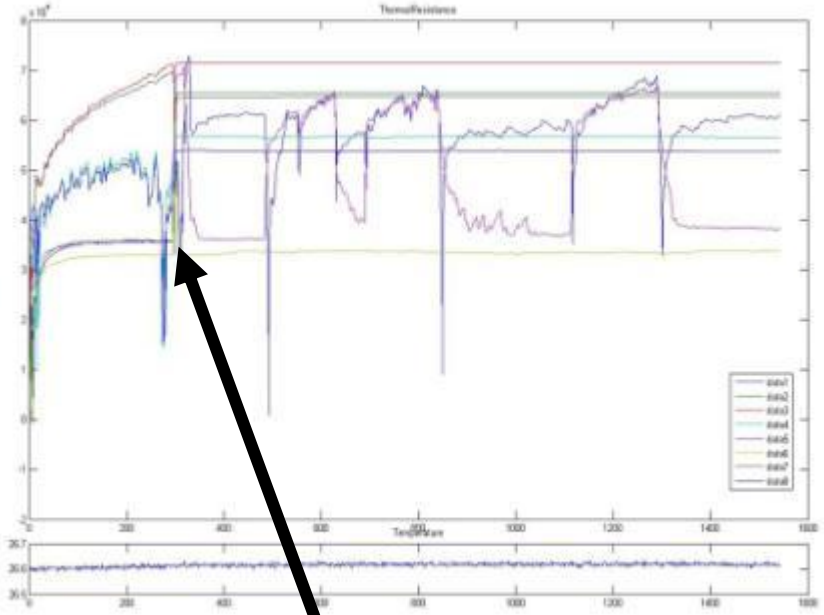
8:OA 7:BAM-OA 6:BAM-OA 5:OA



4: OA 3:BAM-OA 2:BAM-OA 1:OA

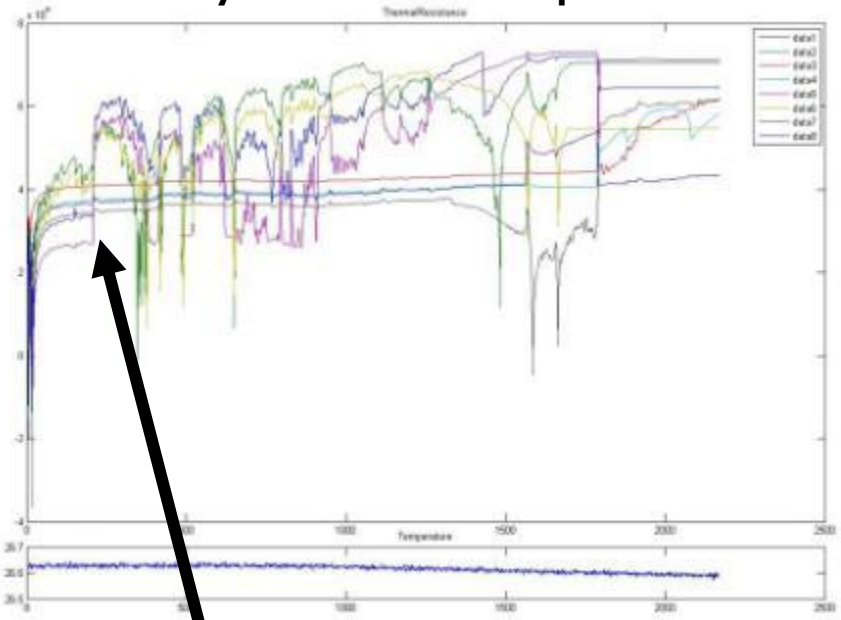
Cantichip8 Data

Cy5 IgG run chip 336



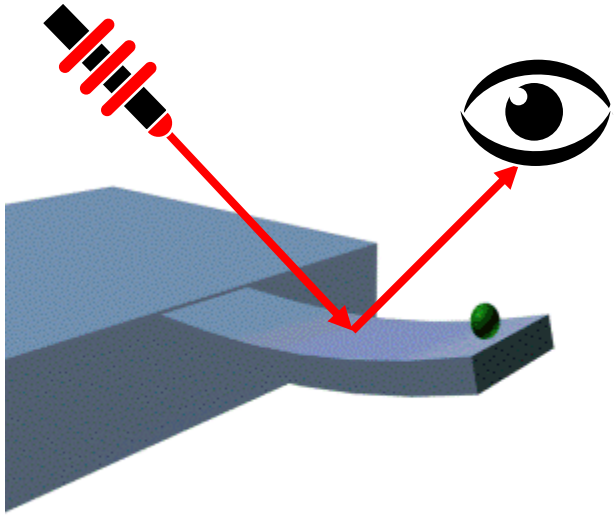
Cy5 IgG mix

Cy3 AntiBAM run chip 336



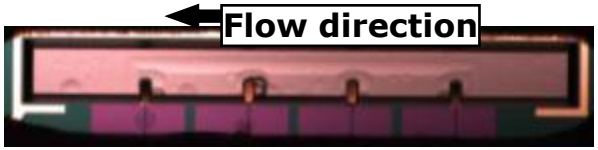
Cy3 Anti BAM

Mass detection of antibodies



- Doppler Vibrometer Polytech MSA 500
- Piezo element vibrates cantilever -> resonance frequency of modes is detected by laser light
- Increase in weight -> Lower resonance frequency

Vibrometer mass change detection results



First mode frequency in KHz; Avg. Of chip 104, 106, 112

	A	B	C	D
Clean	38593	40155	40729	40470
BAM-OA spotted	36817 ↓	38488 ↓	38480 ↓	39315 ↓
After Anti-BAM exp.	39525 ↑	39213 ↑	40731 ↑	40032 ↑

WHY?

Summary

- BAM assay chemistry is working
- Cantilever surface stress signal is not specific enough
- Surface stress induced sensing might be too sensible
- Mass change detection useful for analysis of surface quality

Next

- Repeating Canti4 measurements using 10x lower antibody concentrations
- Working on the new Canti8
- Dynamic mode mass detection in collaboration with Silvan Schmid from Nanoprobes
- Testing repeated stripping and attaching of antibodies

Acknowledgements

- Mogens Havsteen Jakobsen, Supervisor
- Rafael Taboryski, Co- supervisor
- Silvan Schmid, Vibrometer results
- Surface engineering group and Nanoprobes group at DTU