The 15th U.S.-Korea Forum on Nanotechnology

Using Imprinted Polymers to Capture and Detect Bacteria and Viruses

Progress Report

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OBJECTIVE

- Create a general method for screening bacteria and viruses that can be applied to a major infectious disease of global health relevance.

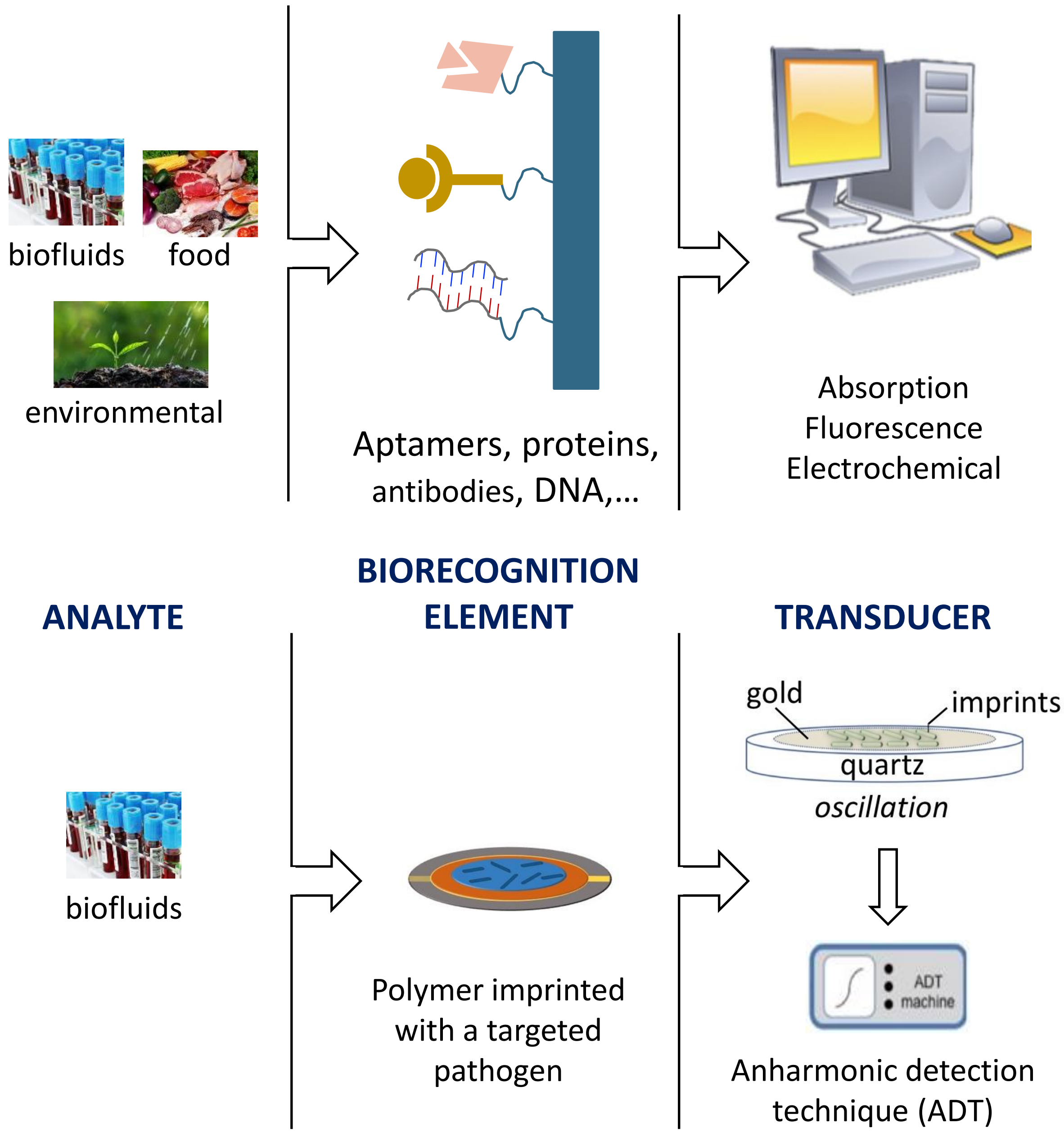
OUR METHOD:

A BIOSENSOR THAT COMBINES A POLYMER AND AN ACOUSTIC TRANSDUCER

A device comprised of a biological element that senses a lock-and-key event and transmits that information into a detectable electrical signal.

Our device is intended to be more than simply a quartz crystal microbalance (QCM) as will be explained.

Components of a biosensor



BACTERIA CAPTURE VISUAL DETECTION

Analyte (model sample):

E. coli-GFP in H_2O

S. typhimurium in H_2O

Biorecognition element:

E. coli-imprinted OSX polymer (bulk)

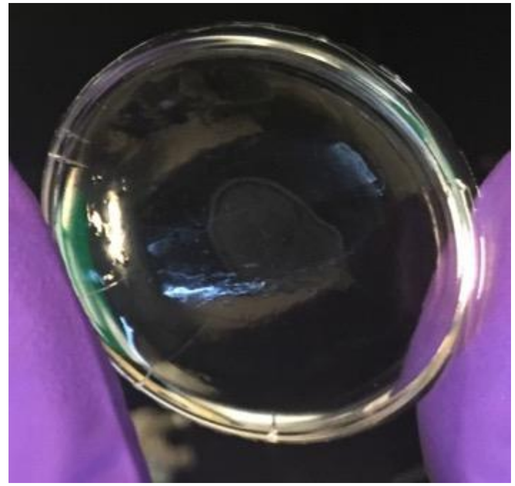
Detection:

Fluorescence imaging

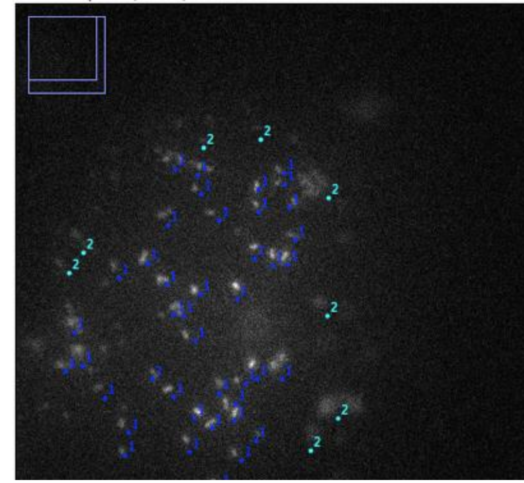
Requirements:

- Labelled or stained bacteria

Imprinted polymer

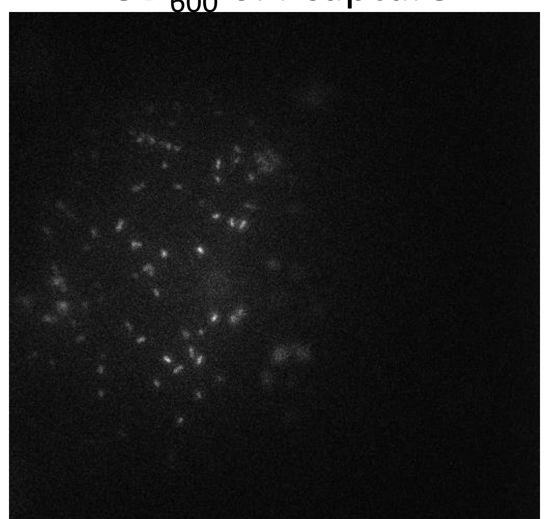


Data processing (ImageJ)



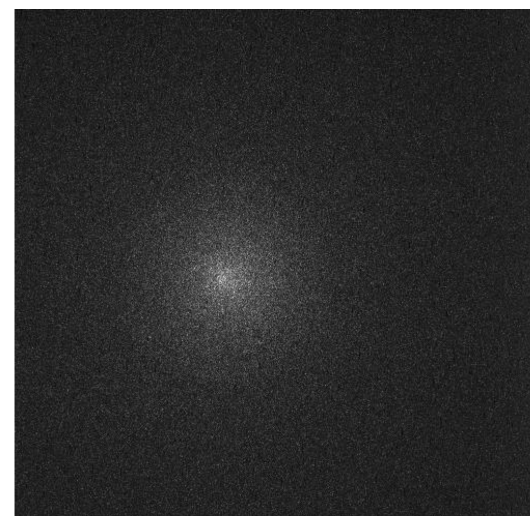
E. coli (targeted)

OD_{600} 0.4 capture



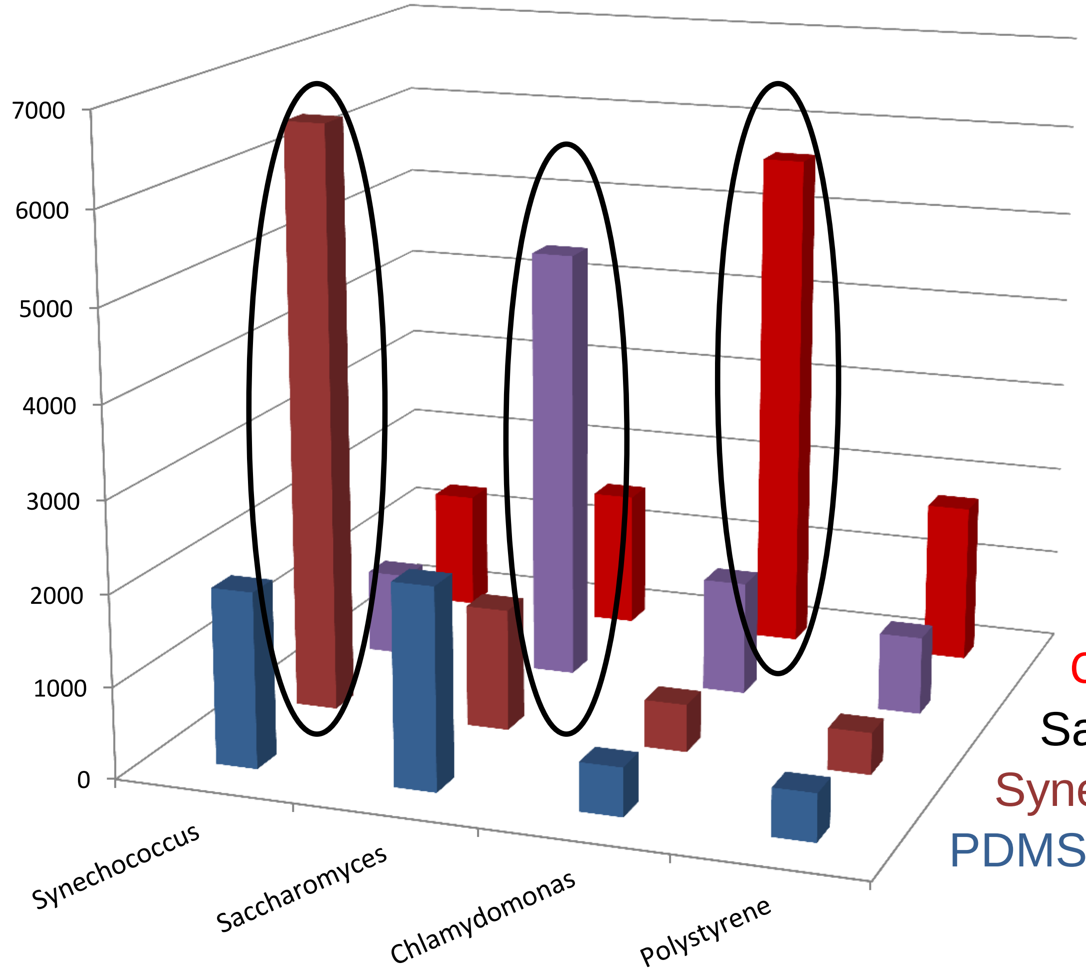
S. typhimurium

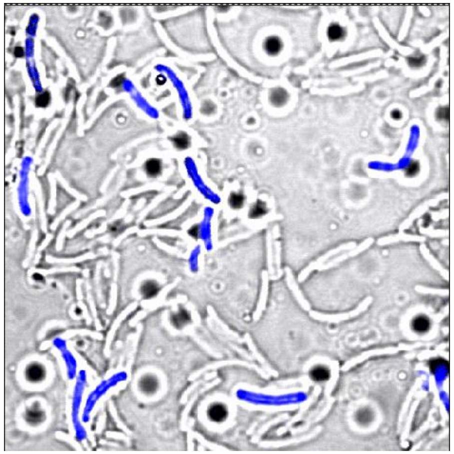
OD_{600} 0.4 capture

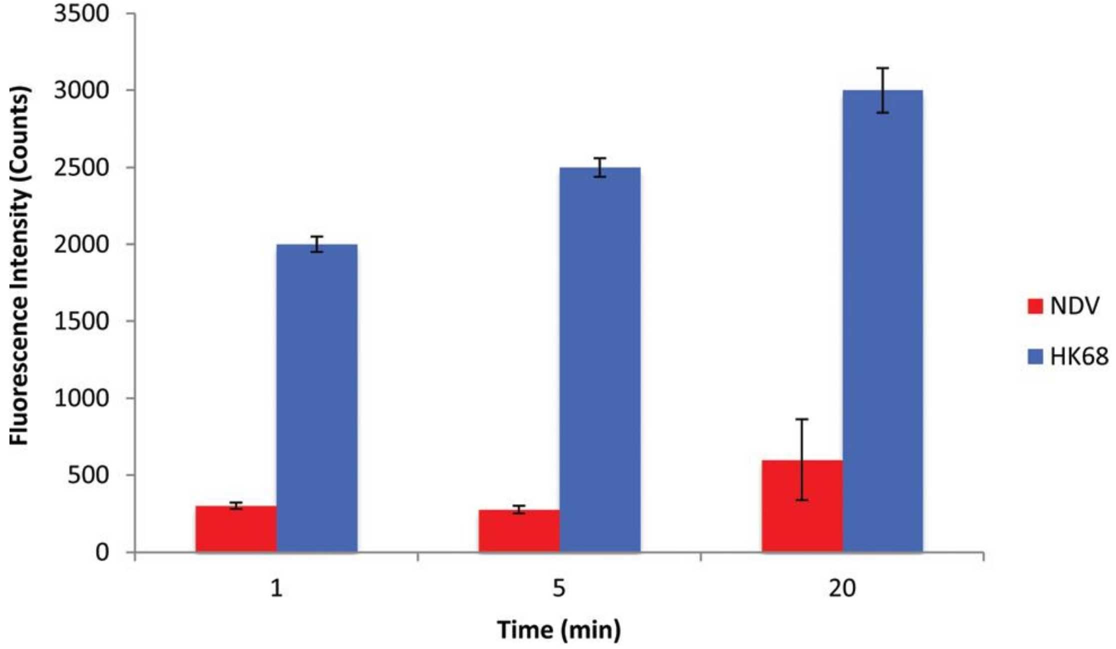


HOW SELECTIVE IS CAPTURE?

Sensing: Selectivity



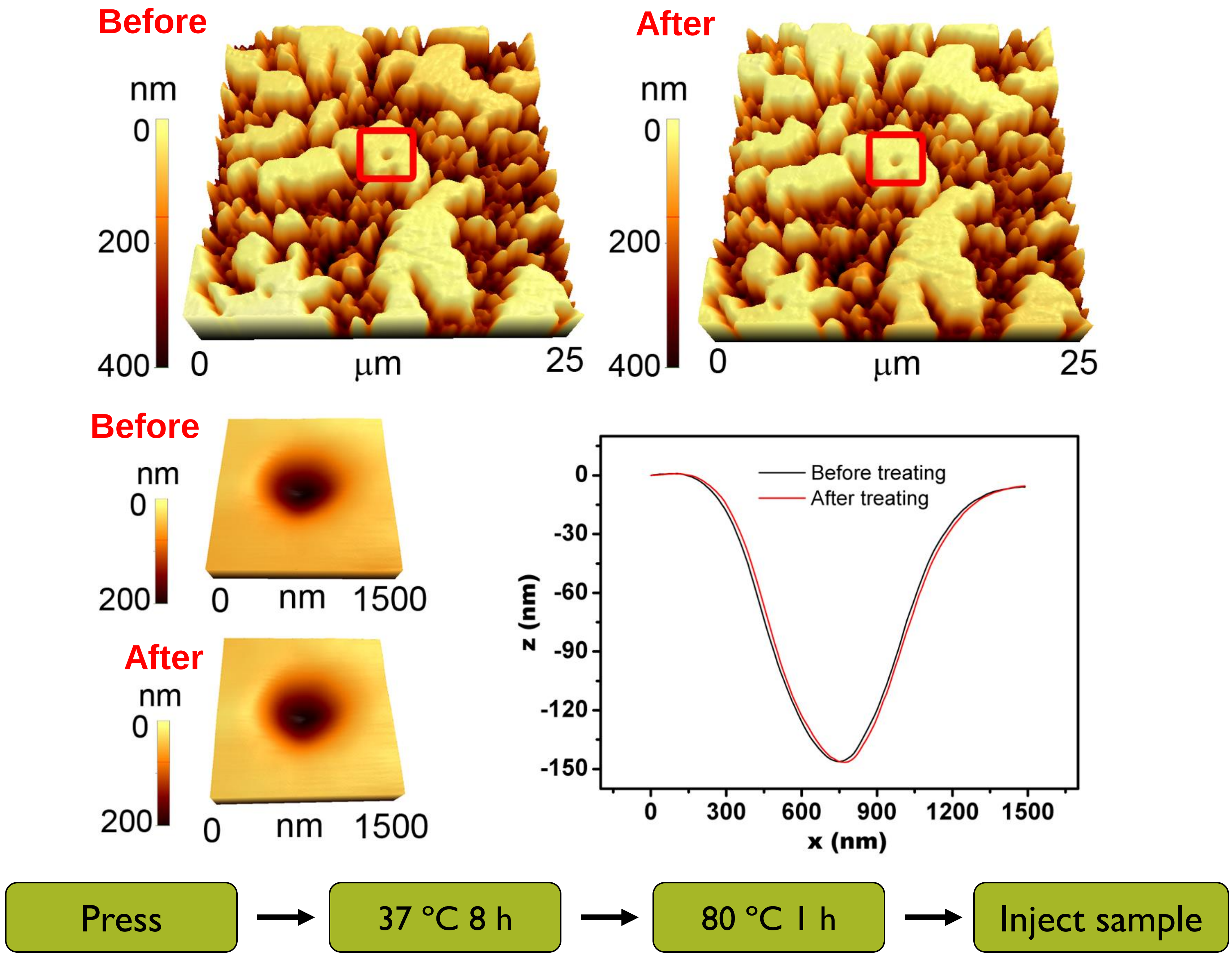




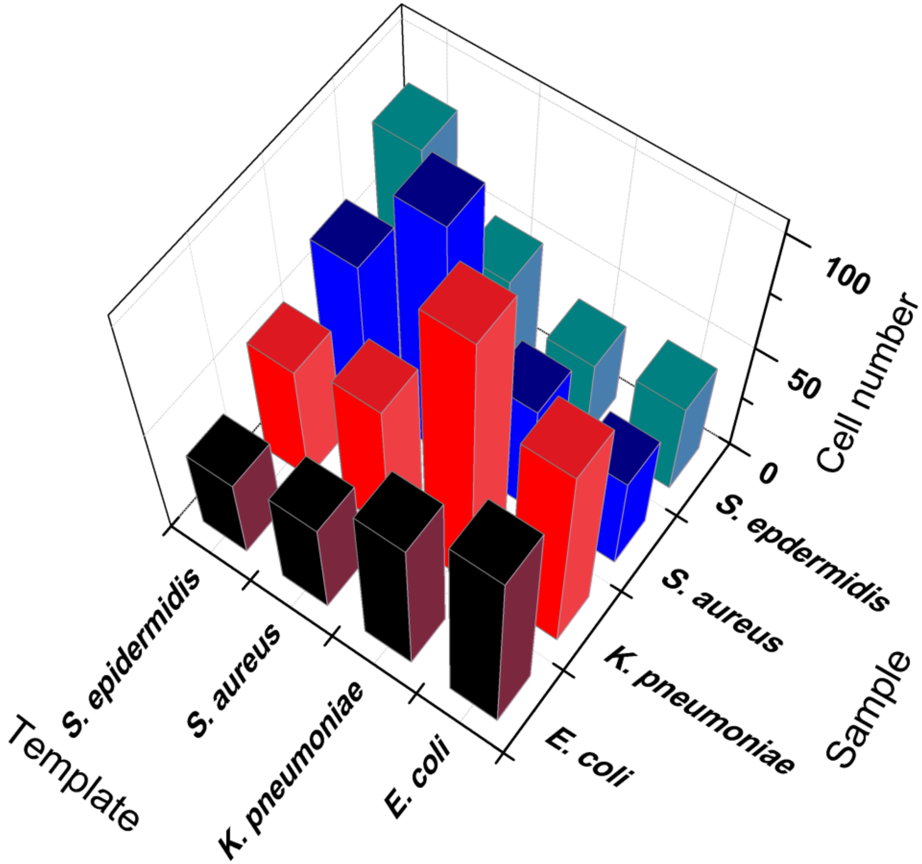
Two inactivated viruses with similar shape, Influenza A (HK68) and Newcastle Disease Virus (NDV), were employed as model pathogens. The polymer film, which was first imprinted with HK68 and exposed sequentially to suspensions containing fluorescently labeled NDV and HK68, was able to preferentially bind HK68 at a capture ratio of 1 : 8.0. When we reversed the procedure and imprinted with NDV, the capture ratio was 1 : 7.

A. Karthik, K. Margulis, K. Ren , R. N. Zare, and L. Leung, "Rapid and Selective Detection of Viruses Using Virus-Imprinted Polymer Films," Nanoscale 7, 18998 - 19003 (2015).

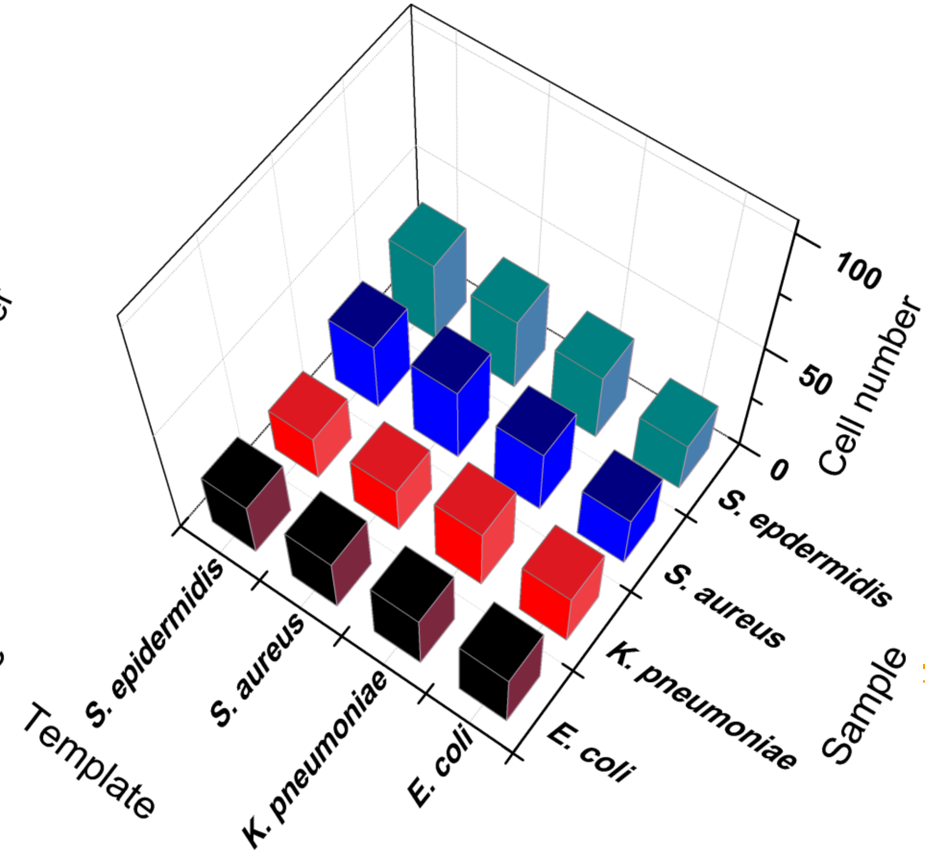
HOW DOES CAPTURE HAPPEN?



Before
nm
0
200
400
0
µm
25
After
nm
0
200
400
0
µm
25
Before
nm
0
200
0
nm
1500
After
nm
0
200
0
nm
1500
Before treating
After treating
z (nm)
x (nm)
0
-30
-60
-90
-120
-150
0
300
600
900
1200
1500
Press
37 °C 8 h
80 °C 1 h
Inject sample



Unmodified



Monolayer Overcoated

K. Ren and R. N. Zare, "Chemical Recognition in Cell-Imprinted Polymers," ACS Nano 6, 4314-4318 (2012).

ANHARMONIC DETECTION TECHNIQUE (ADT)

Collaboration with:

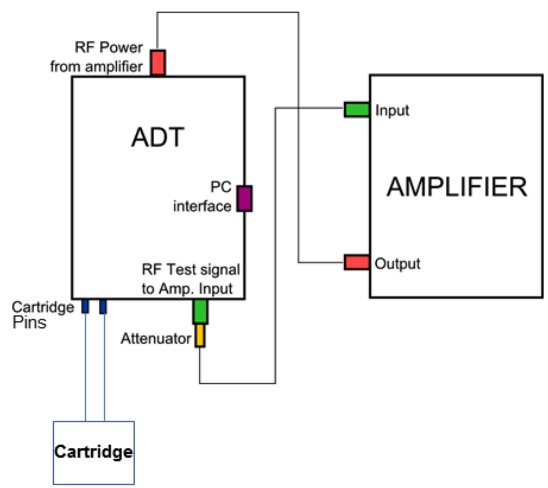
Sourav Ghosh's research group at

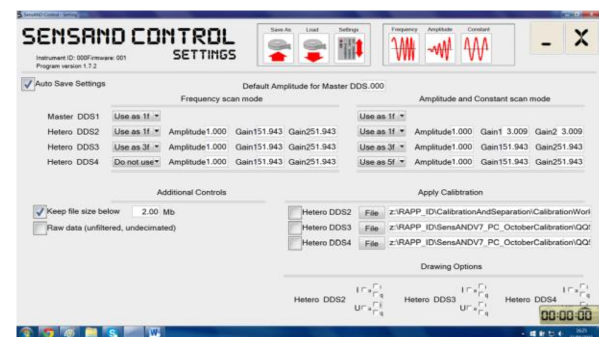
Loughborough University (UK)

Novelty: *nonlinear network analyzer*

- **amplitudes, 0 – 27.5 V**
- **Frequencies, 0.1 to 300 MHz**
- **Records complex current and voltage sensitivity and synchronously at 3 frequencies**
- **Odd harmonics signals are separated from powerful driving signal applied near fundamental resonance frequency by linear passive filtering network**

- Measures a variation in mass by measuring the change in frequency of a quartz crystal resonator
- Resonance is disturbed by the addition or removal of a small mass at the surface of the acoustic resonator
- In our case, bacteria has a certain affinity for the imprinted polymer on the resonator surface which is "functionalized" with recognition sites by virtue of the bacteria-imprinted polymer





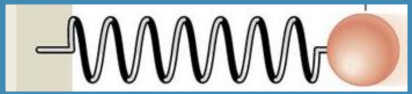
ANHARMONIC DETECTION TECHNIQUE (ADT)

Collaboration with:

Sourav Ghosh's research group at

Loughborough University (UK)

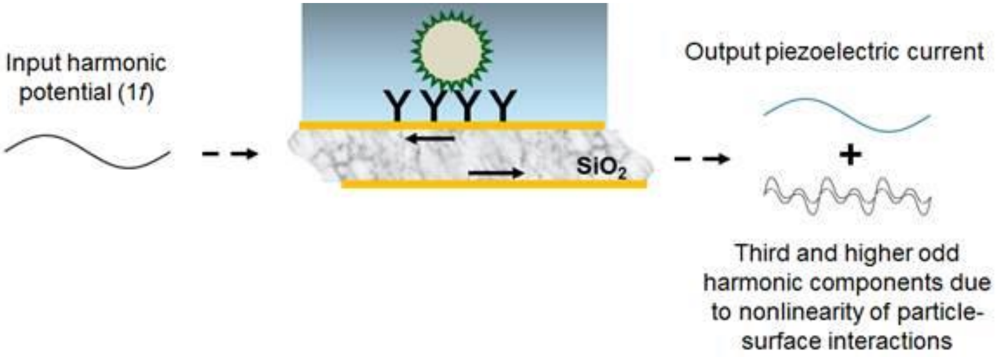
Ball on a spring (harmonic oscillation)



- Amplitude (size of the bounce)
- Bounces back and forth (frequency)

Principle

- Relies on interaction at the surface of a quartz crystal resonator, causing a nonlinear oscillation that introduces distortions in the harmonic (or sinusoidal electric current)
- The distortion is measured from change in magnitude of the third Fourier harmonic (3*f*) current, which is 3 times the driving frequency (3*f*).
- The deviations in higher odd harmonic responses as a function of oscillation amplitude are strongly dependent on the force involved in binding of the analyte under study and the recognition element as well as the size of the analyte.



DEMONSTRATION

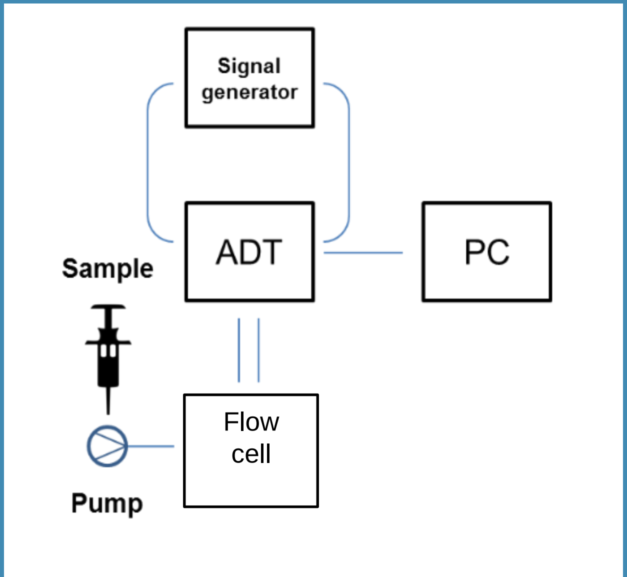
ANHARMONIC DETECTION TECHNIQUE (ADT)

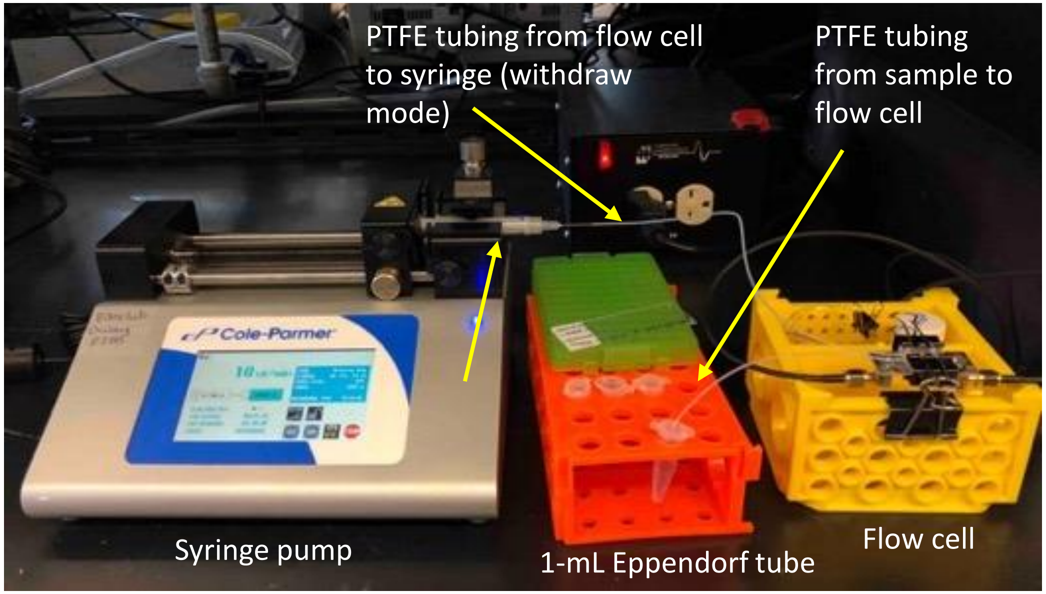
ADT designed and built by:

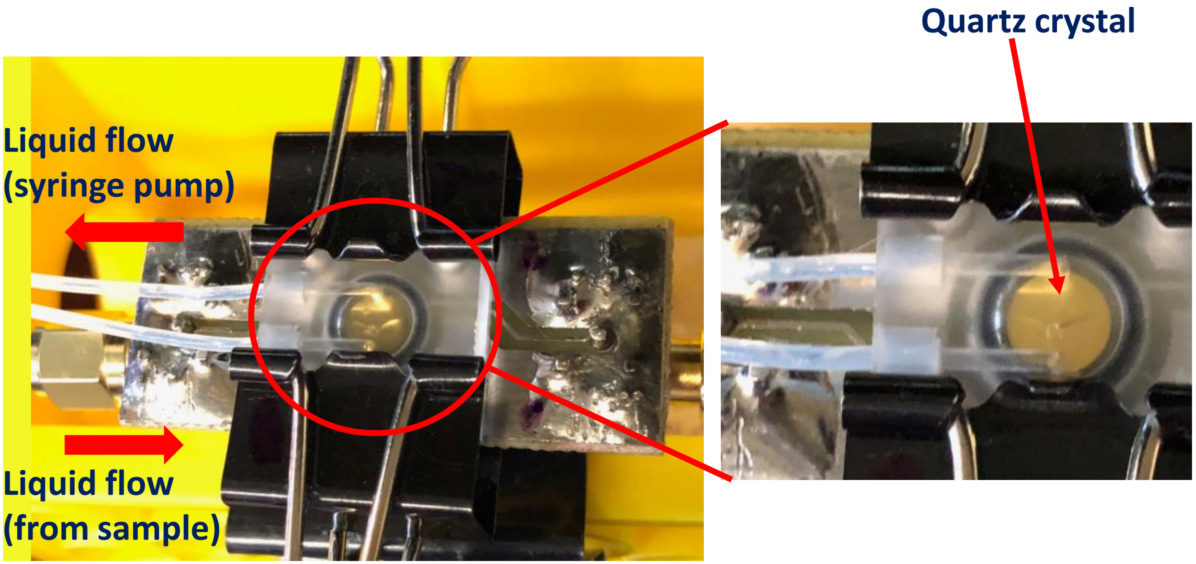
Sourav Ghosh's group at

Loughborough University (UK)

Schematic illustration of experimental setup:

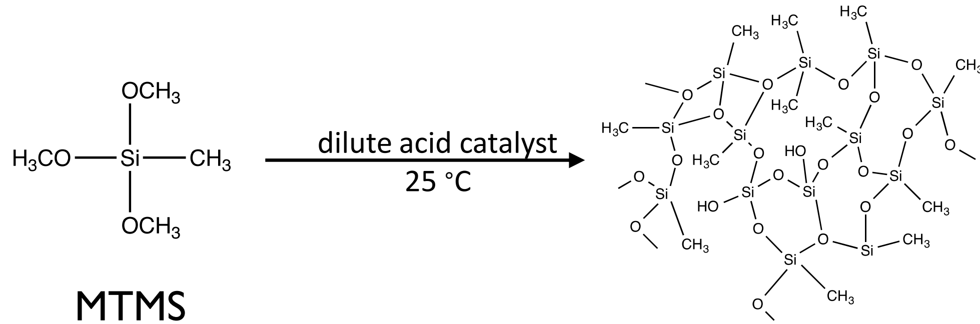






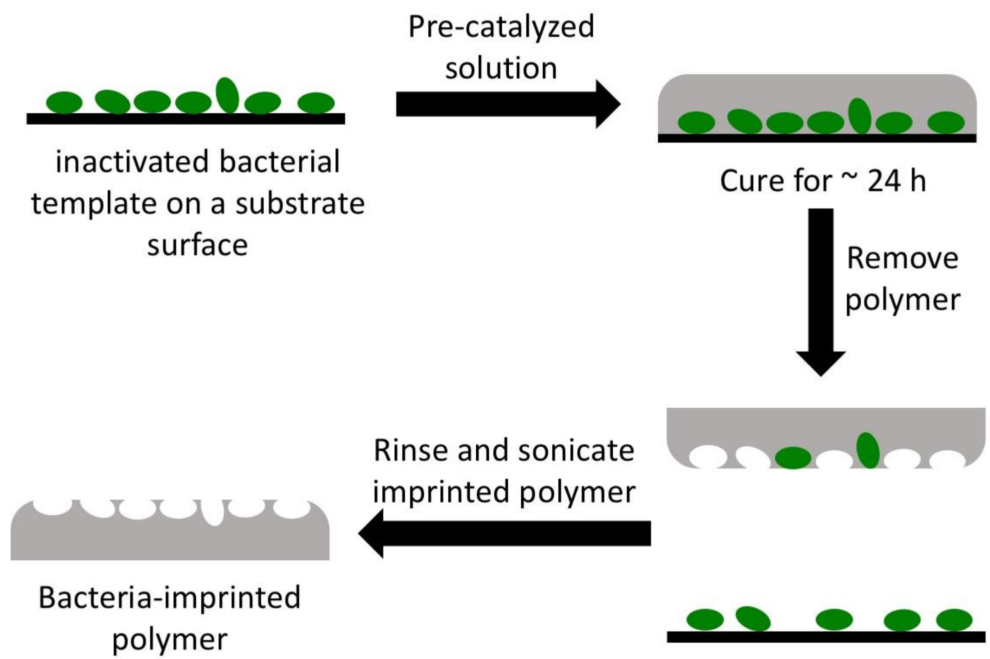
BIORECOGNITION ELEMENT

Preparation of a bulk imprinted OSX polymer



MTMS

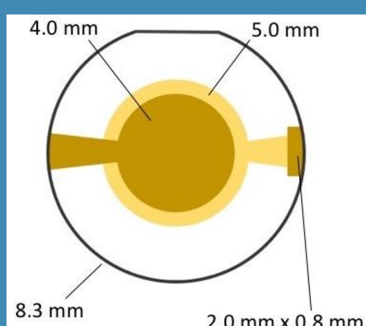
Organosiloxane (OSX) polymer



BIORECOGNITION ELEMENT

A quartz crystal showing the top electrode

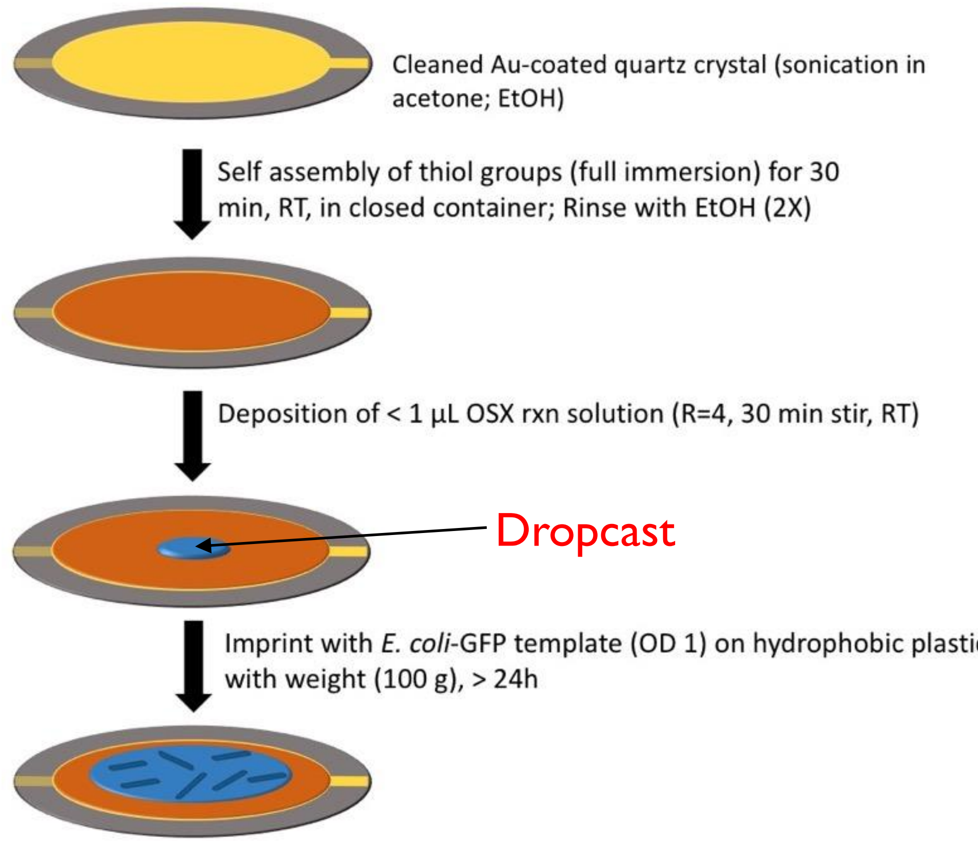
Schematic of quartz crystal (view from bottom electrode)



Frequency range: 14.275 – 14.325 MHz

Aim 1: Optimization of polymer synthesis

Preparation of an imprinted OSX on a quartz crystal by dropcasting method



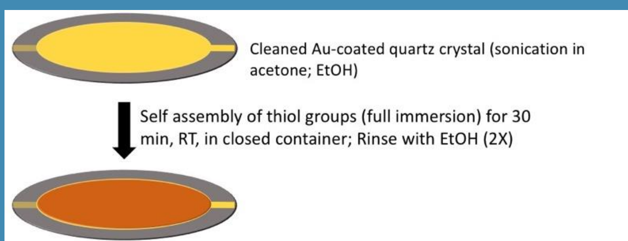
Reported dropcast method for thin film coating on quartz crystal resonators:

(1) T. Cohen et al. *Int. J. Molec. Sci.* **2010**, *11*, 1236-1252. *Whole cell imprinting in sol-gel thin films for bacterial recognition in liquids*.

(2) F.L. Dickert, O. Hayden. *Anal. Chem.* **2002**, *74*, 1302-1306. *Bioimprinting of polymers and sol-gel phases. Selective detection of yeasts with imprinted polymers.*

BIORECOGNITION ELEMENT

STEP 2:
Self-assembly of SH-TMS on gold surface (top electrode)

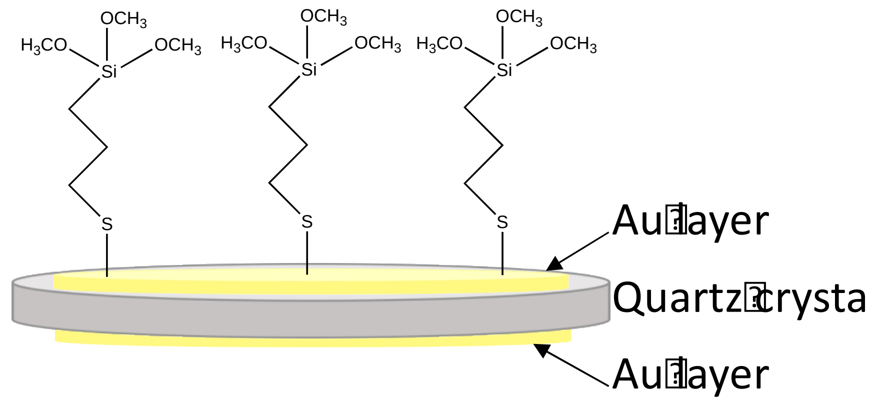


Concentrations tested:
1, 3, 5, 19 mM

Solvents tested:
EtOH, toluene

Deposition times tested:
30 min, 60 min, 2 h

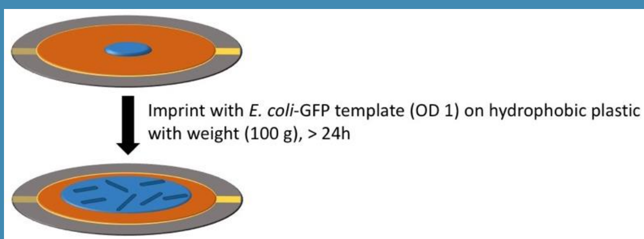
Thiol modification of electrode surface



Crystal surface coating	SH-TMS volume (µL)	Coating Approach
None	---	---
SH-TMS	200	Full immersion
SH-TMS	5	Deposition
SH-TMS	3	Deposition
SH-TMS	1	Deposition

BIORECOGNITION ELEMENT

STEPS 3 and 4:
(3) Deposition of OSX rxn solution on top electrode
(4) Imprinting with *E. coli* (OD 1)



Catalyst concentration tested:
0.12 M, 0.29 M, 0.35 M HCl

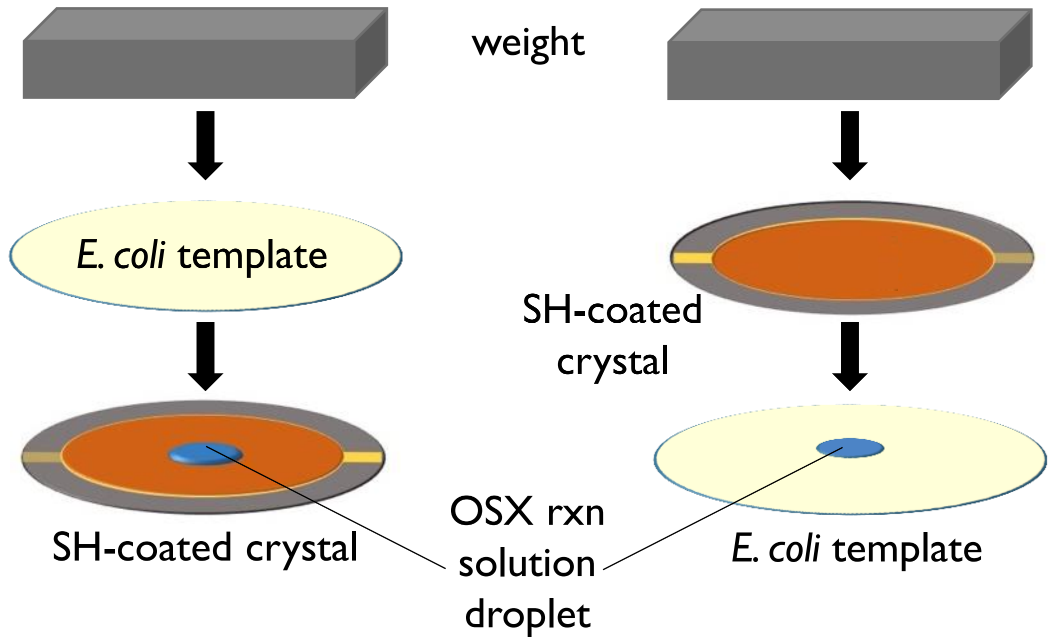
R *values tested:*
1.8, 2.0, and 4.0
R = molar ratio of H_2O to silane

Varied deposition volume
Varied imprinting weights

Preparation of an imprinted OSX on a quartz crystal

IMPRINTING APPROACH #1

IMPRINTING APPROACH #2



R	Deposition volume (µL)	Imprinting weight (g)
1.7 – 4.0	≤ 1 - 5	10 - 350
4.0	≤ 1	100

BIORECOGNITION ELEMENT

STEPS 3 and 4:
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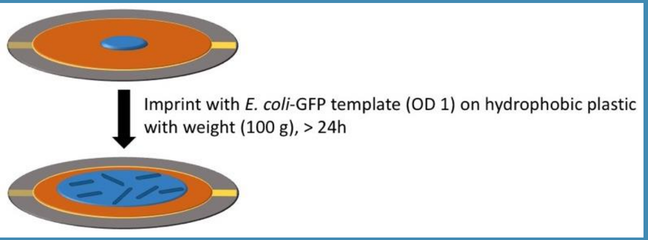
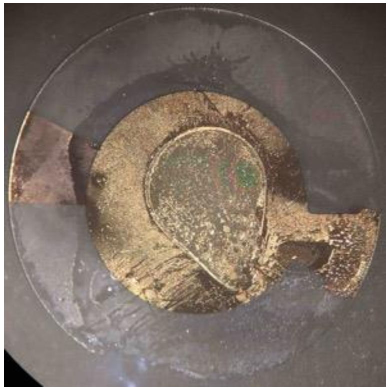


Catalyst concentration tested:
0.12 M, 0.29 M, 0.35 M HCl

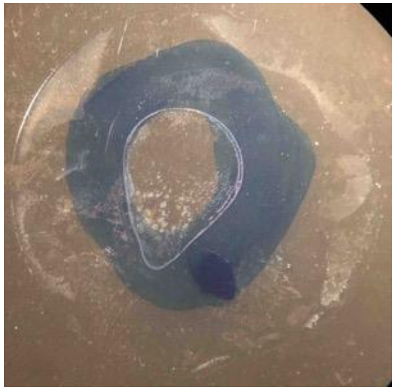
R *values tested:*
1.8, 2.0, and 4.0

Varied deposition volume
Varied imprinting weights

Example of an imprinted OSX on a quartz crystal



E. coli-imprinted OSX polymer on thiol-modified gold-coated quartz crystal



E. coli template used to imprint OSX polymer (left photo)

R	Deposition volume (µL)	Imprinting weight (g)
1.7 – 4.0	≤ 1 - 5	10 - 350
4.0	≤ 1	100

CAPTURE

Morphological similarity:
E. coli and *S. typhimurium* are similar in shape and size: rod-shaped, ~1 µm x 2.5 µm

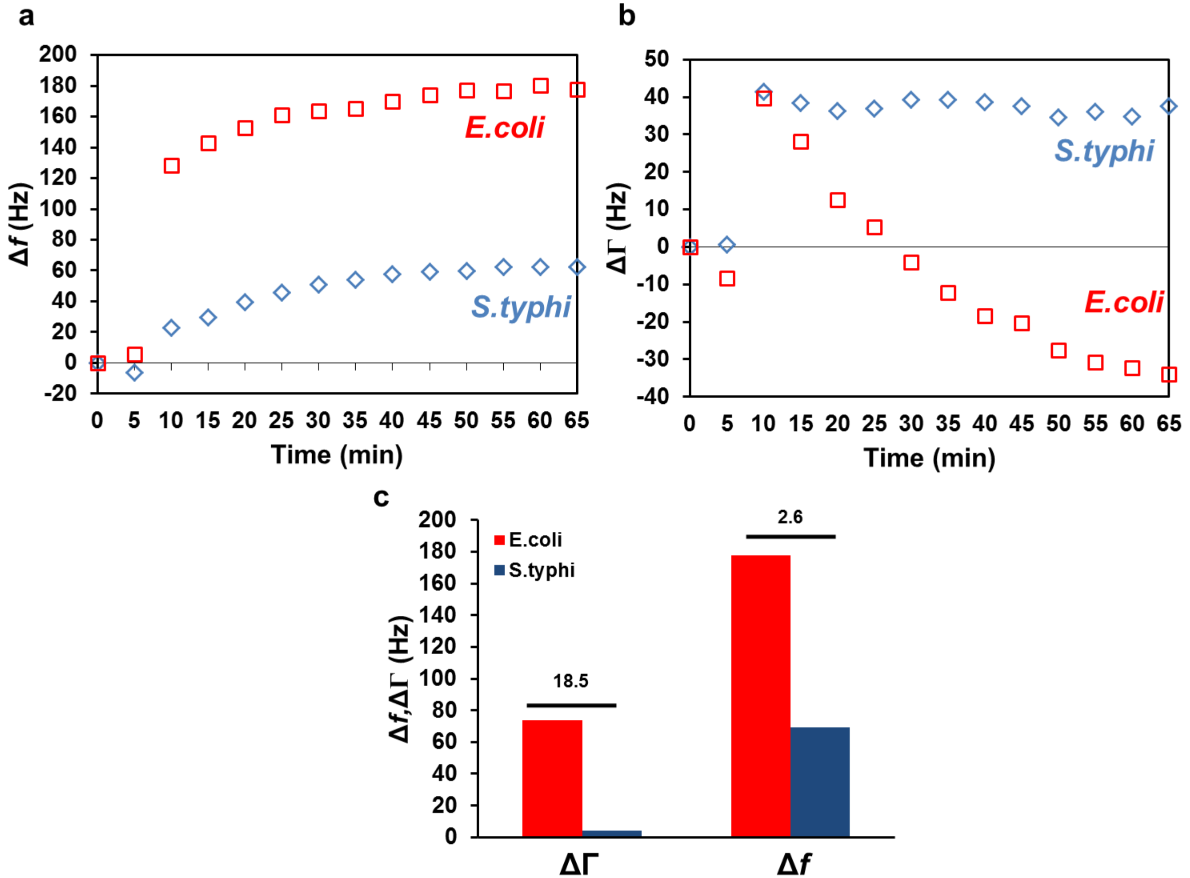
3*f* Measurements:

- Quartz crystal oscillates by short (100 ms) frequency sweeps with discrete increases in voltage (0.25 – 12.5 V)
- Lower signal ratio (at 19.2 V) is likely due to polymer film being too thick.

Experimental:

- *E. coli* and *S. typhimurium* concentrations approximately 1.6 x 10^7 cells/mL
- Capture time: 10 min

Capture of targeted *E. coli* vs non-targeted *S. typhimurium*



Increase in dissipation (ΔΓ) was higher for *E. coli* than *S. typhimurium*: higher selectivity for *E. coli* when compared to frequency shift (Δ*f*)

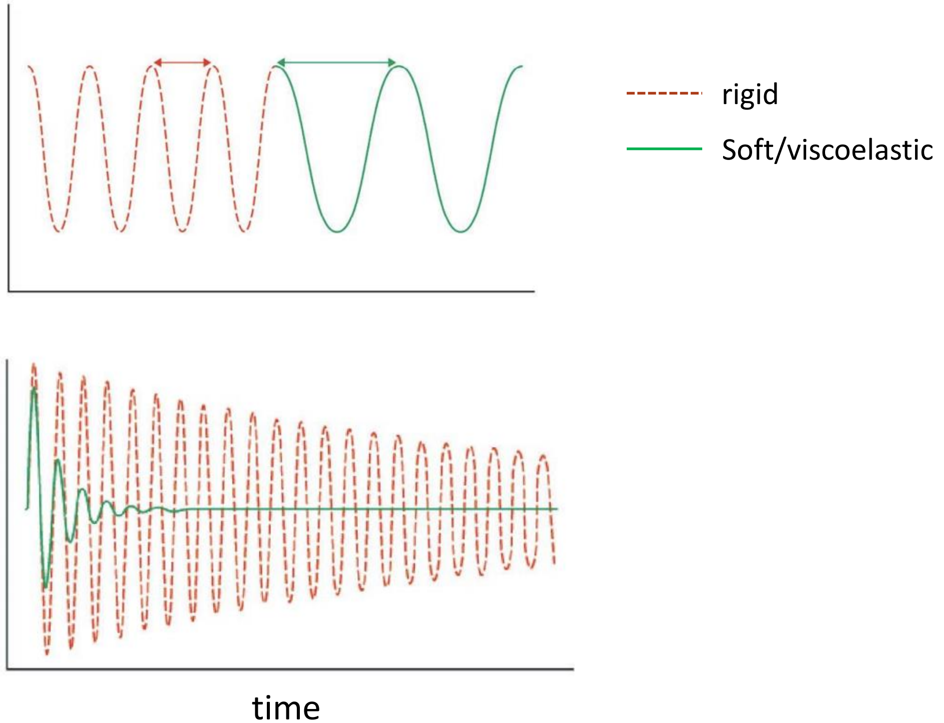
ANHARMONIC DETECTION TECHNIQUE (ADT)

What we are trying to achieve in an imprinted polymer:

- Rigidity
- Strong adherence to the gold surface

Dissipation (damping)

- It gives us an idea of the viscoelasticity of the polymer film on the crystal surface as well as mass information
- It becomes significant when the adsorbed film is not rigid (film and crystal oscillations are not fully coupled)
- When the oscillation stops (potential is turned off), the time needed for the oscillation to stop reflects the viscoelasticity of the film on the surface of the resonator



CAPTURE

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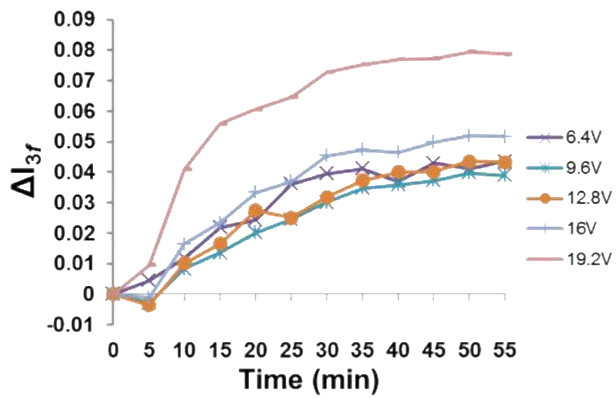
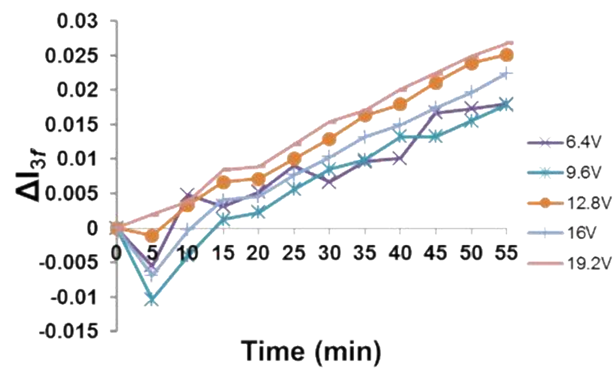


E. coli



S. typhimurium

3*f* signals at different drive potentials. At 19.2 V, *E. coli* signal is 2.9 times higher than for *S. typhimurium*.

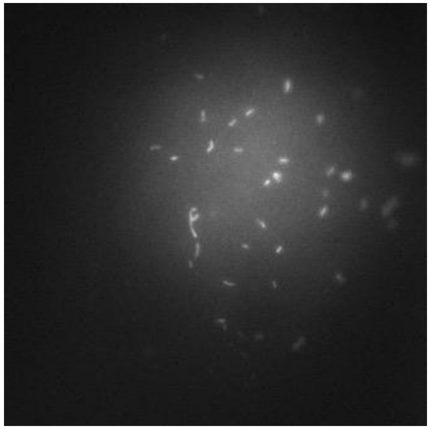
FUTURE DIRECTION:

IMPROVING POLYMER FILM THICKNESS

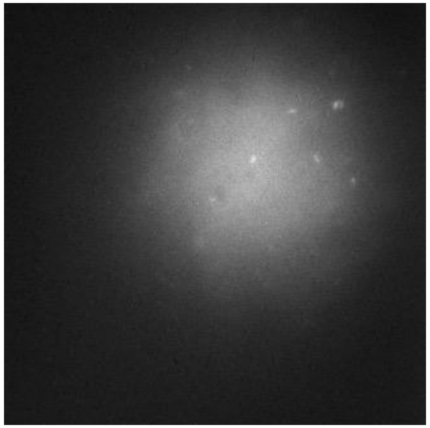
Detection of captured bacteria by fluorescence

Cellulose acetate polymer as an alternative to OSX polymer

Polymer imprinted with glutaraldehyde-inactivated *E. coli-GFP*



Sample: targeted inactivated *E. coli*



Sample: native *E. coli* (non-target)

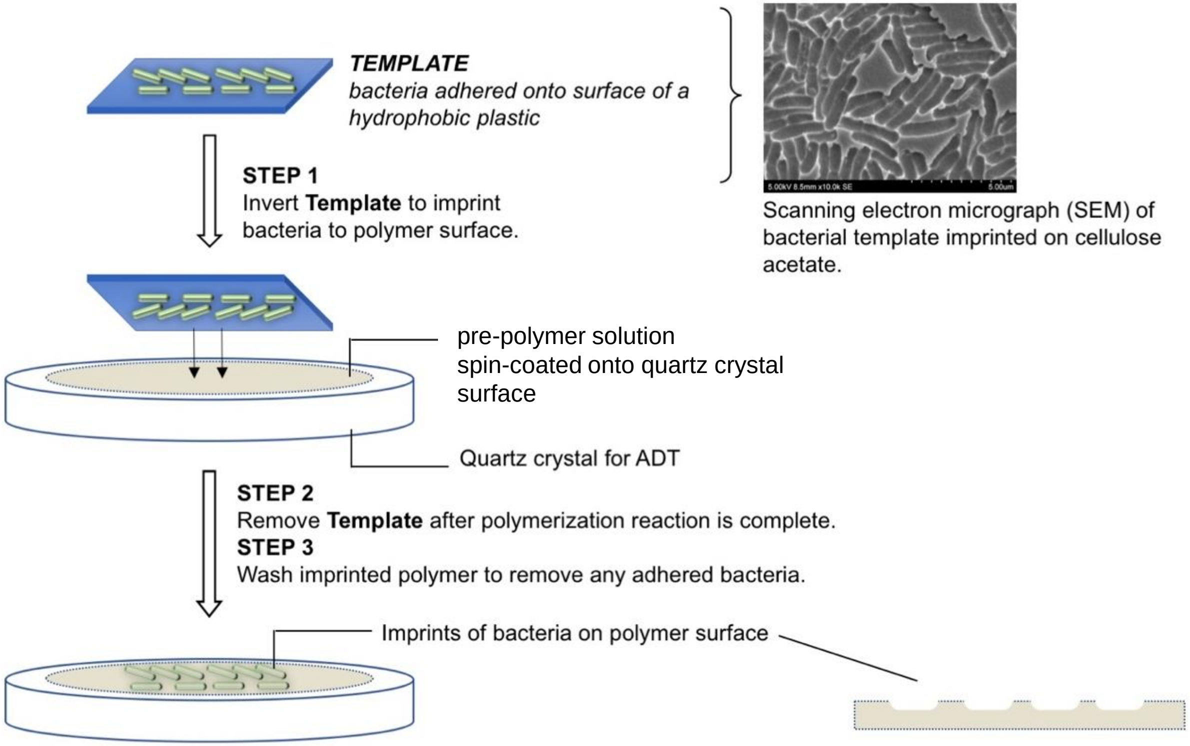
Biosensor	# cell captured	capture time (min)	# cells in sample x 10^7
PDMS / fluorescence	100	30	90
OSX / fluorescence	300	30	90
Cellulose acetate / fluorescence	>300	15	20
OSX / ADT	500	10	1.6

FUTURE DIRECTION:

IMPROVING POLYMER FILM THICKNESS

- **Drop casting** is a challenge in creating uniformly thin polymer films.
- **Spin coating** is a better approach to creating thin polymer films.

Cellulose acetate polymer as an alternative to OSX polymer



ACKNOWLEDGEMENTS

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Alison Mody (HHMI/EXROP Scholar)

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