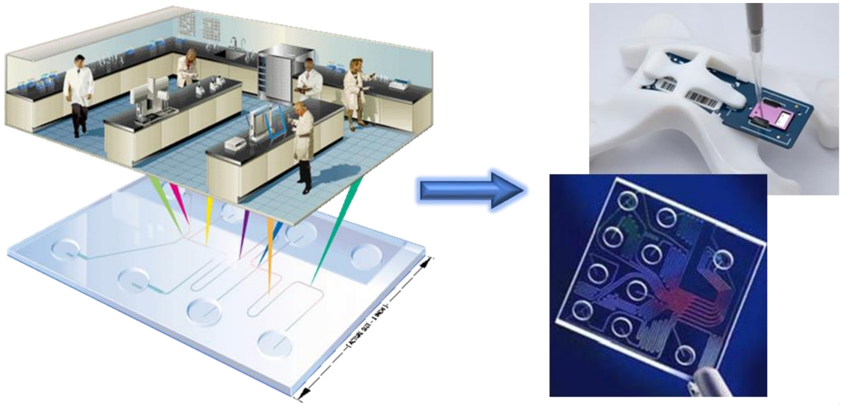
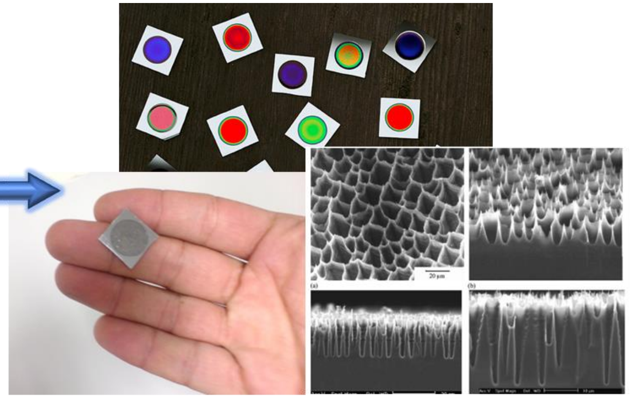
Biosensors and Nanoengineering Lab

Bacterial Contamination and Biomarkers Detection in Milk with Porous Nanosensors

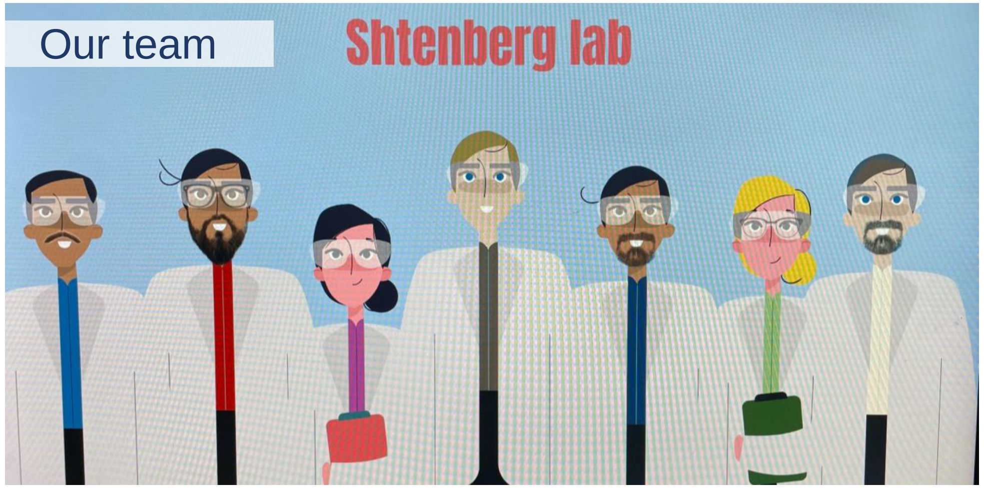


Lab-on-Chip Technology

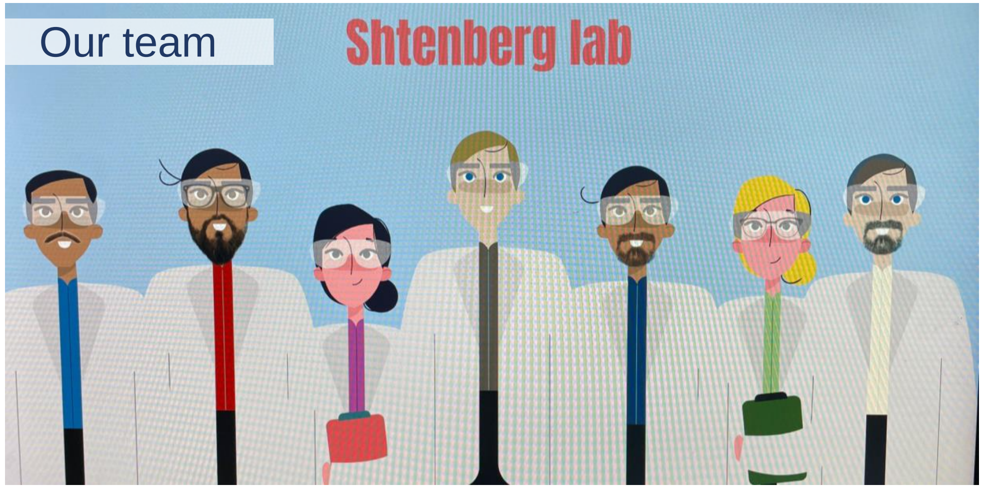


Nano-Porous Si

Giorgi Shtenberg



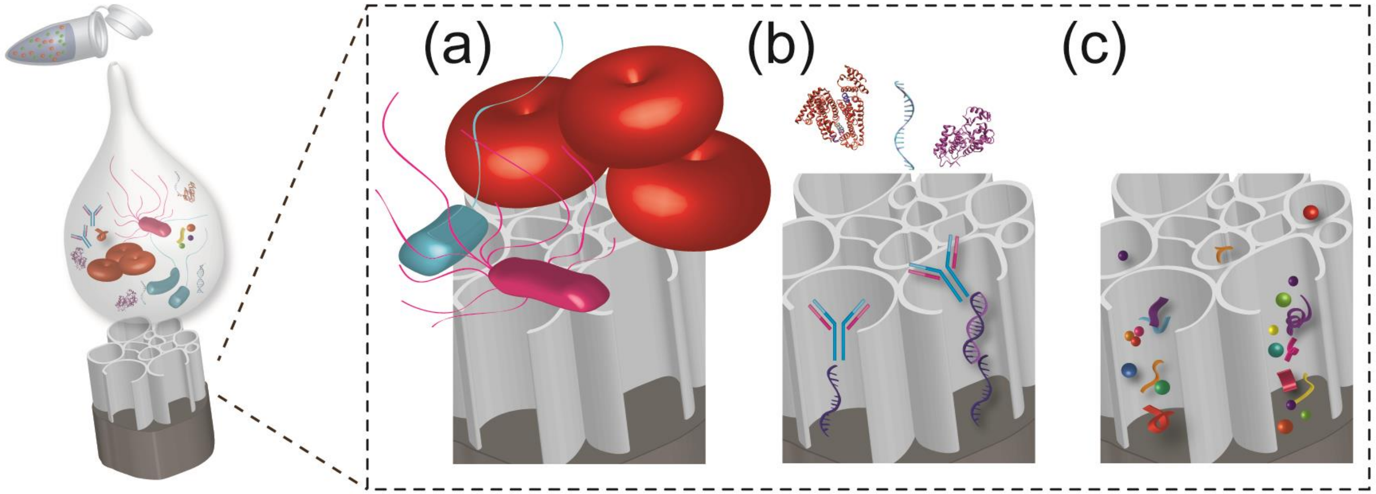
The **Biosensors and Nanoengineering lab** is focused on the development of novel label-free optical biosensors/bioassays based on nanomaterials, nanoparticles and thin-films that will transform from a laboratory-based research into a real on-site “lab-on-chip” platforms for addressing problems in fields of agriculture, animal diagnostics, food safety and environmental monitoring and detection.



This includes:

1. Combined sensing techniques (optical, electrochemical, mass-based transductions) impregnated within a single-device platform (all-in-one) for agricultural applications.
2. Rapid optical bioassays for monitoring environmental pollutants (heavy-metals, pharmaceuticals, pesticides, hormones, toxins).
3. Multifunctional nanoparticles for early diagnosis of animal diseases, field crops quality control and food safety.

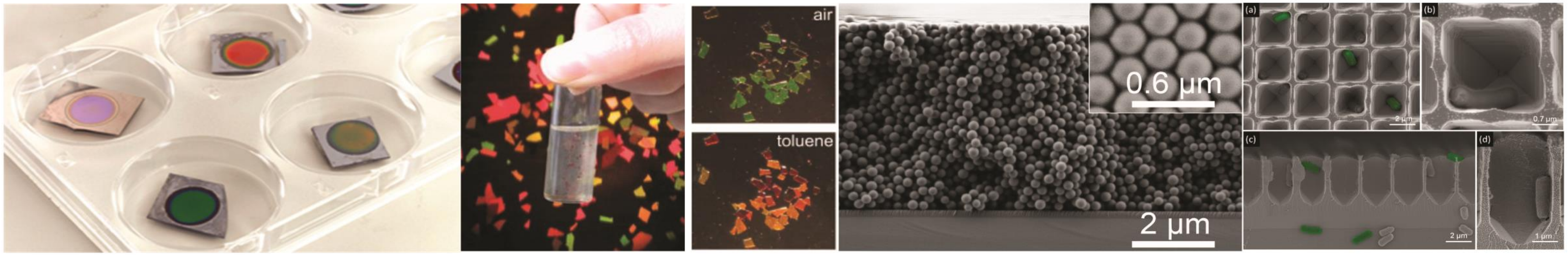
Real-Time & Label-Free Detection



High-throughput
Parallel
Rapid
Label-free
Portable

LOD ~ 10-100nM
Volume ~ 10μL
Time ~ 15 min

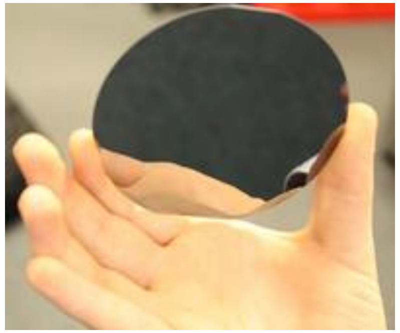
by Nanoengineered Optical Platforms



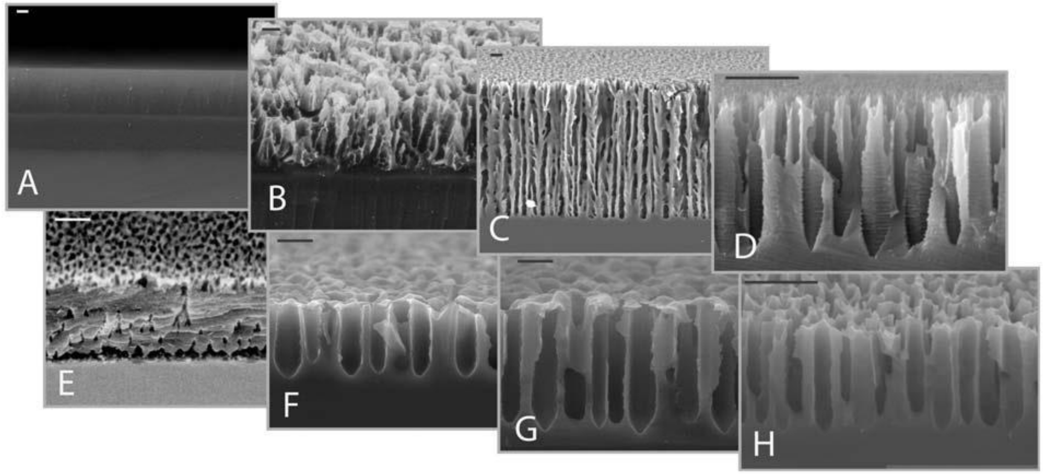
Photonic Crystals | **Optical Nanoparticles** | **Colloidal Crystals** | **Nanoarrays**

Our Chip Technology

Mirror

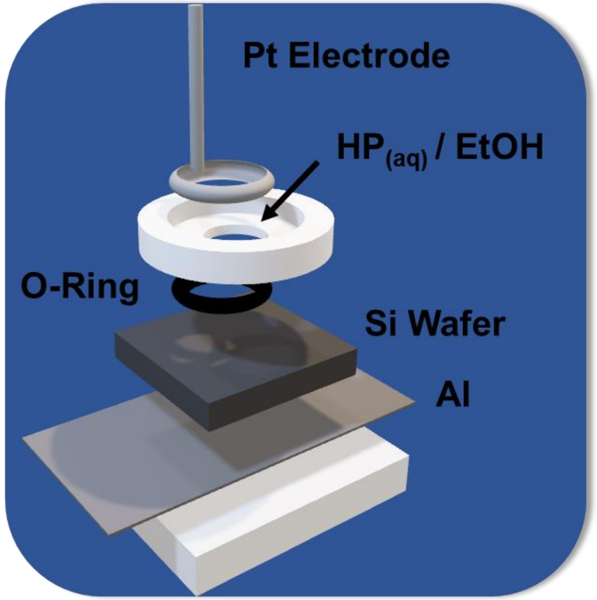


Si Wafer



SEM images of different pore sizes and morphologies of porous Si.

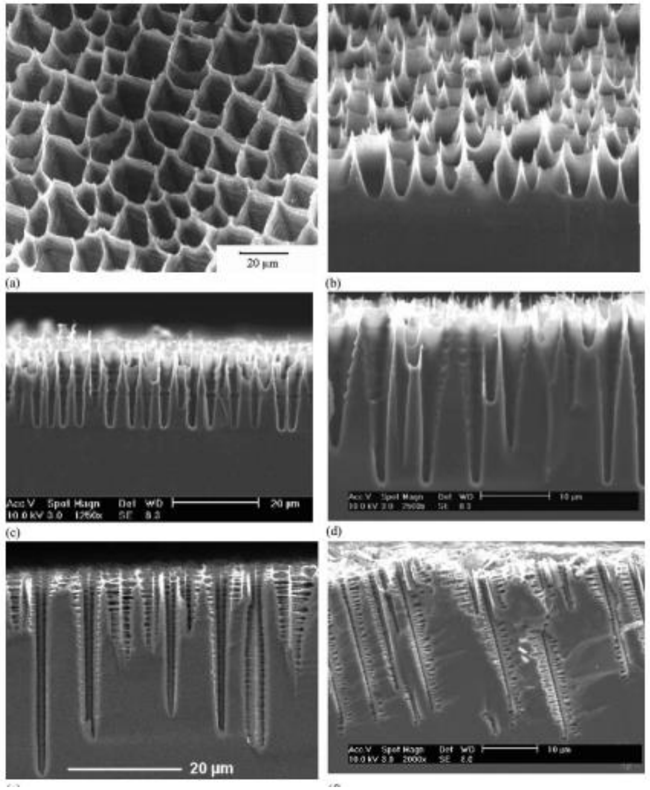
Electrochemical Etching:

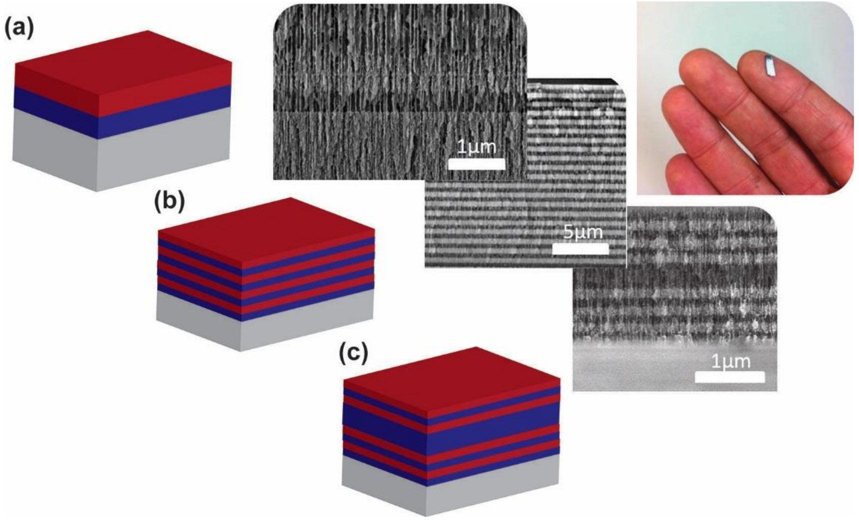


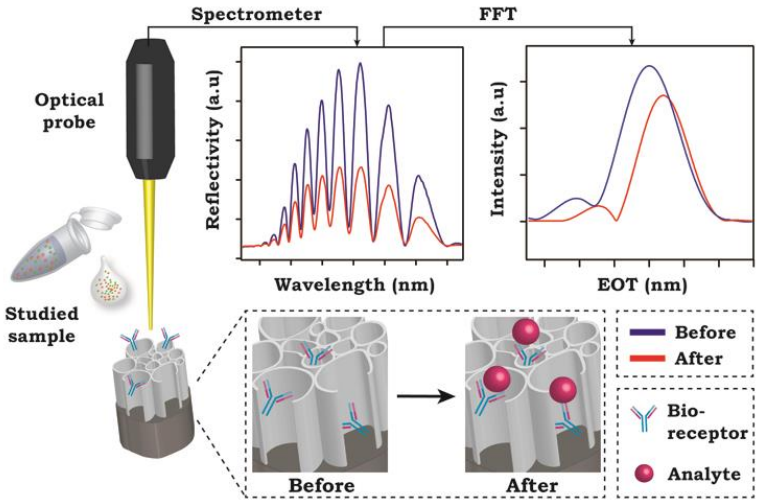
$$2Si + 6\,HF + 2\,h^{+} \longrightarrow \equiv Si{-}H + H_2SiF_6 + 2H^{+} + 1/2\,H_2$$

Porous Si Surface

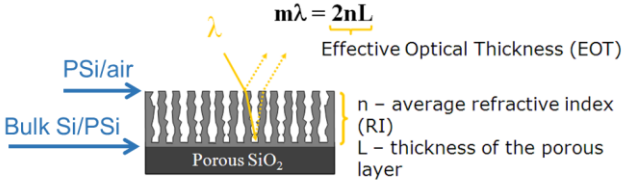
Tuning Physical Properties





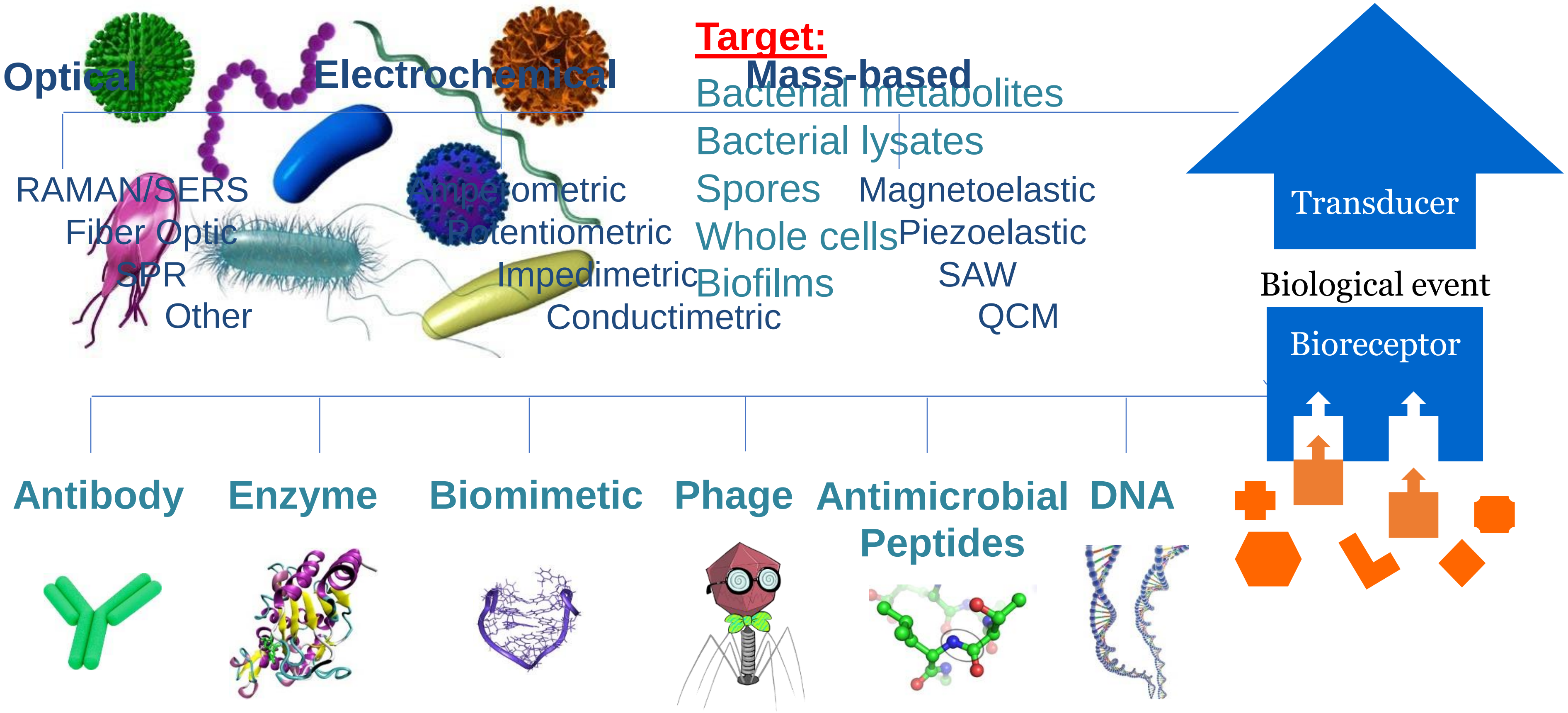


Fabry-Pérot interference:



Biosensors

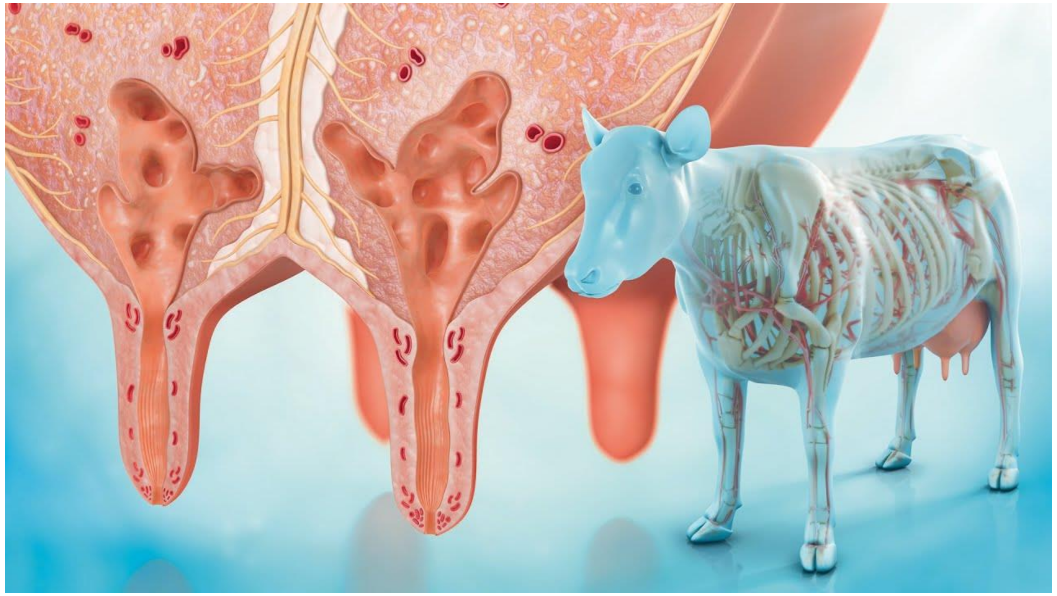
“Analytical device, used for the detection of an analyte, that combines a biological component with a physicochemical detector”



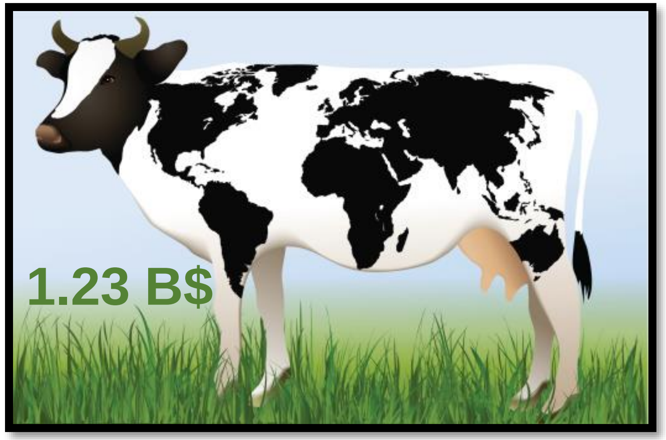
Yao C.Y et al. *World Journal of Gastroenterology* 2014.

Animal Diagnosis

Bovine Mastitis



- Mastitis (mast = breast; itis = inflammation)
- One of the most frequent diseases in dairy cattle
- Results from the inflammation of the mammary gland
- Large adverse effect on farm economics
  - increased treatment costs
  - decreased milk yield
  - escalation of somatic cell counts (SCC) = **$$$/Liter**
  - increased risk of removal from the herd
  - death
- The projected <u>annual</u> losses caused by mastitis are



Bovine Mastitis

- Classification (Healthy, Sub-Clinical, Clinical & Chronic)
  - nature of the causative pathogen and on the age, breed, immunological health and lactation state of the animal

Classification	SCC (cells/mL)
Healthy	≤200,000
Sub-clinical	>200,000
Clinical	>1,000,000

<u>Sub-Clinical</u>

~90-95% of all mastitis cases

Udder/Milk appears normal

Elevated SCC

Lower milk output

Longer duration

→

Bacteria (70%)

Yeast & Molds (~2%)

Unknown (28%)

→

- *Corynebacterium bovis*
- *CNS*
- *S. aureus*
- *Str. dysgalactiae*
- *Str. agalactiae*
- *Str. uberis*
- *Enterococcus spp.*
- *E. coli*
- *Klebsiella spp.*
- *Trueperella pyogenes*

Diagnostic Techniques of Bovine Mastitis

Lab approaches

- Fossomatic SCC
- FACS – differential counts of leucites
- Culture tests
- Coulter Counter
- Rapid tests (kits, ELISA, etc.)

Cow side

- California Mastitis Test (CMT)

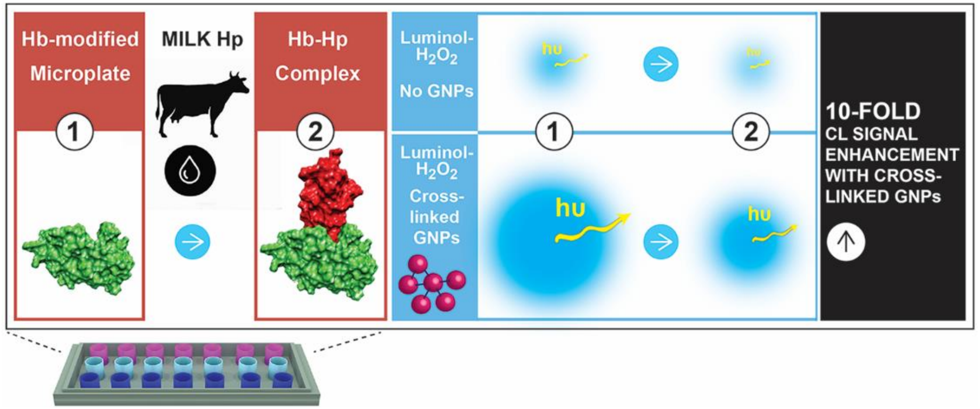
On Farm

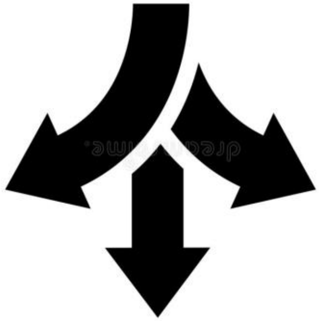
- Electrical conductivity (EC) test
- pH test

Therefore, there is an urgent need to develop rapid, non-destructive, accurate, cost effective, simple and portable method to analyze new cases of BM.

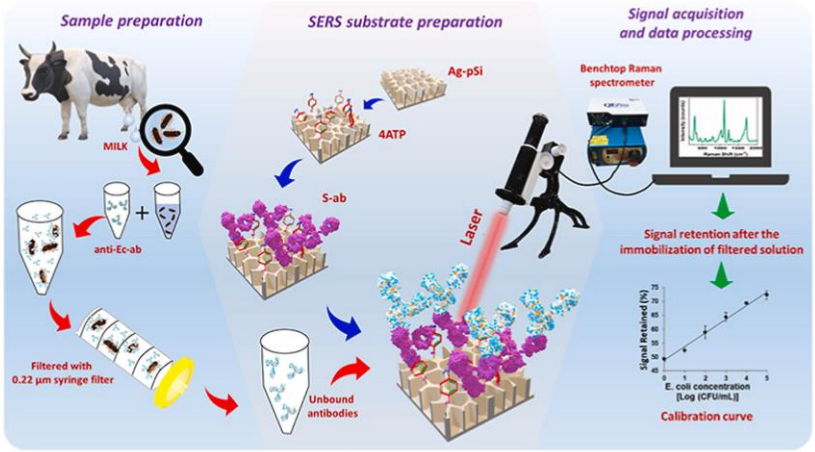
Bacteria Detection

Chemiluminescence



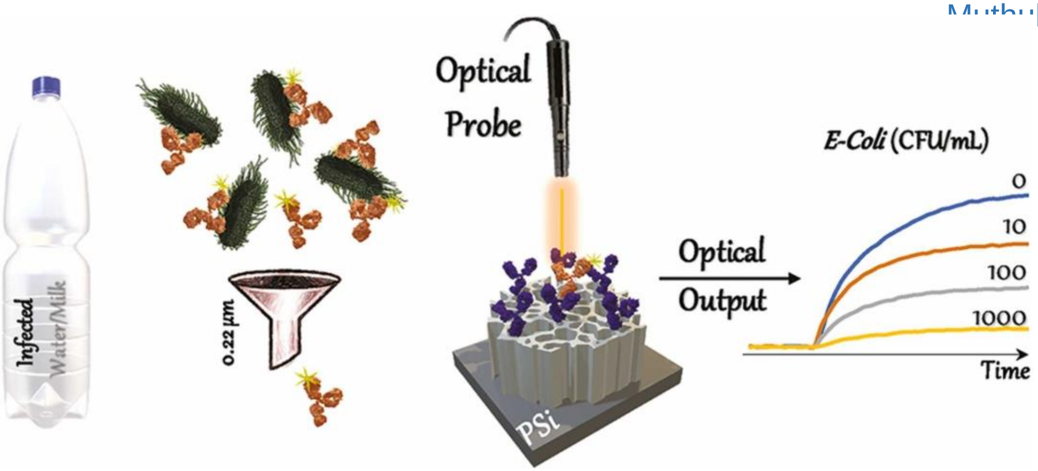


Raman



Muthukumar D. et al. *Talanta* 2023

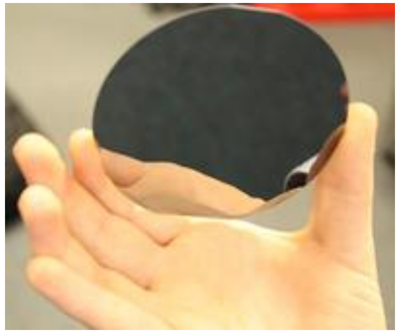
Reflectance



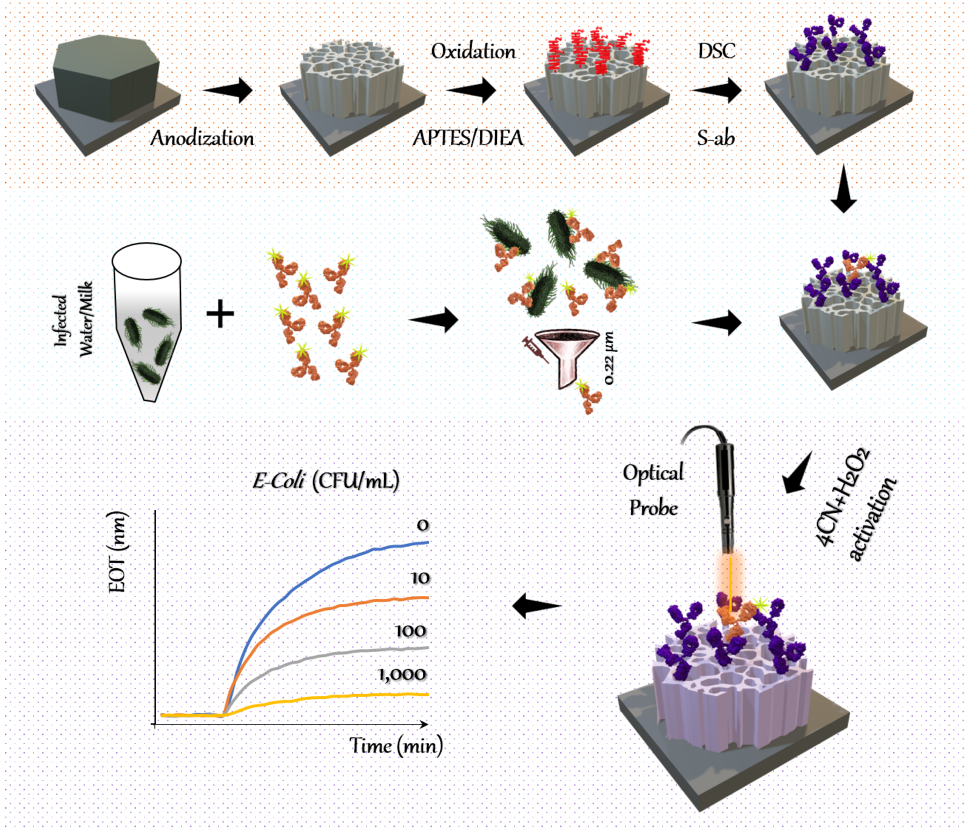
Muthukumar D. et al. *Sensors and Actuators B.* 2023

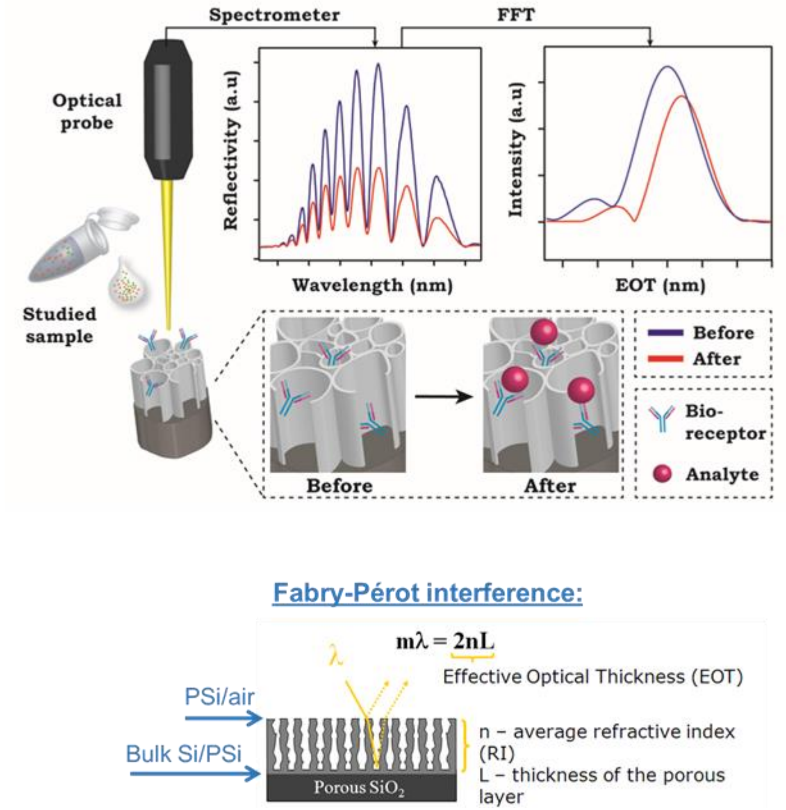
E. coli Detection by RIFTS

(Reflective interferometric Fourier transform spectroscopy)



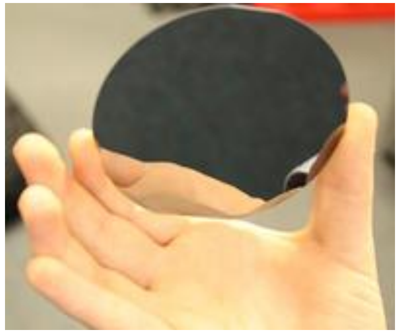
Si Wafer



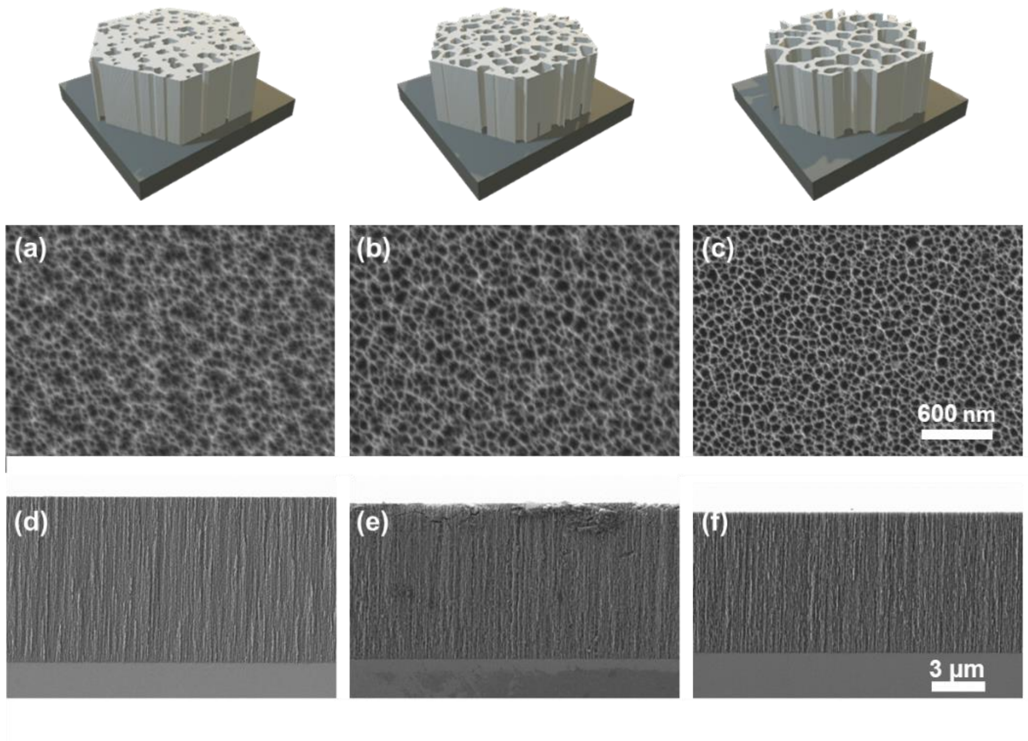


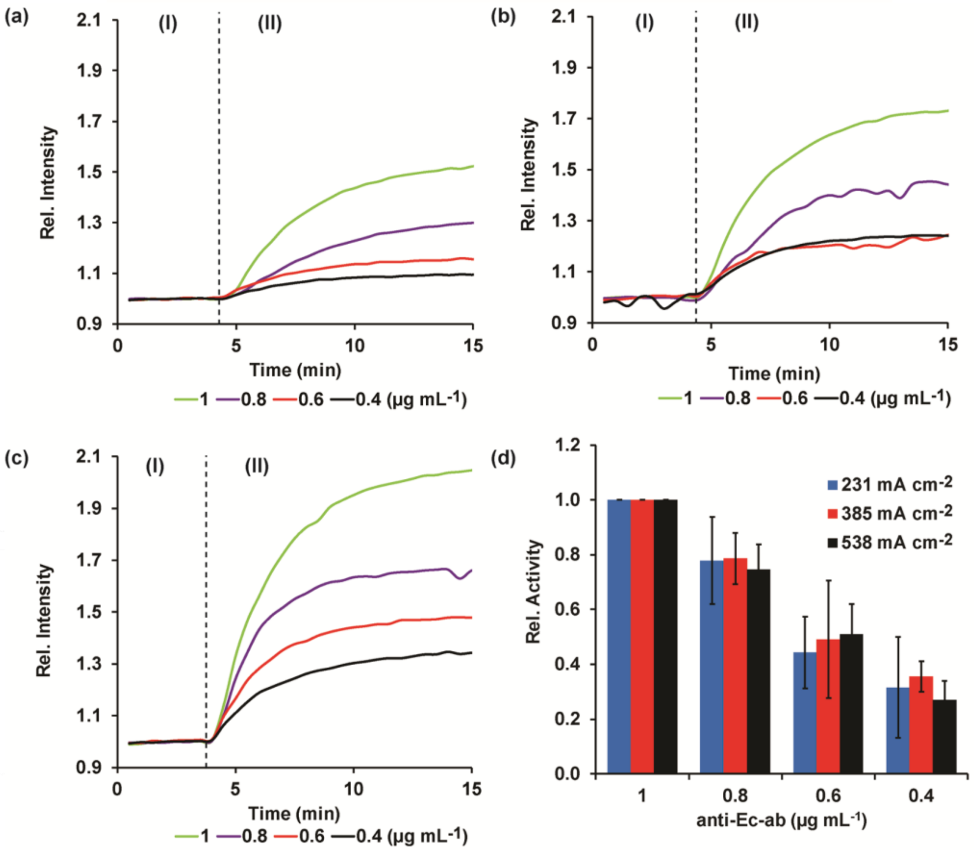
E. coli Detection by RIFTS

(Reflective interferometric Fourier transform spectroscopy)



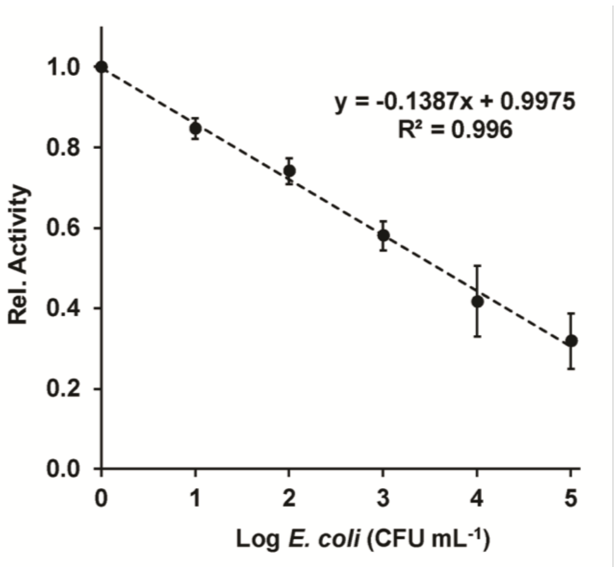
Si Wafer



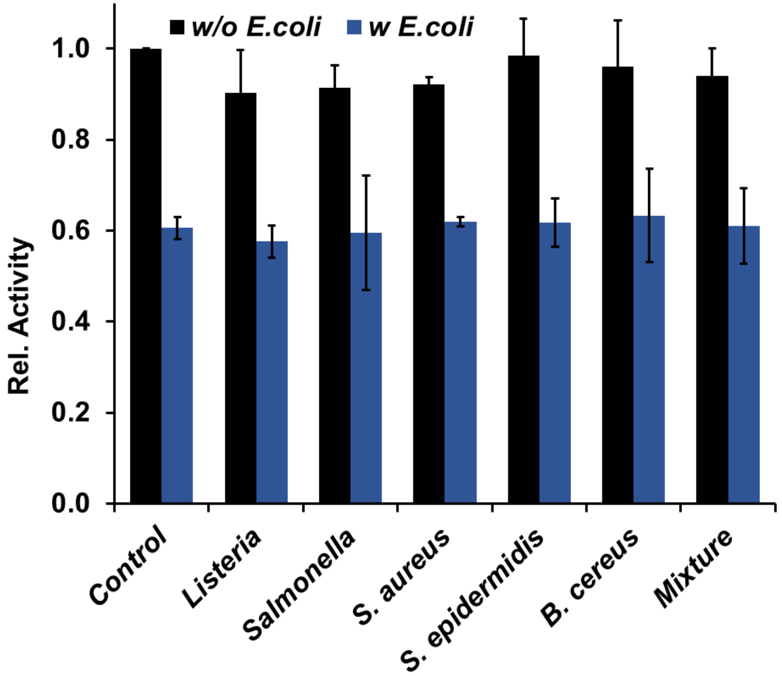


E. coli Detection by RIFTS

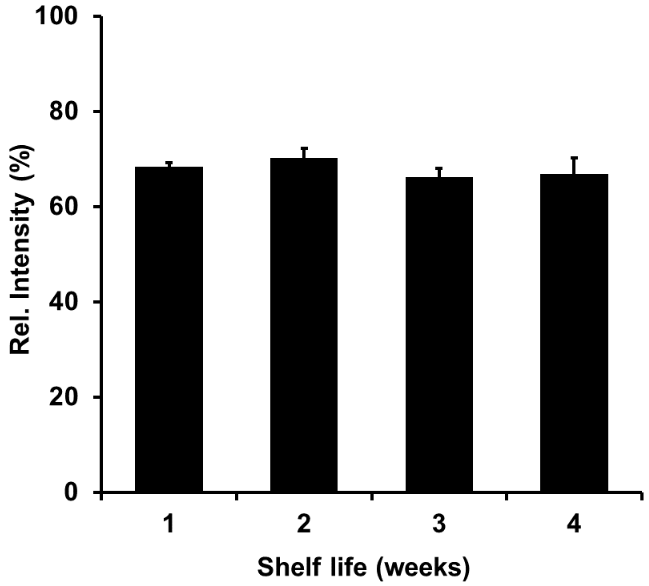
LoD 2 CFU/mL, 80 min



Specificity & Selectivity



Shelf-life assessment



E. coli Detection by RIFTS

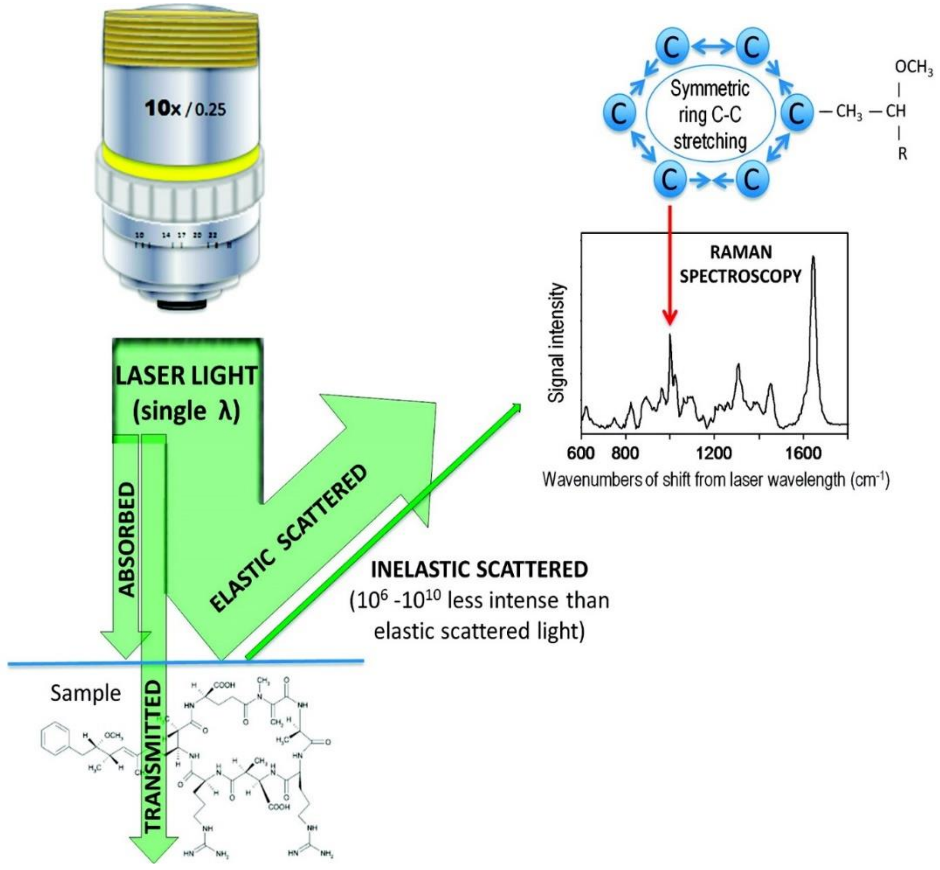
Table 1. Real samples assessment through RIFTS and plate counting methods.

Sample	Spiked conc. (CFU mL^{-1})	Relative intensity	Log (CFU mL^{-1})		Recovery (%)	RSD (%)
			RIFTS detection	Plate counting		
Ground water	50	0.74±0.04	1.89±0.28	1.88±0.16	100	12
	500	0.59±0.05	2.96±0.35	2.79±0.40	107	7
Irrigation water	50	0.69±0.02	2.25±0.14	2.42±0.45	93	14
	500	0.59±0.04	2.90±0.31	2.94±0.06	99	9
River water	50	0.73±0.05	1.96±0.34	1.95±0.22	100	13
	500	0.65±0.02	2.54±0.18	2.70±0.07	94	8
Raw milk	50	0.60±0.01	2.89±0.09	3.11±0.33	93	11
	500	0.59±0.15	2.91±0.20	2.82±0.08	103	7
Pasteurized milk	50	0.65±0.07	2.49±0.48	2.43±0.45	103	2
	500	0.52±0.13	3.44±0.93	3.75±1.07	92	4

Data are presented as mean ± SD ($n \geq 3$).

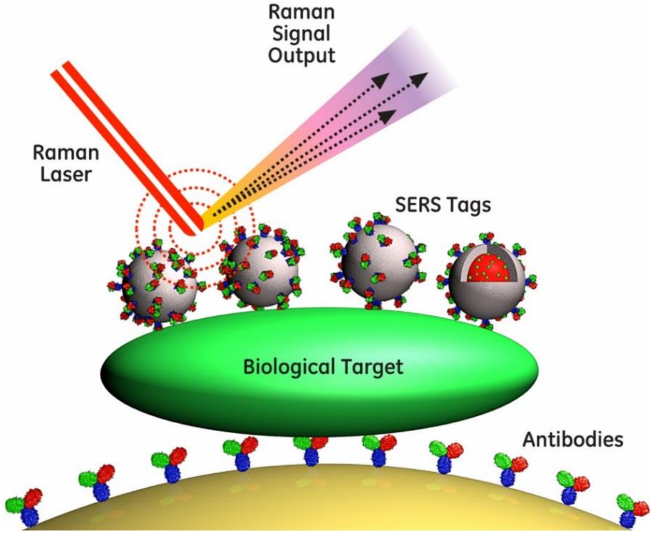
Surface Enhanced Raman Spectroscopy (SERS)

Alternative approach

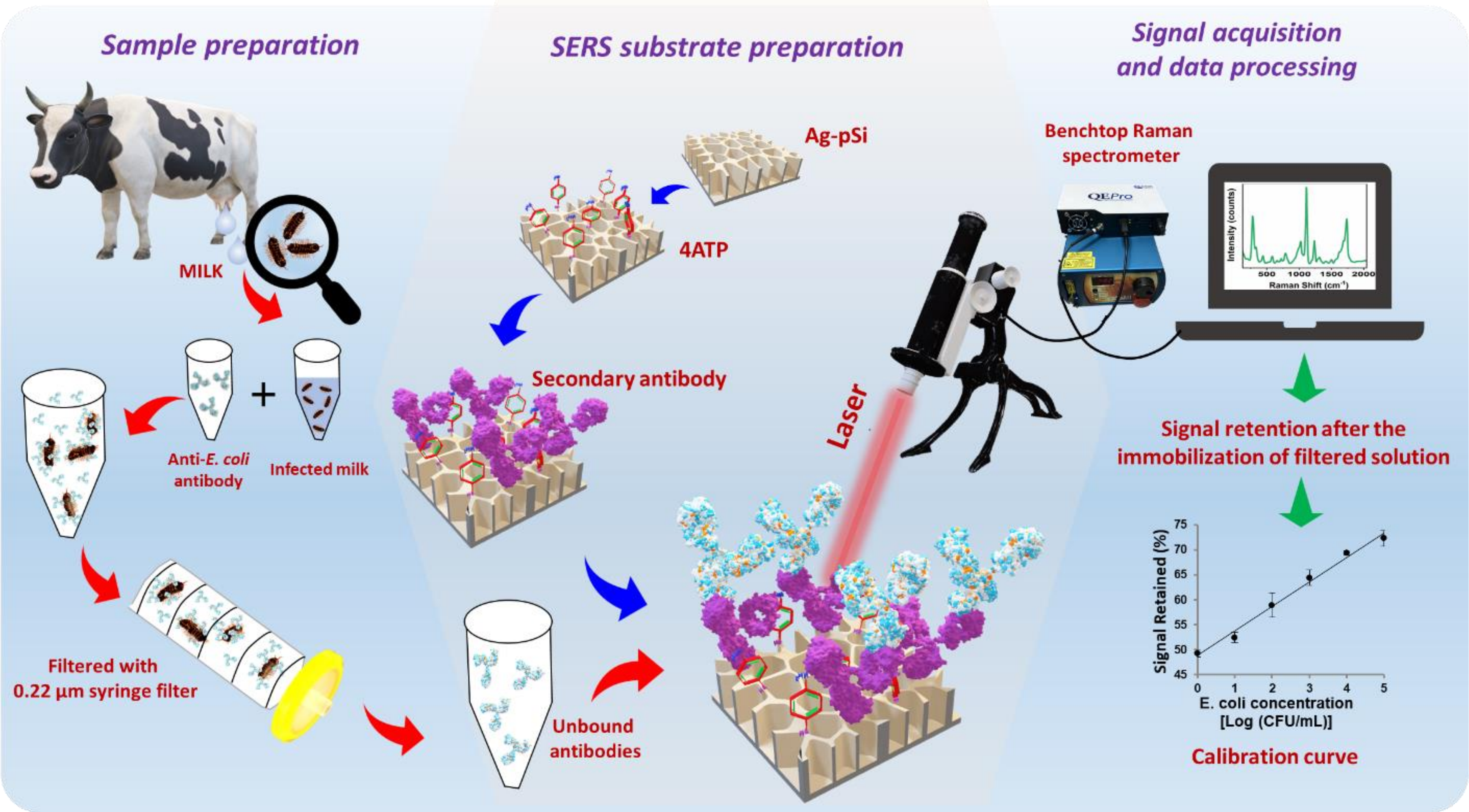


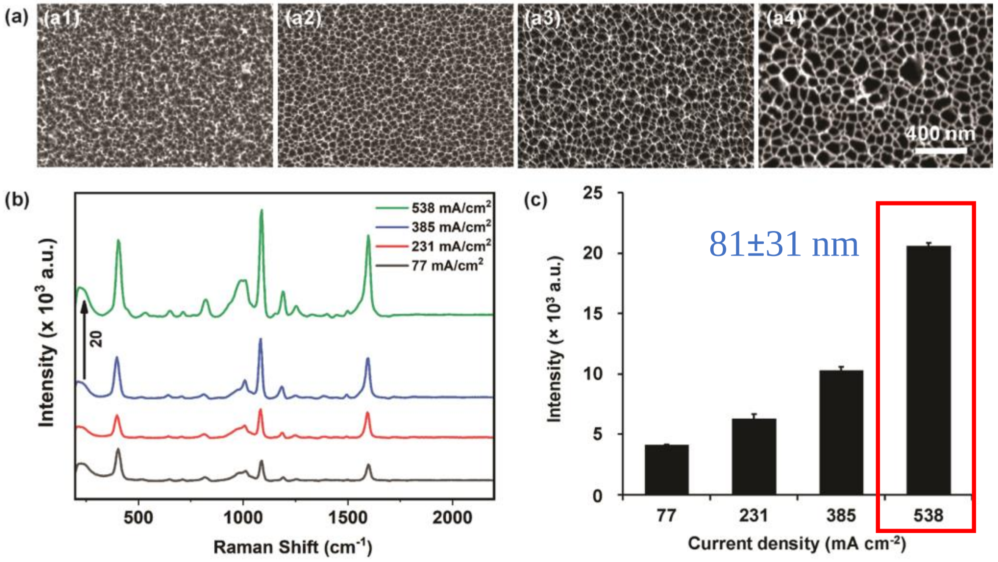
Amplification:

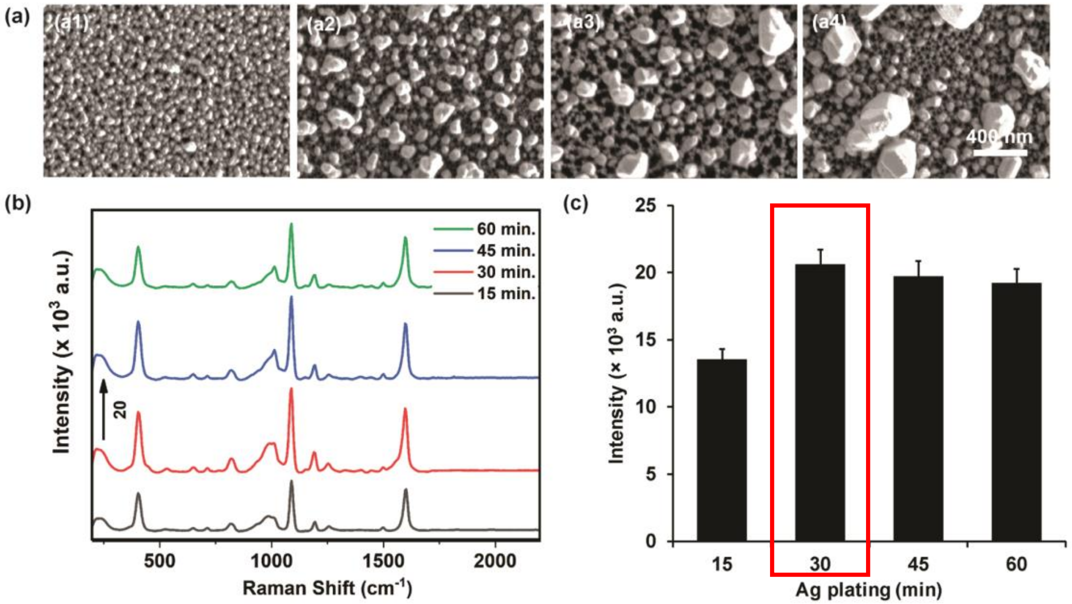
Ag NPs shape & content

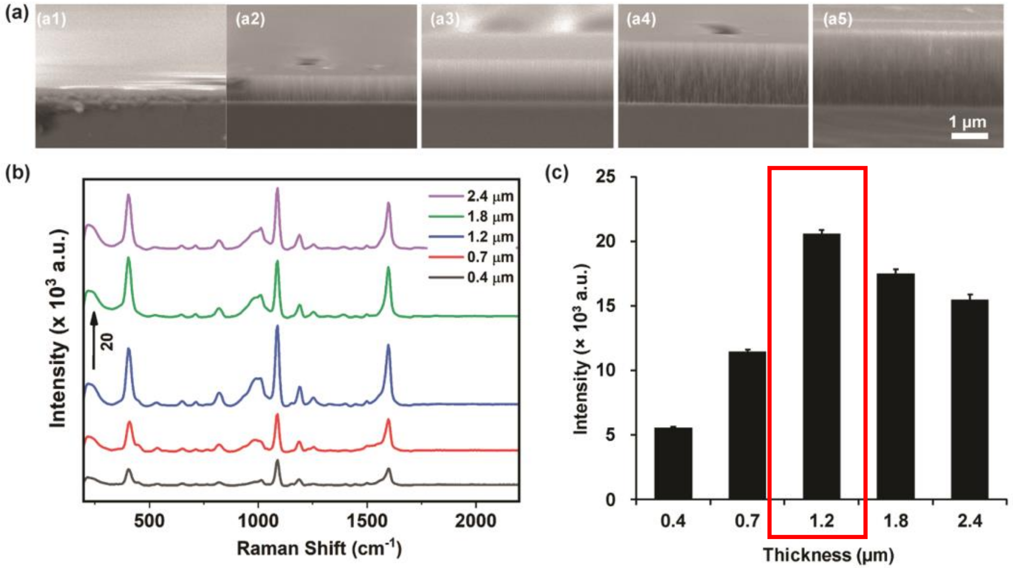


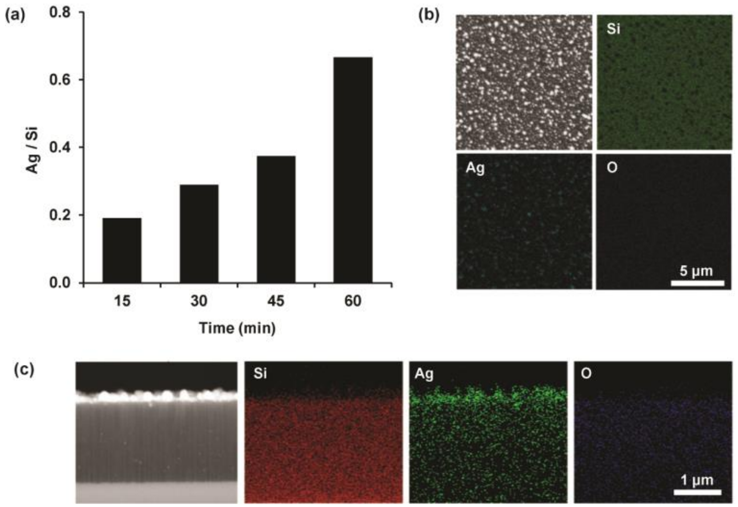
Surface Enhanced Raman Spectroscopy (SERS)

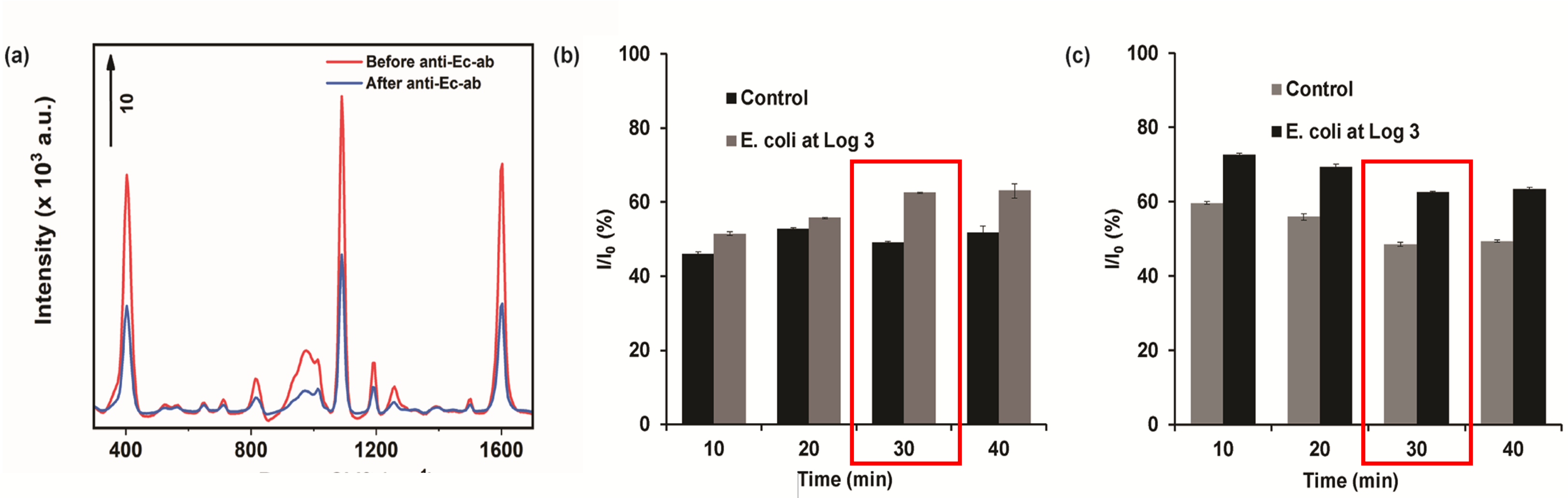


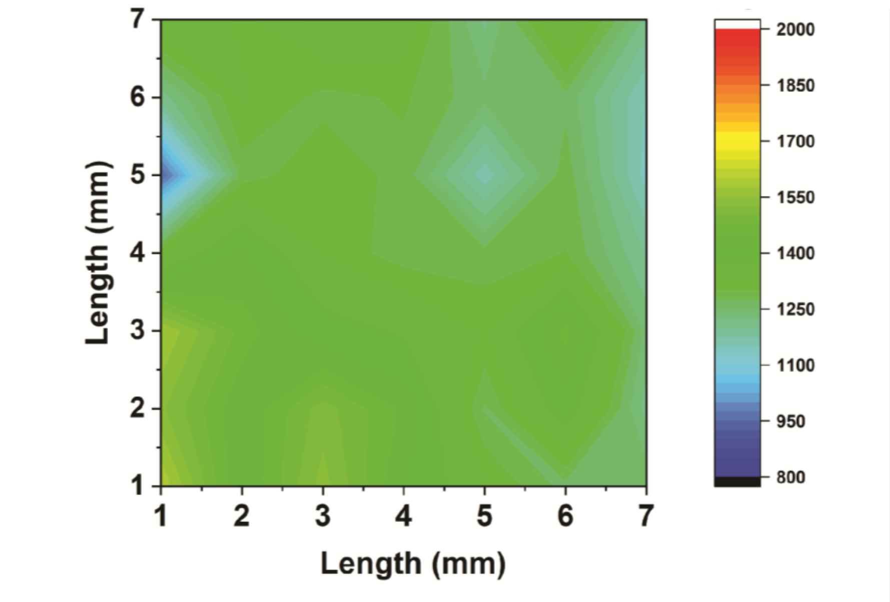

(a) (a1) (a2) (a3) (a4)
400 nm
(b)
538 mA/cm²
385 mA/cm²
231 mA/cm²
77 mA/cm²
Intensity (x 10³ a.u.)
Raman Shift (cm⁻¹)
(c)
81±31 nm
Intensity (× 10³ a.u.)
77 231 385 538
Current density (mA cm⁻²)

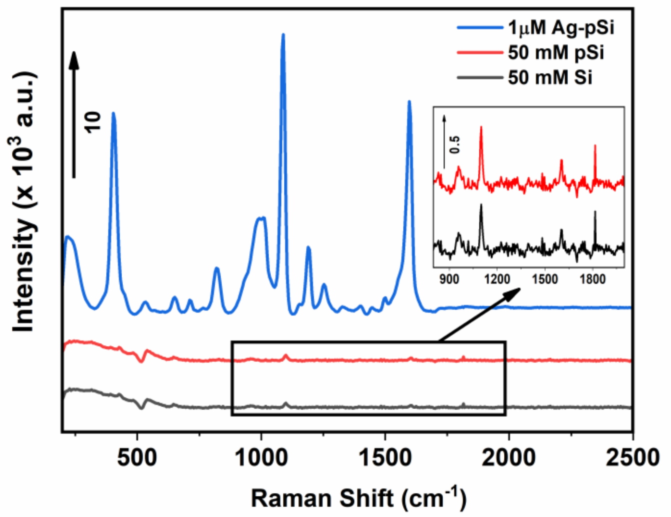

(a) (a1) (a2) (a3) (a4)
400 nm
(b)
60 min.
45 min.
30 min.
15 min.
Intensity (x 10³ a.u.)
Raman Shift (cm⁻¹)
(c)
Intensity (× 10³ a.u.)
15 30 45 60
Ag plating (min)


(a) (a1) (a2) (a3) (a4) (a5)
1 µm
(b)
2.4 µm
1.8 µm
1.2 µm
0.7 µm
0.4 µm
Intensity (x 10³ a.u.)
Raman Shift (cm⁻¹)
(c)
Intensity (× 10³ a.u.)
0.4 0.7 1.2 1.8 2.4
Thickness (µm)


(a)
Ag / Si
15 30 45 60
Time (min)
(b)
Si
Ag
O
5 µm
(c)
Si
Ag
O
1 µm

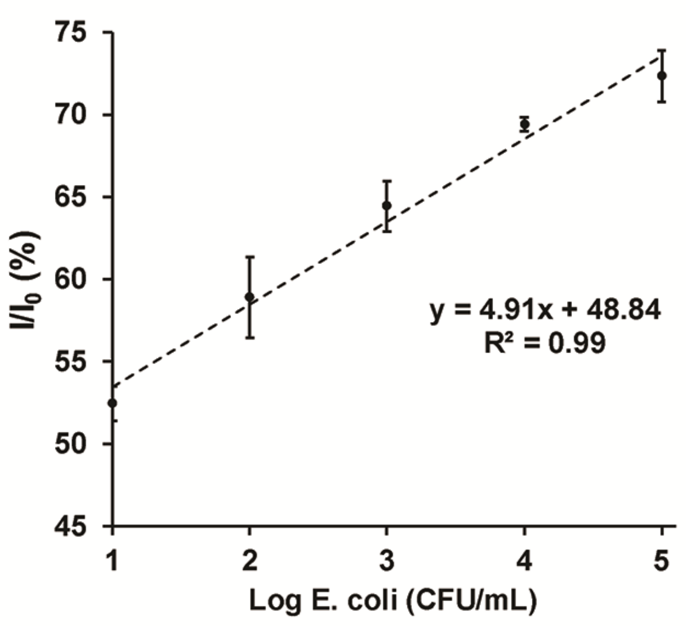




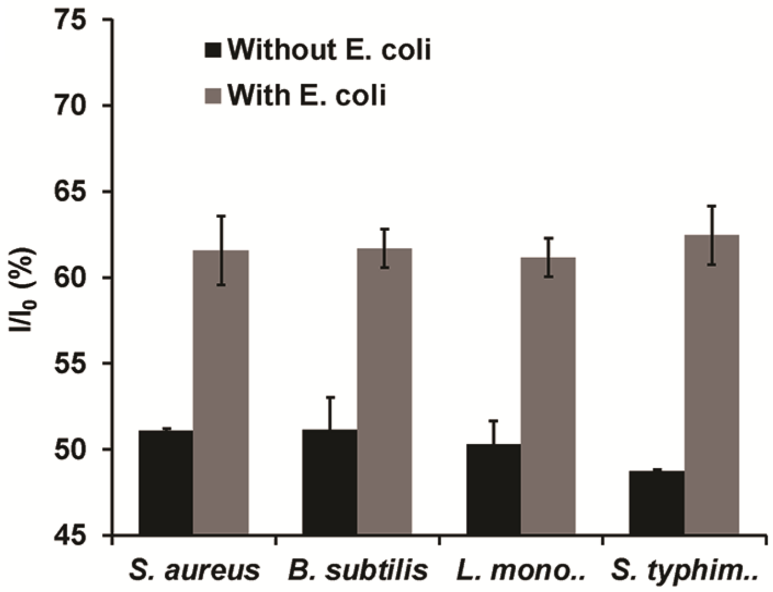


$EF_{4ATP/Ag\text{-}pSi} \sim 5.6\times10^{7}$

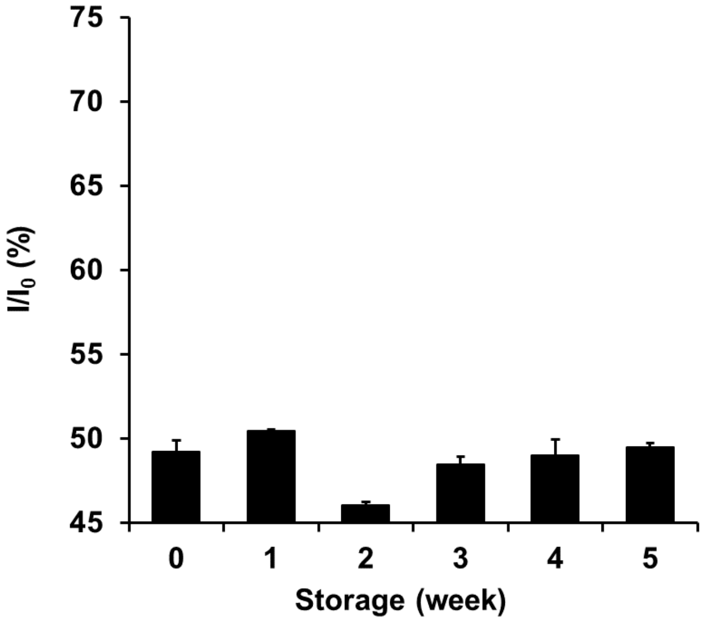
LoD 3 CFU/mL, 75 min



Specificity & Selectivity

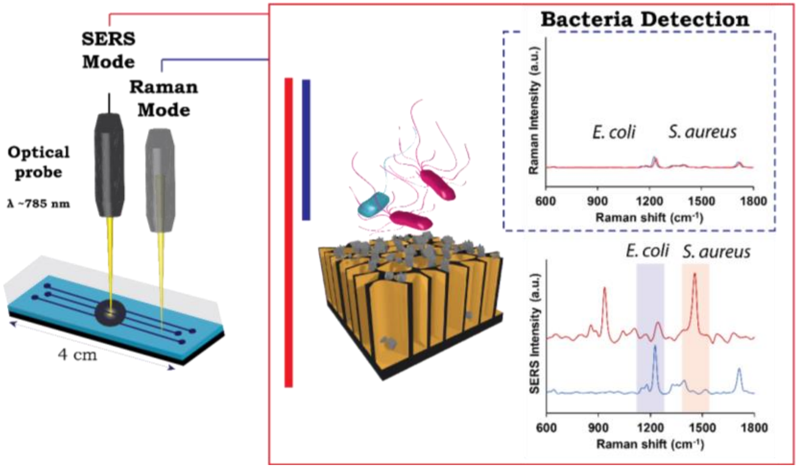


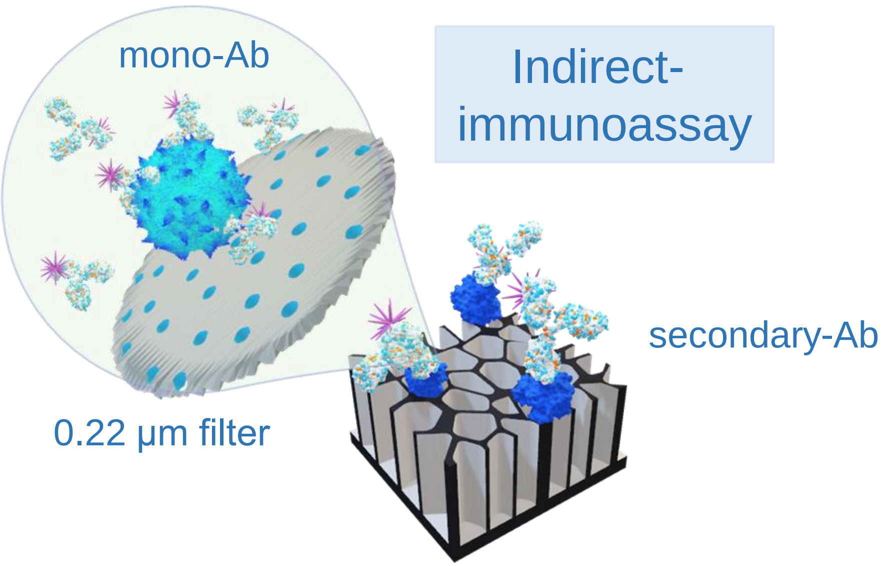
Shelf-life assessment



L. monocytogenes and *S. typhimurium* were generously received from the Department of Clinical Bacteriology and Mycology at the Kimron Veterinary Institute, managed by **Dr. Shlomo E. Blum**.

Bacteria Detection by SERS

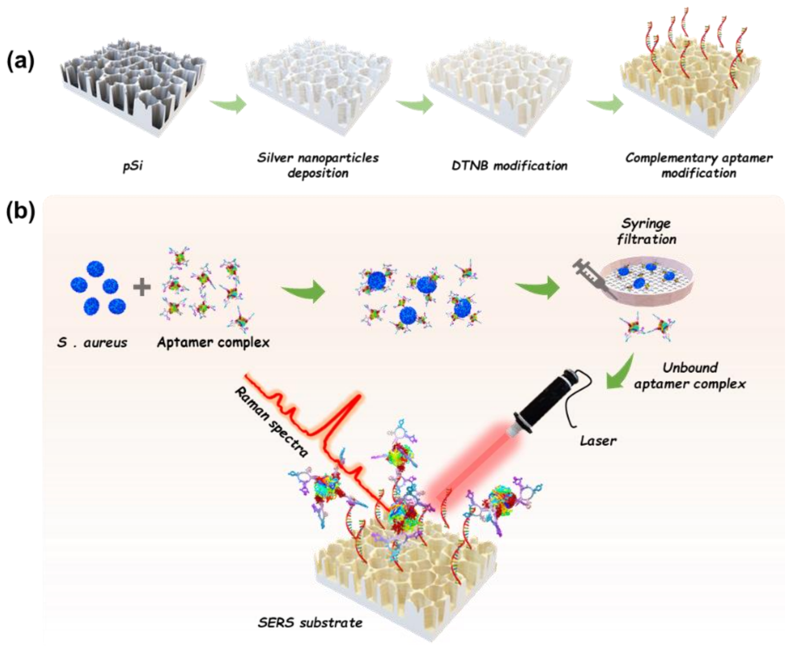




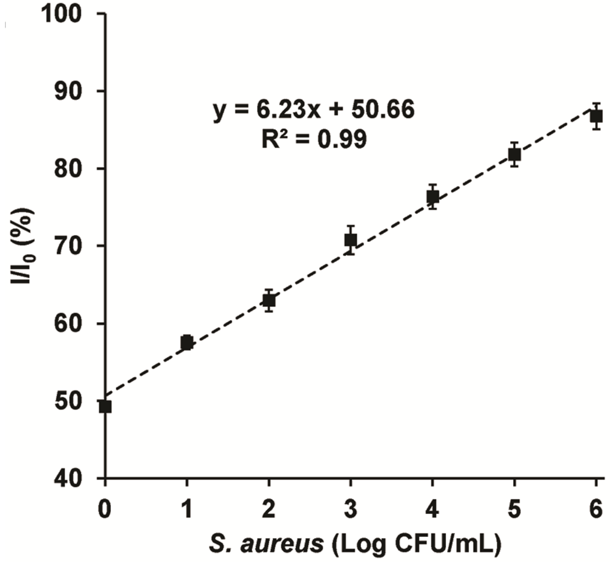
Milk sample	Status	Incubation (hr)	Plate (CFU/mL)	SERS (CFU/mL)	Recovery (%)
Commercial Bovine	Spiked E. coli	0	150	120 ± 14	80
		1	500	391 ± 187	78
		2	1,600	1,693 ± 497	106
		3	16,000	13,369 ± 1,962	84
Whole Bovine	Spiked E. coli	0	80	65 ± 4	81
		1	160	131 ± 15	82
		2	820	845 ± 168	103
		3	2,000	1,958 ± 398	98
Whole Sheep	Spiked E. coli	0	60	58 ± 20	97
		1	170	138 ± 24	81
		2	1,500	1,439 ± 434	96
		3	18,000	18,538 ± 5,712	103
Whole Goat	Spiked E. coli	0	80	69 ± 20	86
		1	180	152 ± 27	84
		2	1,100	950 ± 50	86
		3	10,000	11,481 ± 3,153	115

@ t = 0 hr, 100 CFU/mL

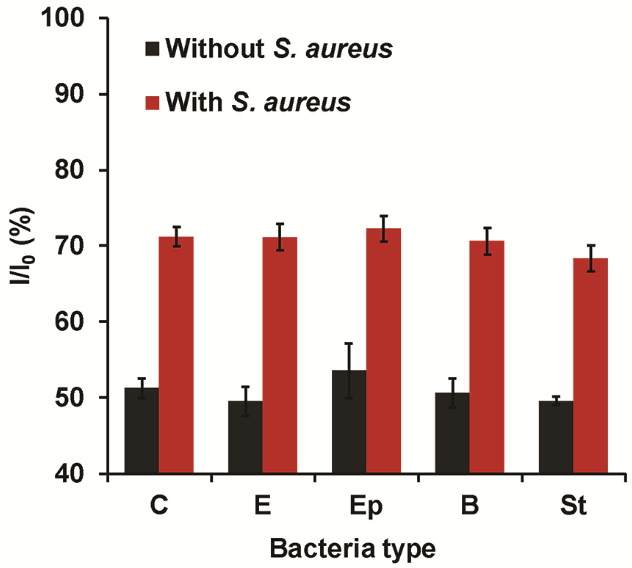
***Milk obtained by Mr. Joseph Lepar



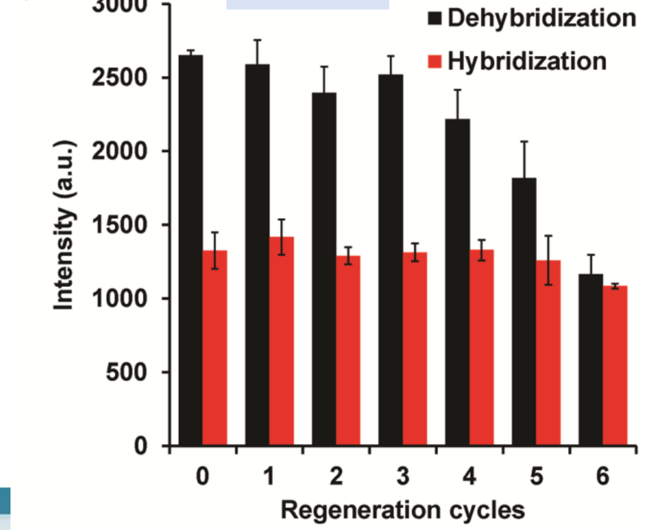
LoD 2 CFU/mL, 75 min



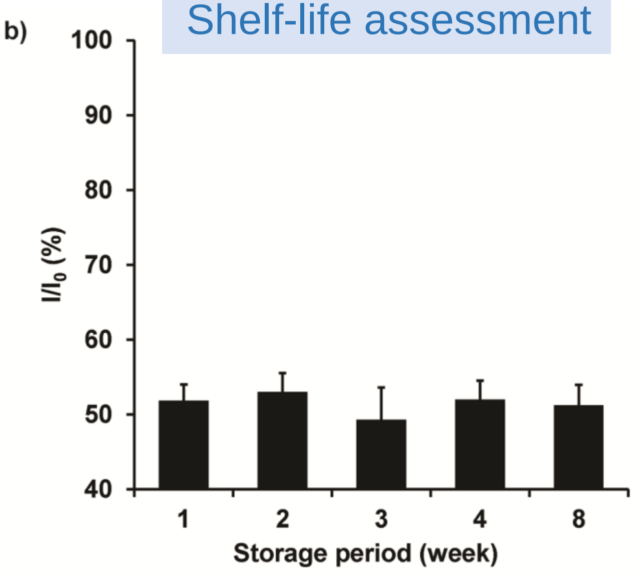
Specificity & Selectivity



Reuse



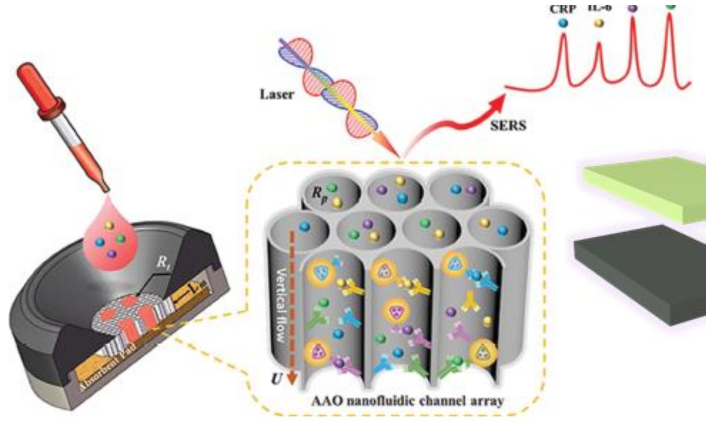
Shelf-life assessment

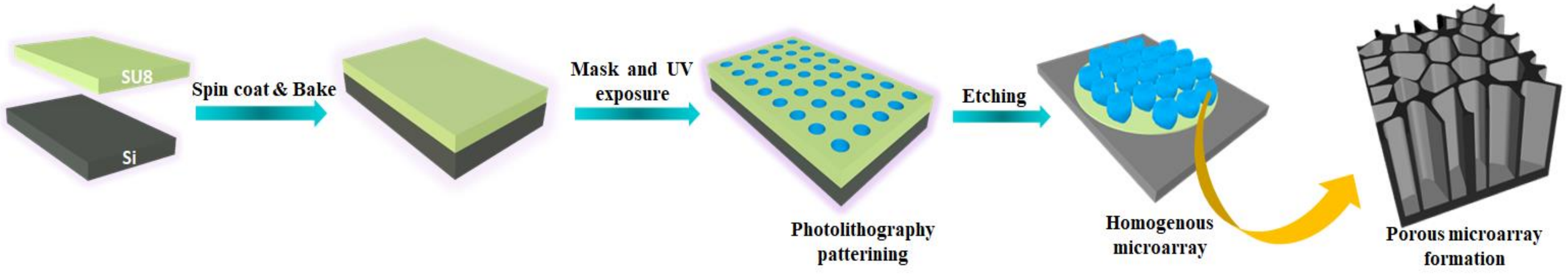


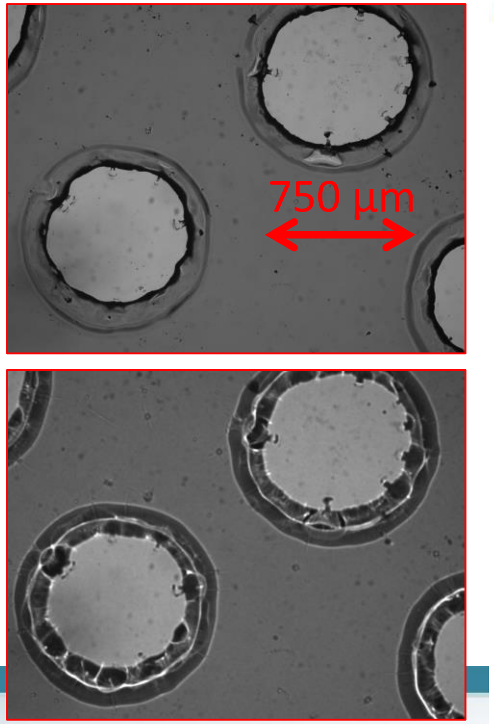
Bacteria Detection by SERS

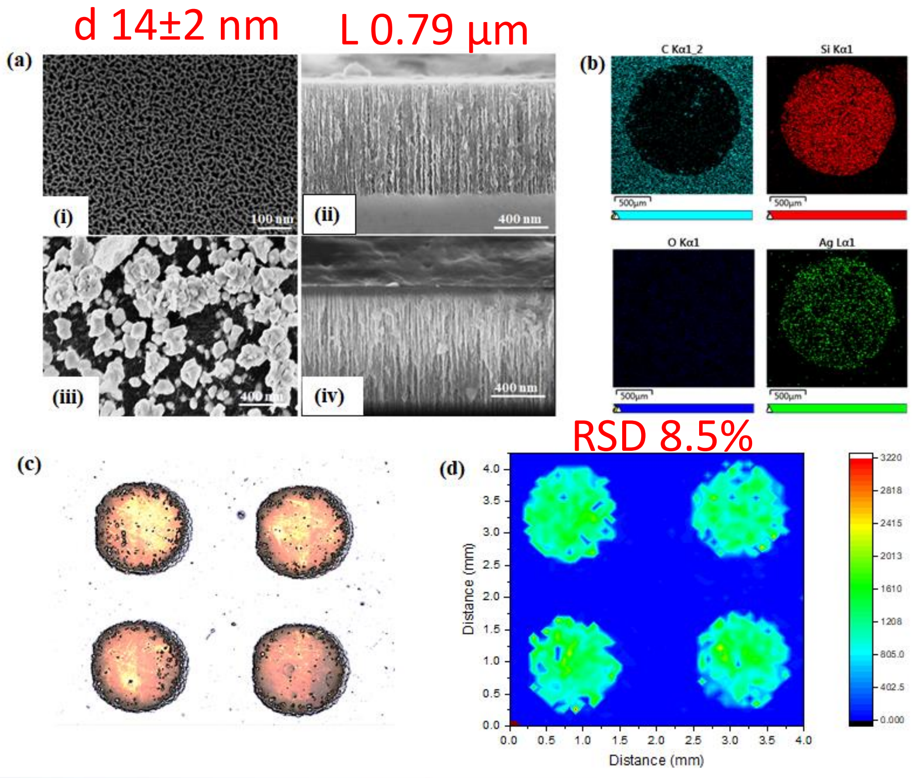
Sample	Spiked (Log CFU mL^{-1})	I/I_0 (%)	SERS (Log CFU mL^{-1})	Plate (Log CFU mL^{-1})	Recovery (%)
Fish	0	54.02±1.86	0.54±0.30	0.00±0.00	-
	2	63.01±1.26	1.98±0.20	2.14±0.08	93
	3	70.59±2.06	3.20±0.33	3.05±0.03	105
	4	78.28±1.95	4.43±0.31	4.18±0.01	106
Pasteurized Milk	0	52.76±1.84	0.34±0.29	0.00±0.00	-
	2	63.57±1.57	2.07±0.25	2.20±0.04	94
	3	72.90±0.90	3.57±0.14	3.24±0.07	110
	4	74.83±2.23	3.88±0.36	4.10±0.03	95
Ground Water	0	52.50±1.32	0.30±0.21	0.00±0.00	-
	2	64.08±2.13	2.15±0.34	2.31±0.10	93
	3	69.48±2.87	3.02±0.46	3.24±0.03	93
	4	77.31±2.03	4.13±0.34	4.13±0.01	100
Tahini	0	52.50±0.67	0.25±0.11	0.00±0.00	-
	2	64.35±1.38	2.20±0.22	1.99±0.07	110
	3	69.48±3.16	3.02±0.51	3.04±0.08	99
	4	78.78±1.88	4.51±0.30	4.11±0.07	110

Multiplex SERS - Microarray

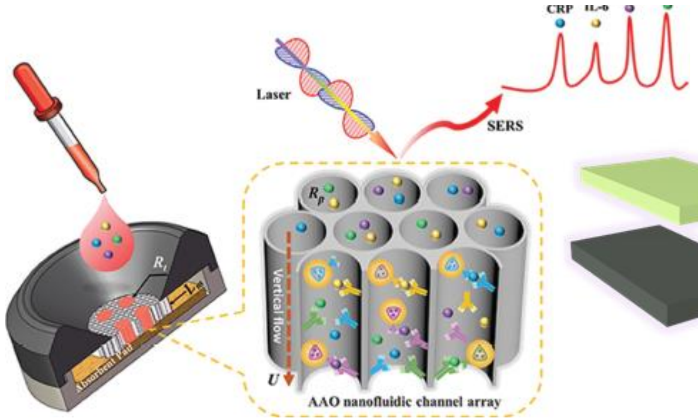


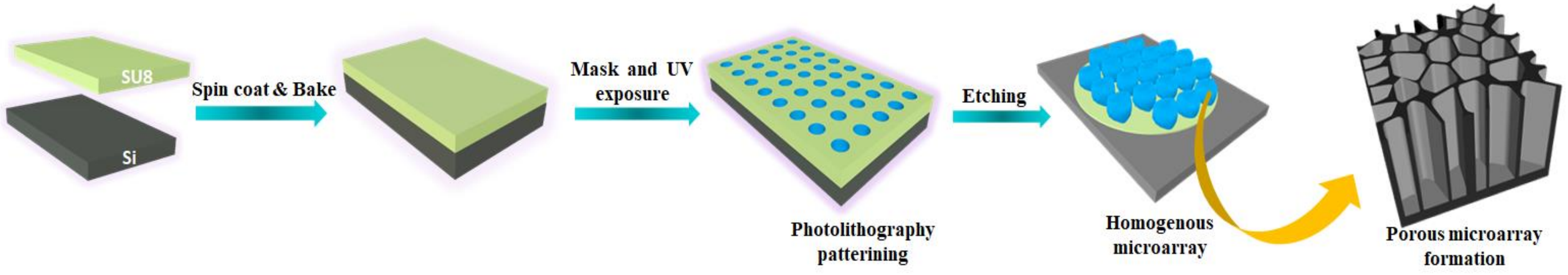


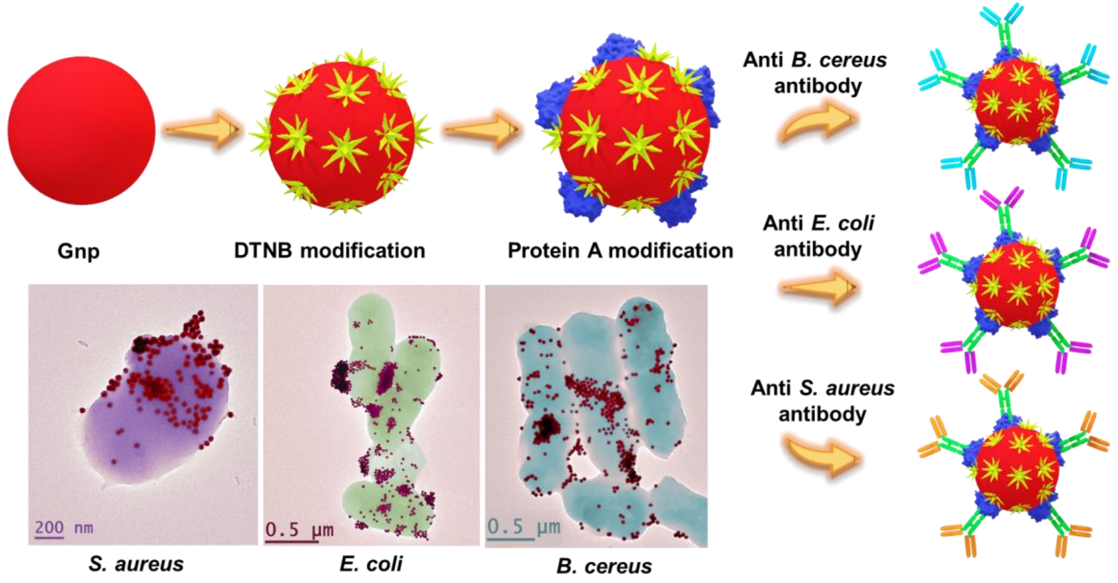


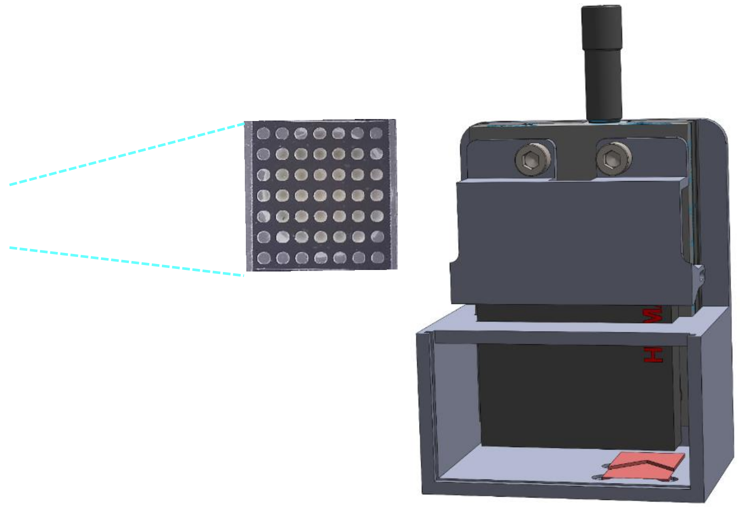


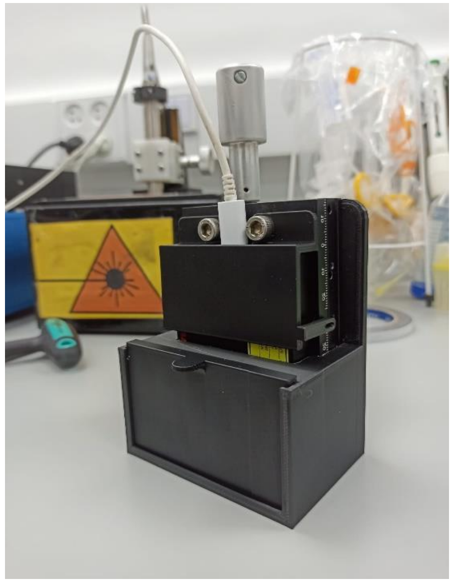
Multiplex SERS - Microarray



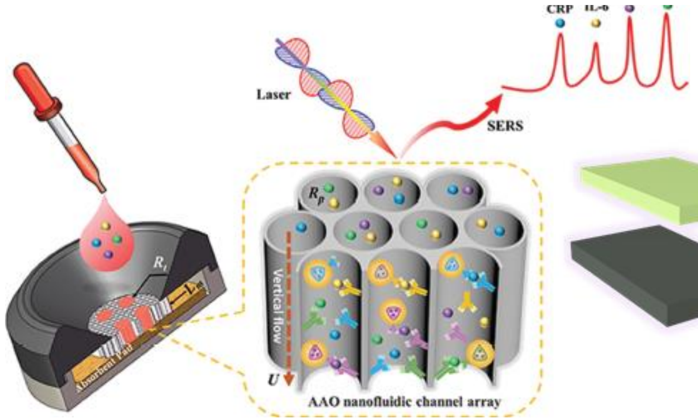


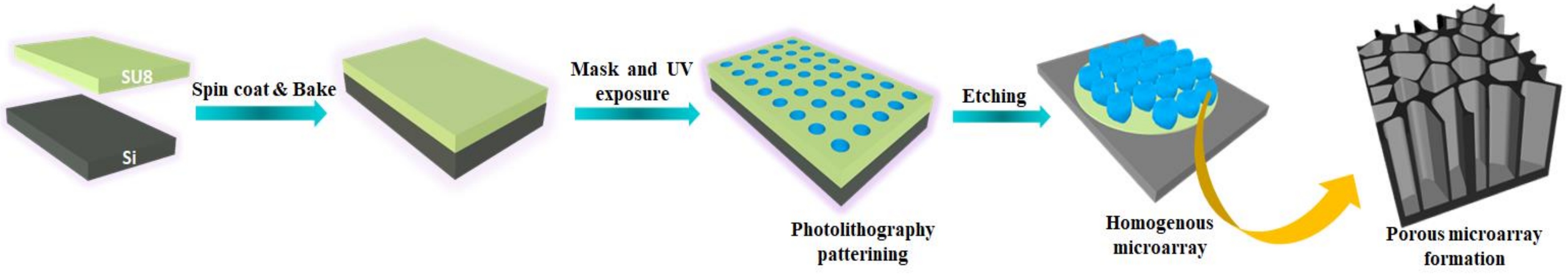


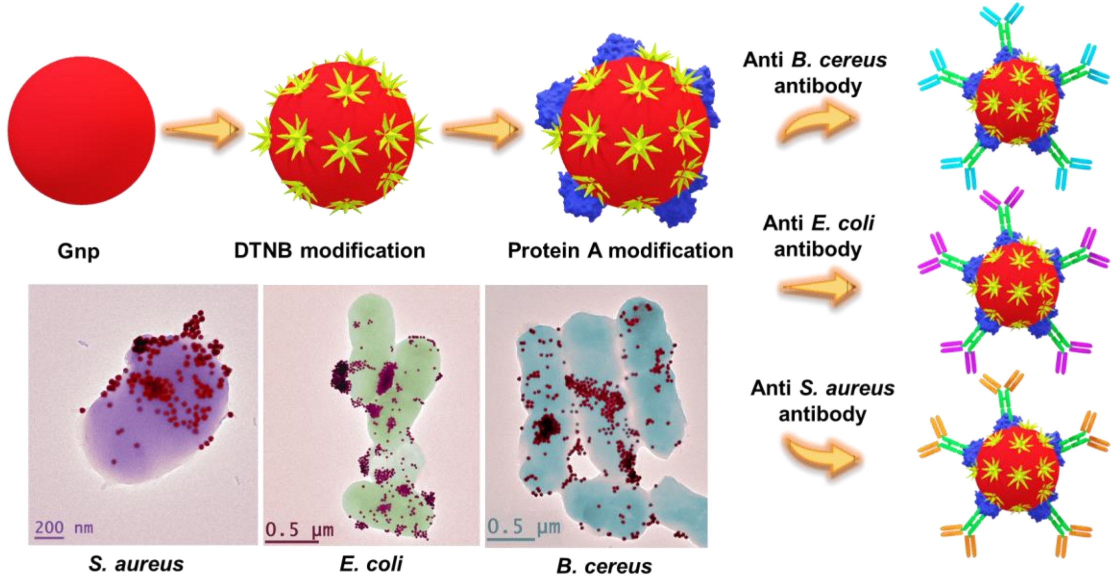




Multiplex SERS - Microarray

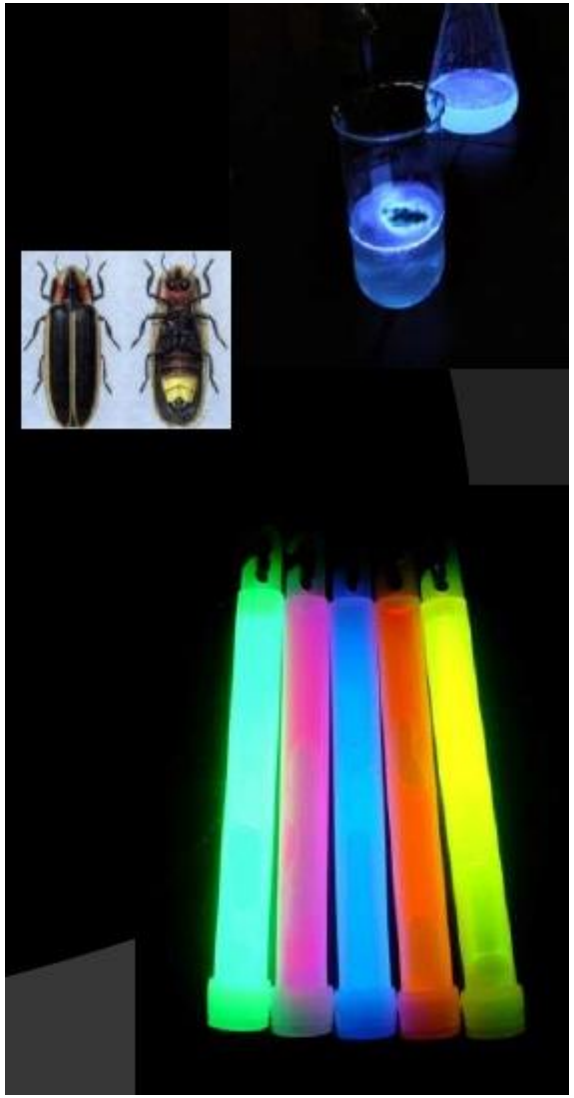






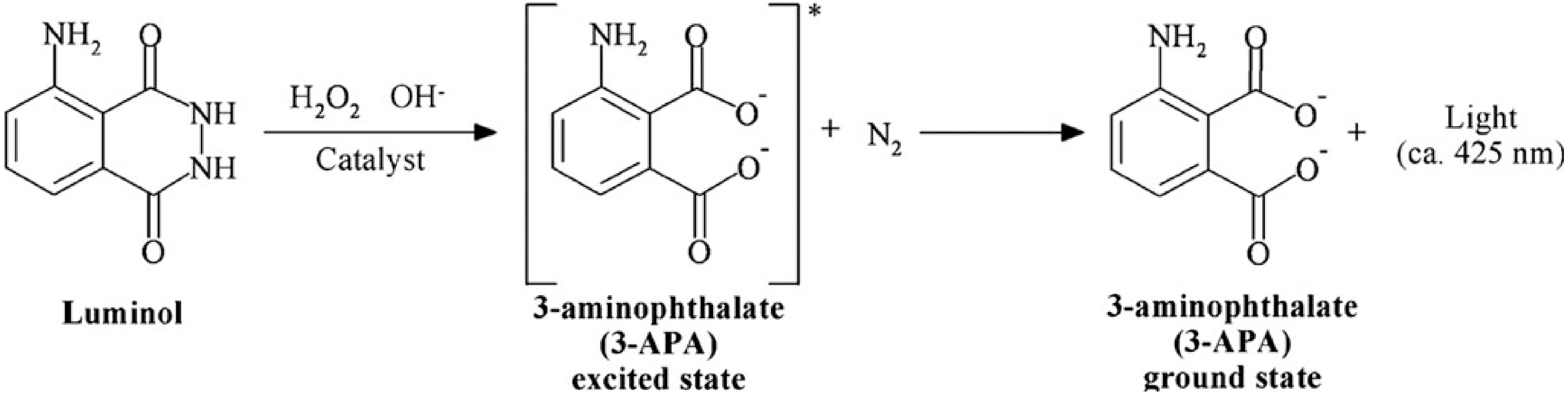
Pathogen	Spiked: Plate counting (Log CFU mL^{-1})	Measured: SERS I/Ic (%)	Measured: SERS (Log CFU mL^{-1})	Recovery (%)
Water				
B. cereus	1.9±0.1	72.5±0.9	1.7±0.1	89.3
E. coli	2.4±0.0	71.7±3.4	2.0±0.1	81.4
S. aureus	2.4±0.0	77.9±5.7	2.0±0.1	81.2
Lettuce				
B. cereus	1.9±0.1	64.8±7.2	1.7±0.0	93.2
E. coli	2.4±0.0	69.6±3.5	2.1±0.1	86.2
S. aureus	2.4±0.0	66.7±1.1	2.2±0.1	91.4
Rice				
B. cereus	1.3±0.0	78.9±1.5	1.3±0.1	104.0
E. coli	2.3±0.1	63.0±1.7	2.4±0.1	102.6
S. aureus	2.4±0.1	63.0±1.7	2.5±0.1	106.8
Chicken				
B. cereus	1.9±0.0	66.9±3.4	1.8±0.1	90.8
E. coli	2.2±0.0	73.1±1.6	1.7±0.1	80.6
S. aureus	2.0±0.0	74.3±0.6	1.6±0.1	80.3

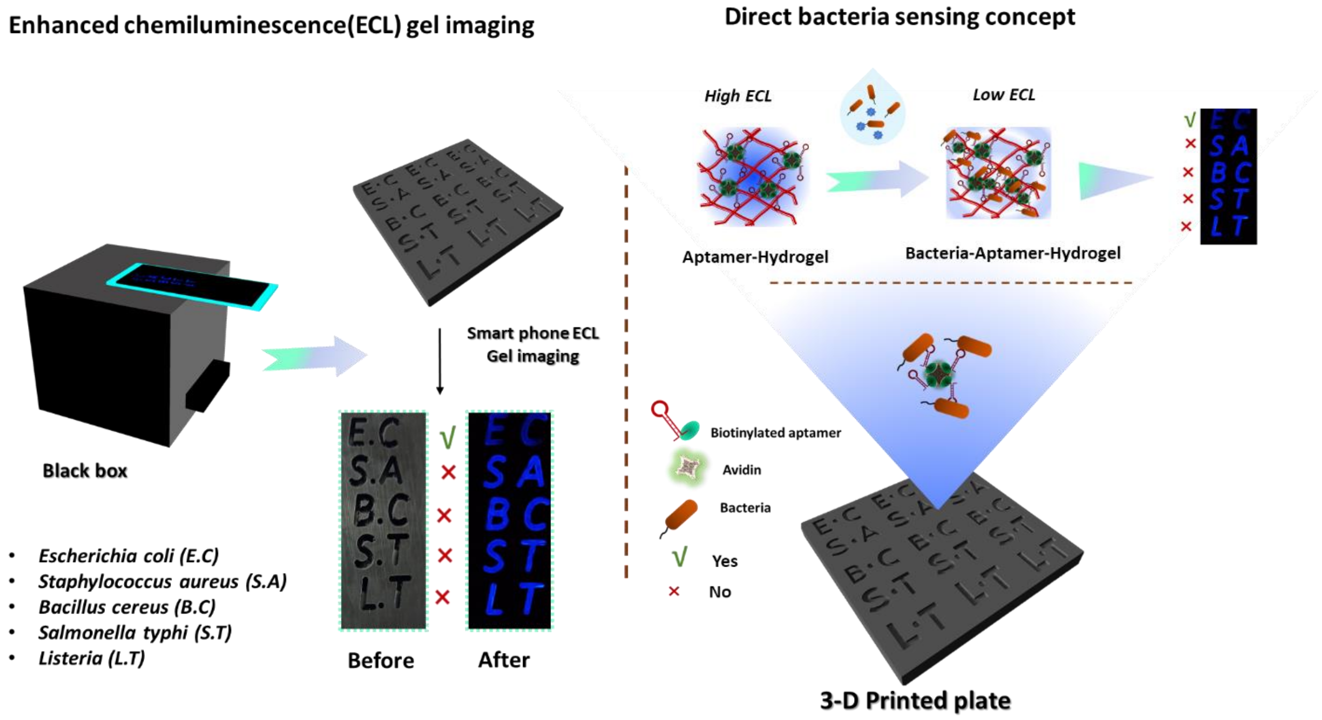
Chemiluminiscence (CL)?

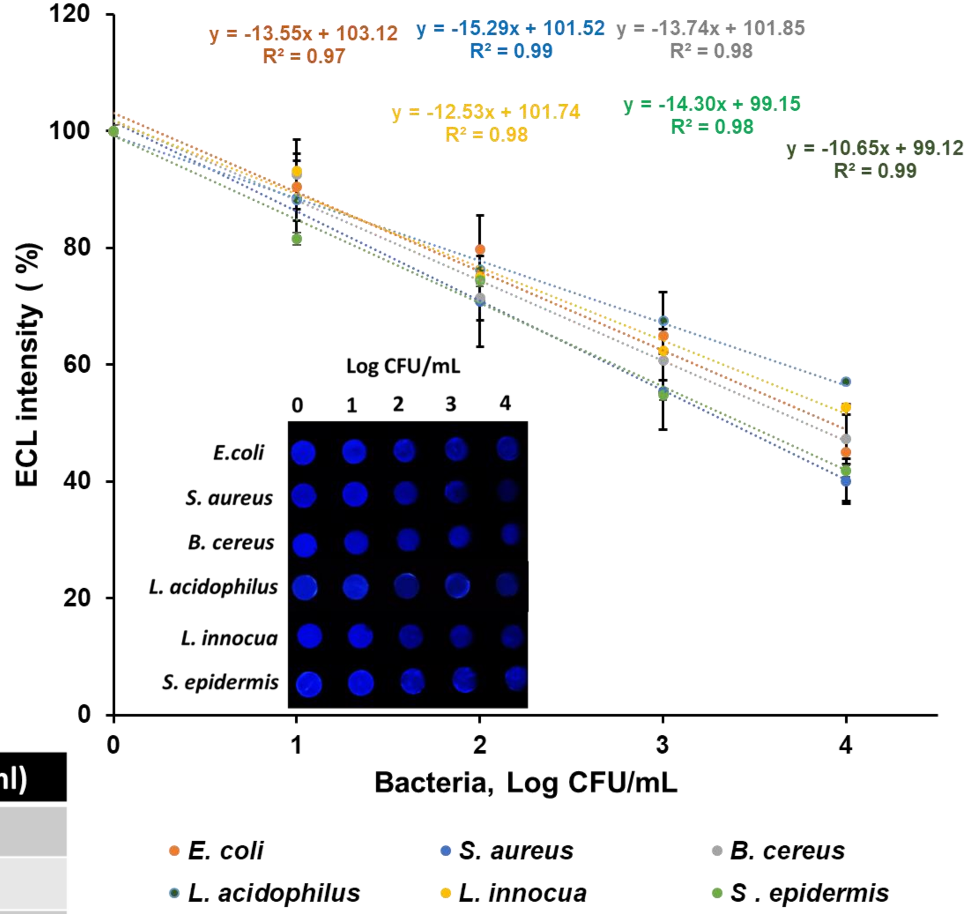


Chemically is a property of a substance undergoing a chemical reaction and releases energy in the form of light.

<u>Luminol</u> is an organic compound upon oxidation – emits blue light
Formula: $C_8H_7N_3O_2$
Discovered in the late 19th century

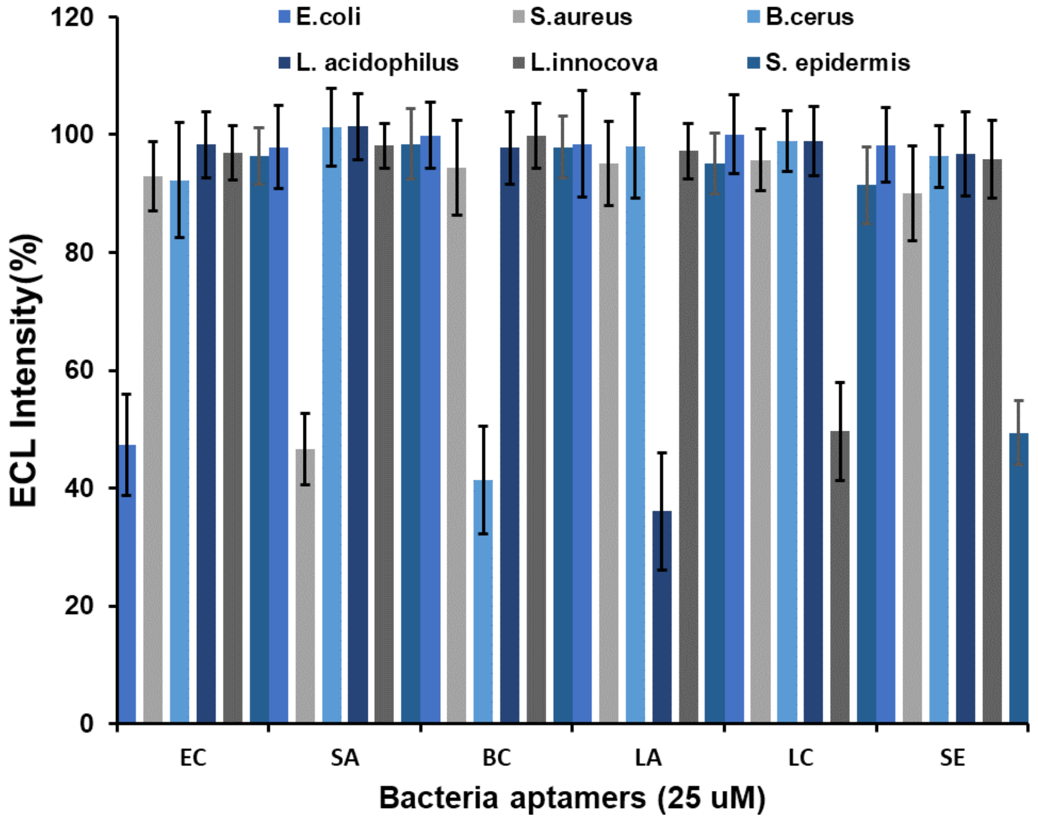






Bacteria	LOD (cfu/ml)
E.coli	10
S. aureus	4
B. cereus	5
L. innocua	10
L. acidophilus	12
S.epidermis	13

Specificity & Selectivity



Food samples with spike bacteria		ECL imaging quantification (Log CFU/mL)	Plate count (Log CFU/mL)	Recovery (%)
Lettuce	***E. coli***	2.09±0.50	1.99±0.06	**105**
	S. aureus	1.89±0.13	2.00±0.07	**94**
Rice	***B. cereus***	2.10±0.35	2.01±0.07	**104**
	L. innocova	1.95±0.20	1.97±0.10	**99**
Yogurt	***L. acidophilus***	1.86±0.10	1.98±0.06	**94**
	E. coli	2.02±0.68	1.93±0.09	**105**
Fish	***S. epidermis***	2.00±0.41	1.97±0.06	**102**
	L. innocova	1.83±0.41	1.99±0.08	**92**

Biomarkers for diagnosis of mastitis

J. Dairy Sci. 104:2106–2122
https://doi.org/10.3168/jds.2020-18539

The value of the biomarkers cathelicidin, milk amyloid A, and haptoglobin to diagnose and classify clinical and subclinical mastitis

L. Wollowski,[1] W. Heuwieser,[1*] A. Kossatz,[1] M. F. Addis,[2] G. M. G. Puggioni,[3] Laurent Meriaux,[4] and S. Bertulat[1]

Journal of Dairy Science
Volume 104, Issue 2, February 2021, Pages 2106-2122

Research

The value of the biomarkers cathelicidin, milk amyloid A, and haptoglobin to diagnose and classify clinical and subclinical mastitis

L. Wollowski [1], W. Heuwieser [1], A. Kossatz [1], M.F. Addis [2], G.M.G. Puggioni [3], Laurent Meriaux [4], S. Bertulat [1]

Review

Cell PRESS

Mastitis detection: current trends and future perspectives

Caroline Viguier[1,2], Sushrut Arora[1,3], Niamh Giln
Richard O'Kennedy[1,3]

Nirala et al. J Nanobiotechnol (2020) 18:6
https://doi.org/10.1186/s12951-019-0569-9
Journal of Nanobiotechnology

RESEARCH Open Access

Check for updates

Ultrasensitive haptoglobin biomarker detection based on amplified chemiluminescence of magnetite nanoparticles

Narsingh R. Nirala[1], Yifat Harel[2], Jean-Paul Lellouche[2] and Giorgi Shtenberg[1*]

ology
REVIEW
published: 31 July 2019
doi: 10.3389/fbioe.2019.00186

Check for updates

ors for On-Farm Diagnosis of

[1,2*], Verónica C. Martins[1,2], Filipe A. Cardoso[1], José Germano[1], Mónica Rodrigues[1,3], Carla Duarte[2,4†], Ricardo Bexiga[4], Susana Cardoso[2] and Paulo P. Freitas[2,5]

Veterinary Research Communications (2022) 46:329–351
https://doi.org/10.1007/s11259-022-09901-y

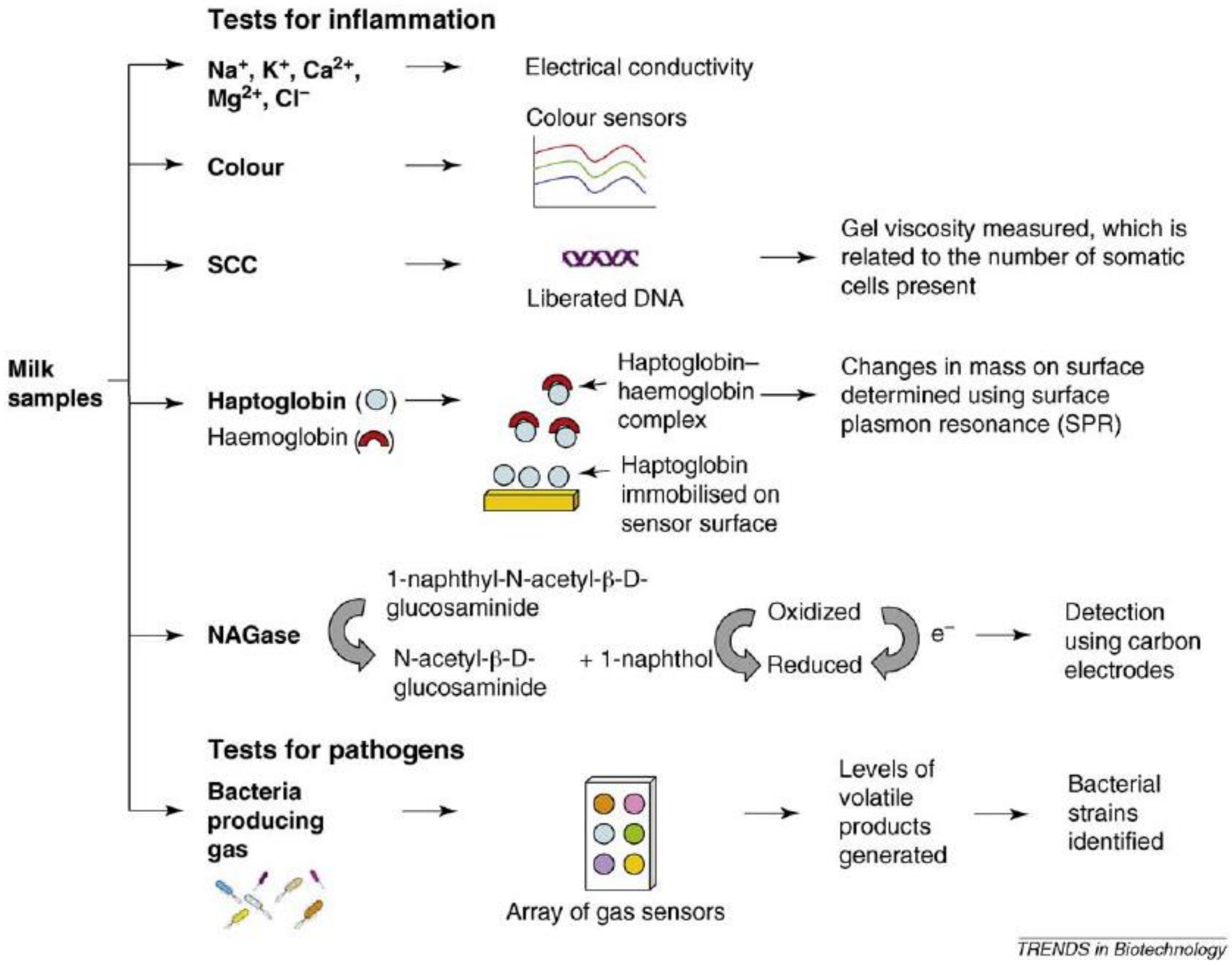
REVIEW ARTICLE

Milk proteins as mastitis markers in dairy rur review

Anna Giagu[1,2,3] · Martina Penati[4] · Sara Traini[4] · Simone Dore[2] · Maria Filippa Addis[4]

Received: 26 September 2021 / Accepted: 8 February 2022 / Published online: 23 February 2022

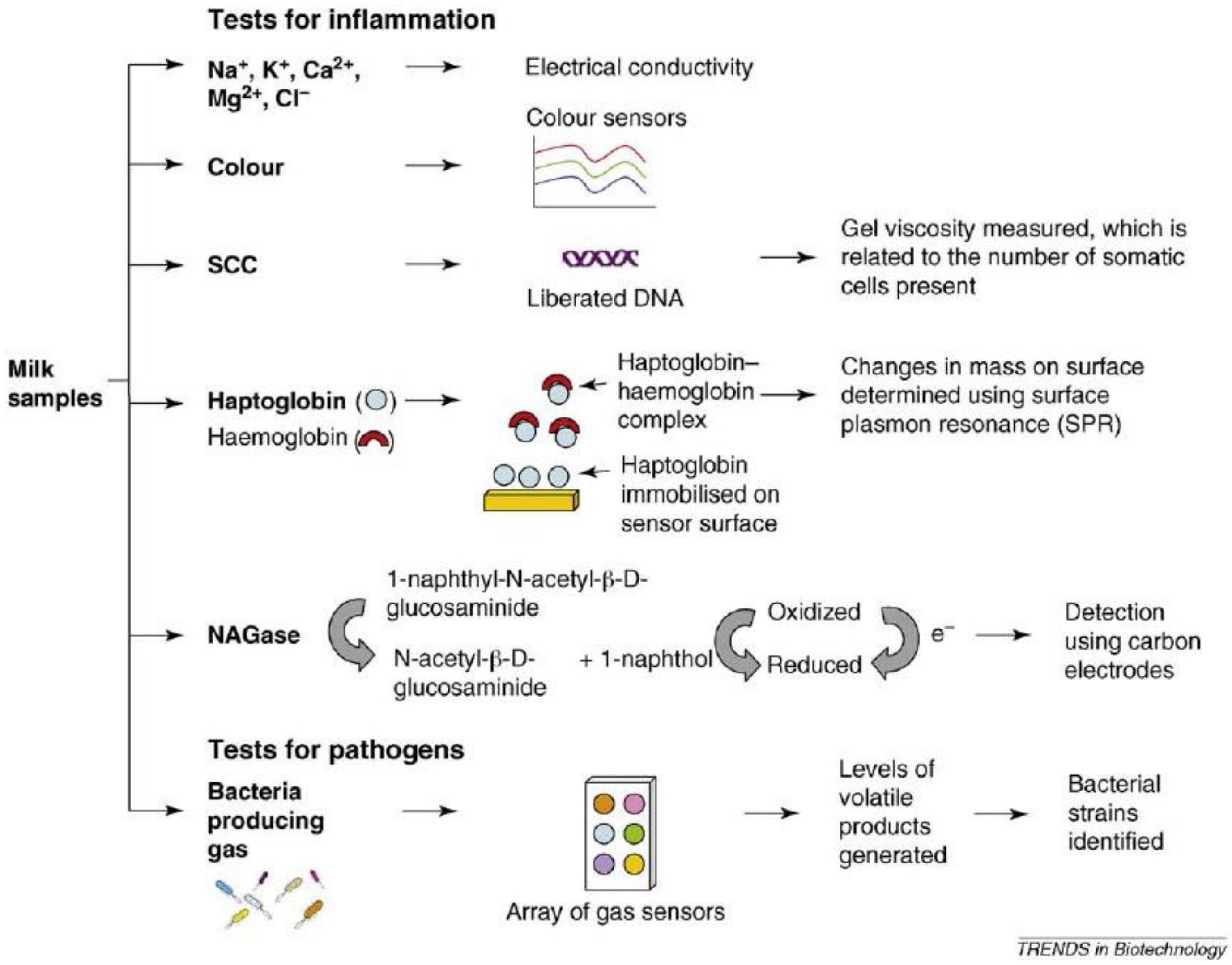
Biomarkers for diagnosis of mastitis



Haptoglobin - acute phase protein used as predicting diagnostic biomarker both in humans (i.e., diabetes, ovarian cancer, some neurological and cardiovascular disorders) and in animals (e.g., bovine mastitis).

NAGase - a prominent inflammatory indicator widely used in correlation to SCC values. This intracellular lysosomal glycosidase is released from damaged epithelial cells of the mammary tissue and is associated with cell lysis, hence indicating tissue destruction

Biomarkers for diagnosis of mastitis



Concentration increased

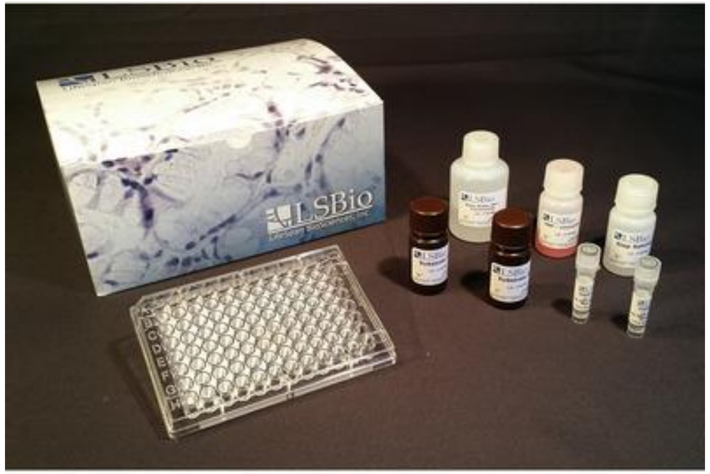
10-100 folds

during disease/ inflammation

Biomarkers for diagnosis of mastitis

ELISA - Enzyme-Linked Immunosorbent Assay

NOVUS BIOLOGICALS
a bio-techne brand



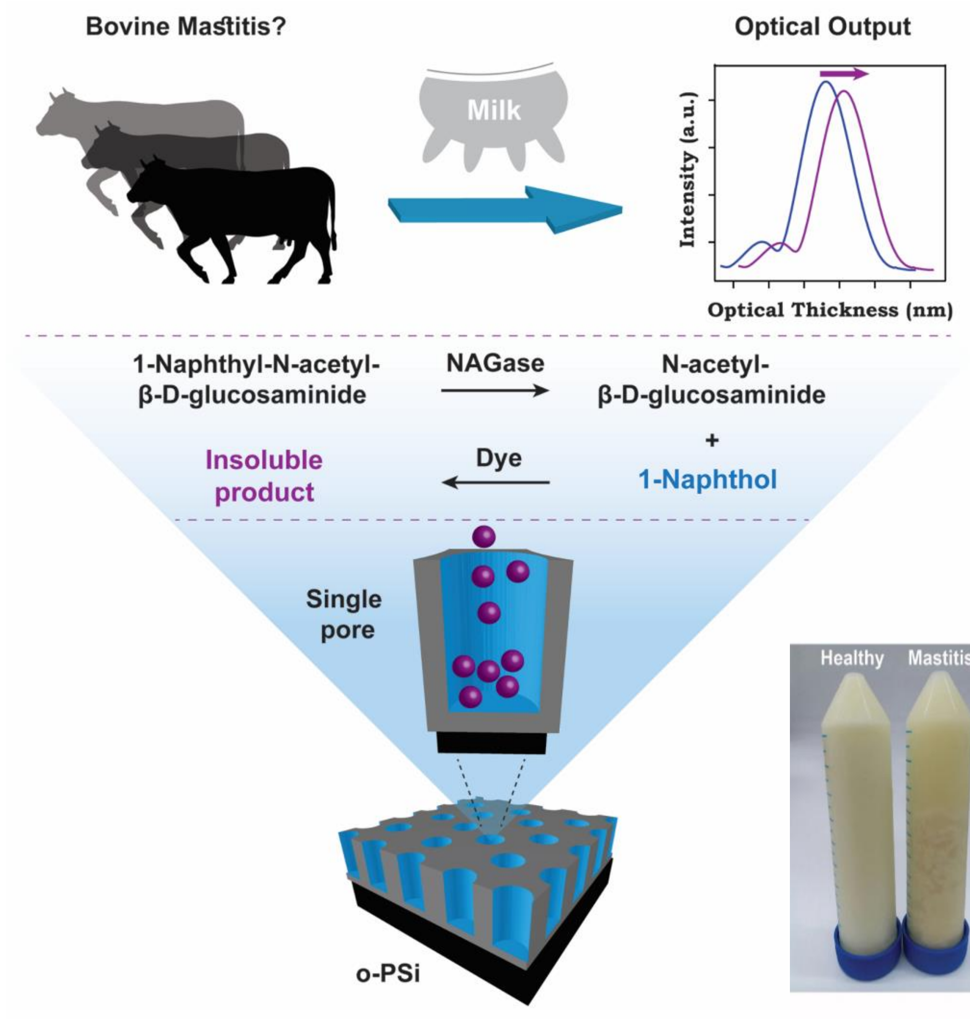
~ 843$ kit
+
Lab Equipment

Biomarkers for diagnosis of mastitis

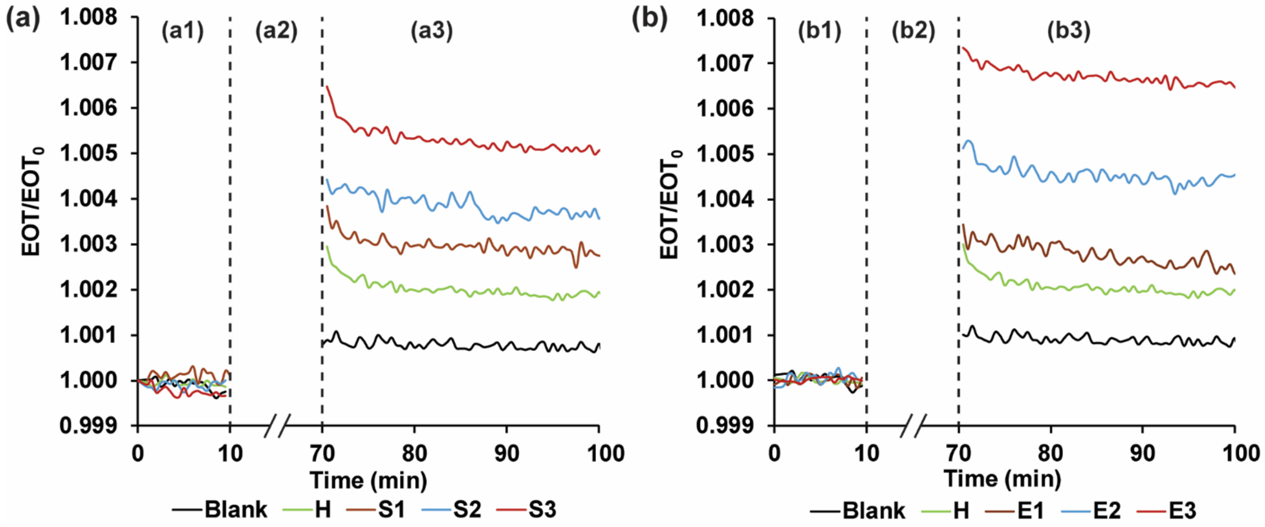
Haprodia eProCheck Rapid tester
Haptoglobin and Progesterone

ELISA kit
+
Portable Equipment

NAGase Indicator of Bovine Mastitis



S. dysgalactiae | *E. coli*



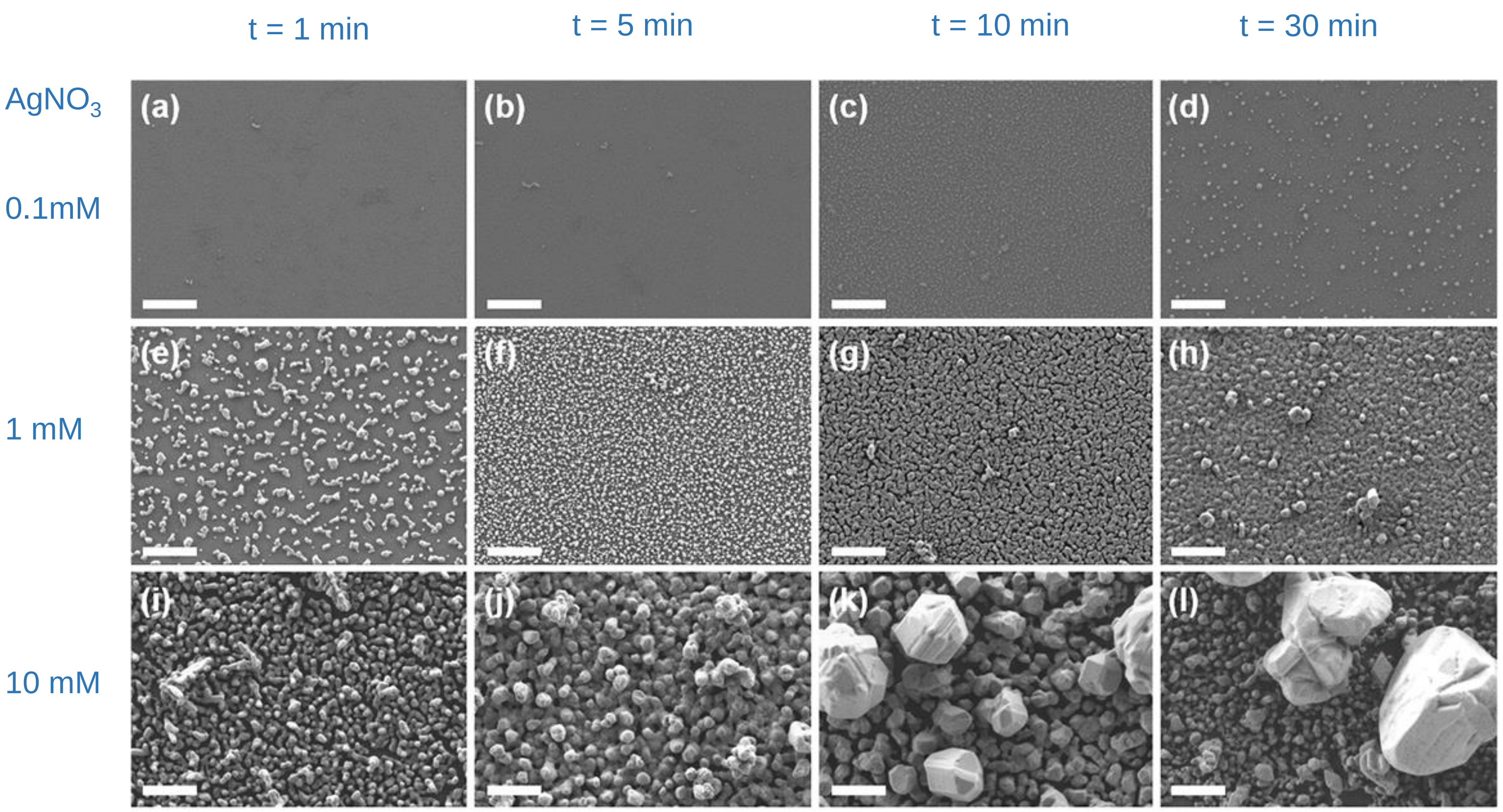
Sample	SCC (X1000) cells mL^{-1}	Pathogen	RIFTS (μM min^{-1})	FL assay (μM min^{-1})
H	71	N/A	1.03 ± 0.18	1.41 ± 0.01
S1	353	*S. dysgalactiae*	1.46 ± 0.03	1.60 ± 0.07
S2	495	*S. dysgalactiae*	2.71 ± 0.21	2.72 ± 0.17
S3	>1000	*S. dysgalactiae*	3.73 ± 0.15	3.32 ± 0.04
E1	300	*E.coli*	1.43 ± 0.02	1.96 ± 0.02
E2	636	*E.coli*	2.99 ± 0.03	3.24 ± 0.18
E3	>1000	*E.coli*	4.44 ± 0.50	4.00 ± 0.11

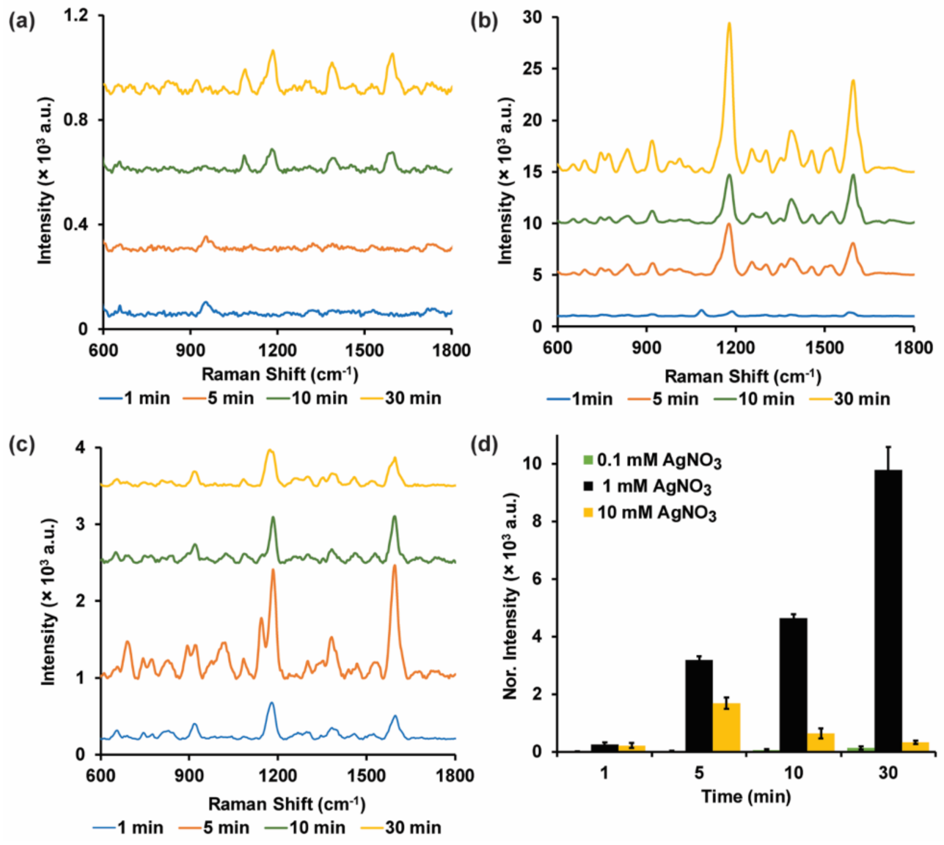
Data are reported as mean ± SD (n ≥ 3).

LoD 31 μM

Kumar N.D. et al. *ACS Sensors* 2020

NAGase Detection by SERS



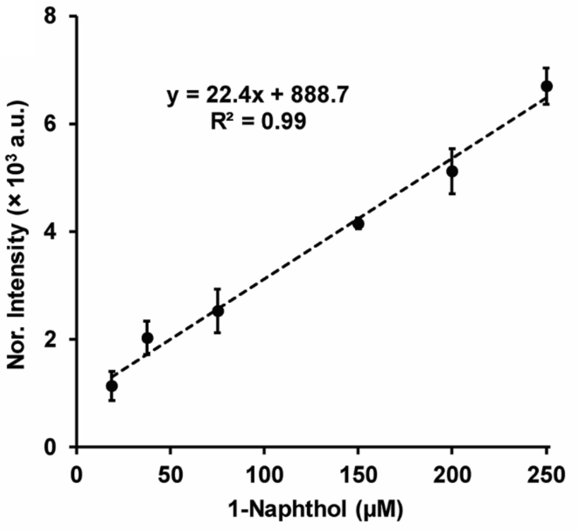


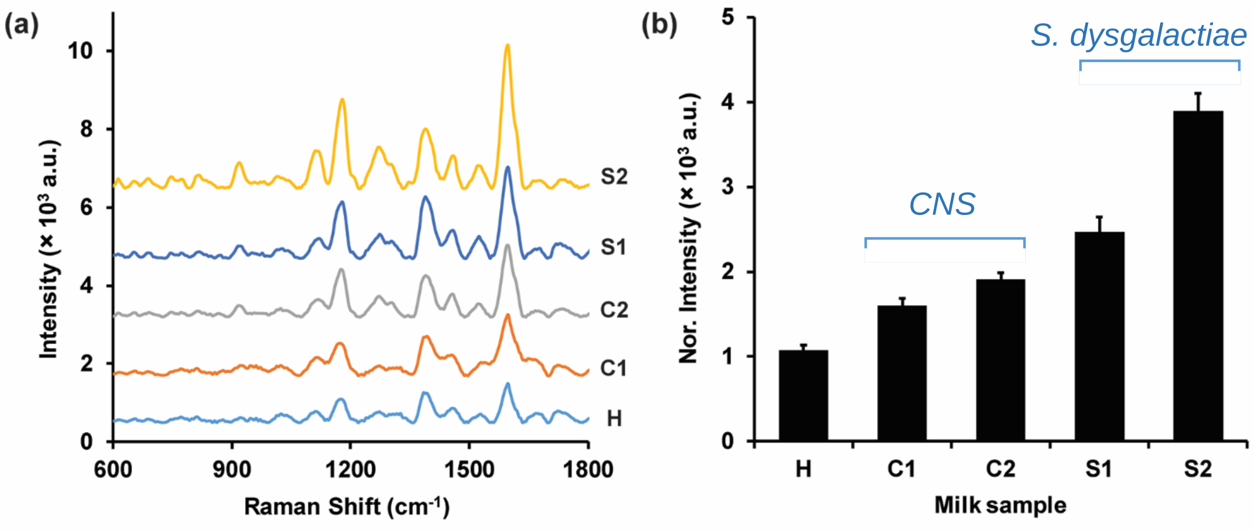
PSi-H Thickness 1.38 μm Diameter 20-30 nm

Enhancement factor 1.06×10^5

Nirala R.N. et al. *Appl. Surf. Sci.* 2021

NAGase Detection by SERS





Overall assay 20-30 min

Milk sample	Bacteria	SCC ($\times 10^3$ cells mL^{-1})	SERS ($\mu M\ min^{-1}$)	FL assay ($\mu M\ min^{-1}$)	Recovery (%)
H	N/A	70	0.27±0.09	0.30±0.01	90
C1[a]	CNS	300	1.06±0.13	1.20±0.11	88
C2[b]	CNS	> 1,000	1.53 ±0.26	1.80±0.17	85
S1[a]	*S. dysgalactiae*	300	2.36±0.26	2.40±0.07	98
S2[b]	*S. dysgalactiae*	> 1,000	4.48±0.31	5.30±0.34	86

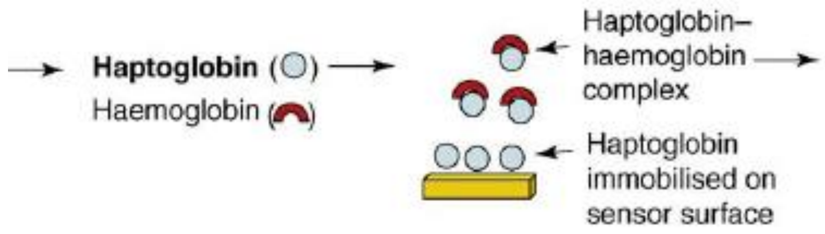
[a] Subclinical BM milk sample.

[b] Clinical BM milk sample.

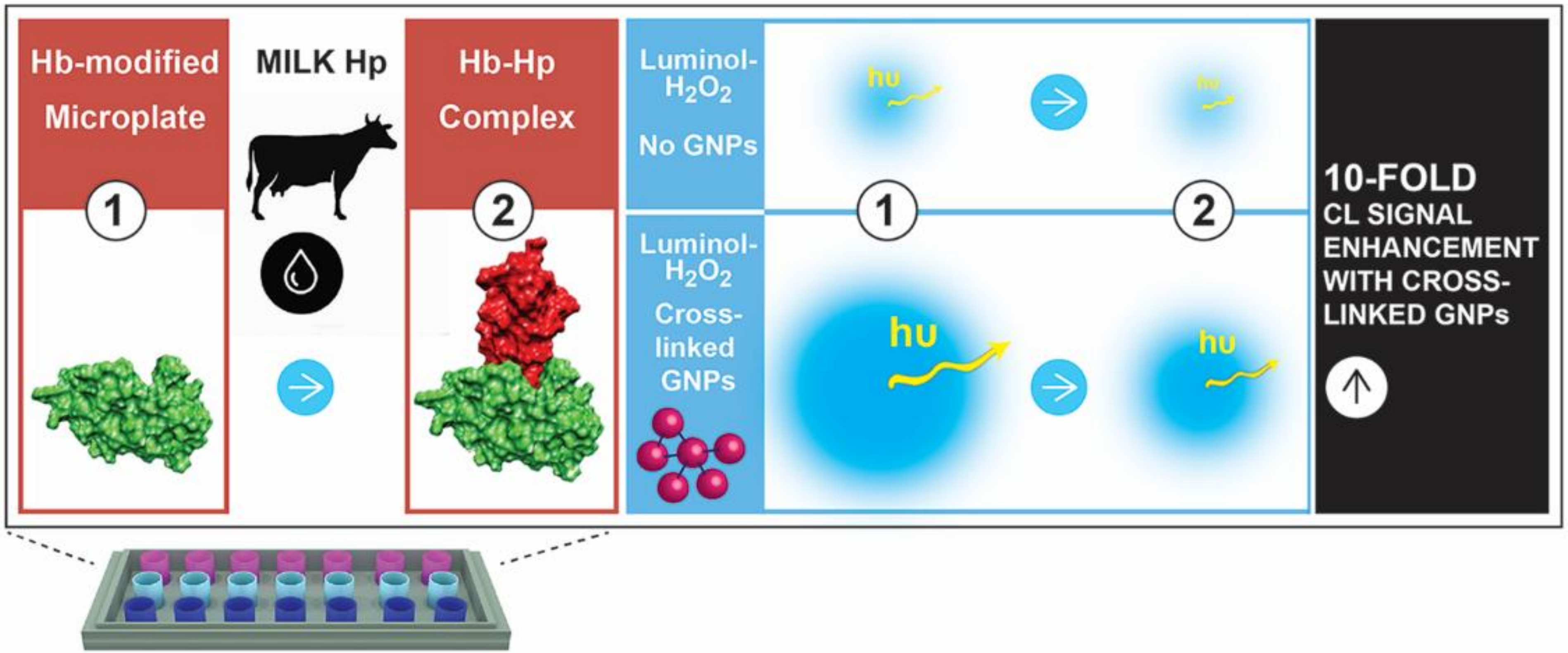
Data are reported as mean ± SD ($n \geq 3$).

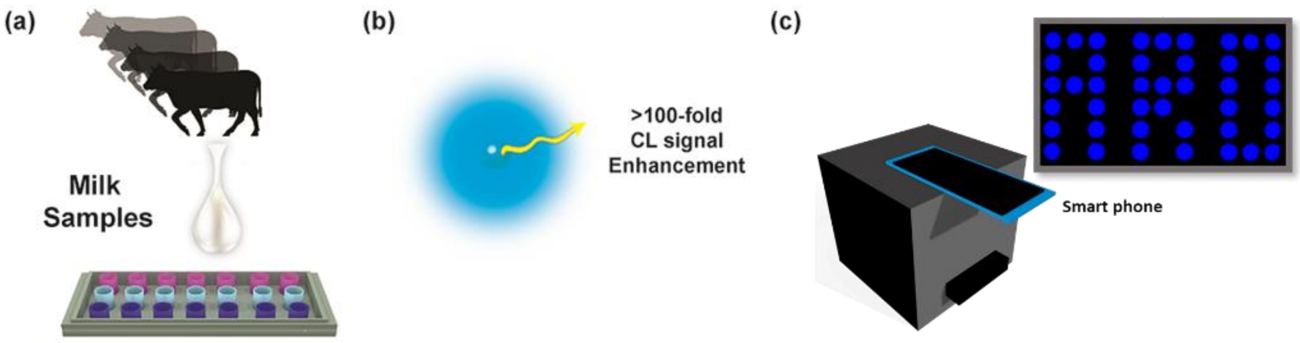
Nirala R.N. et al. *Appl. Surf. Sci.* 2021

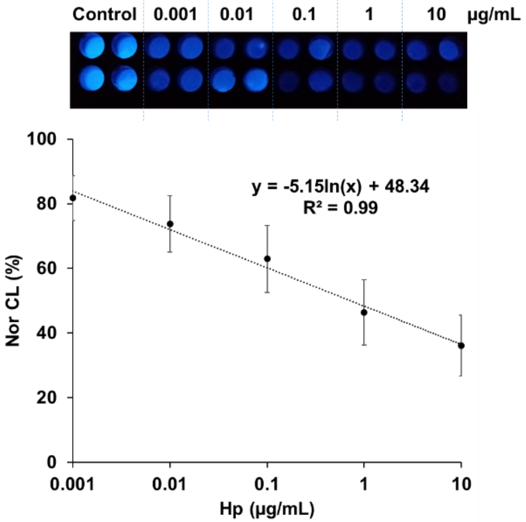
Biomarkers for diagnosis of mastitis

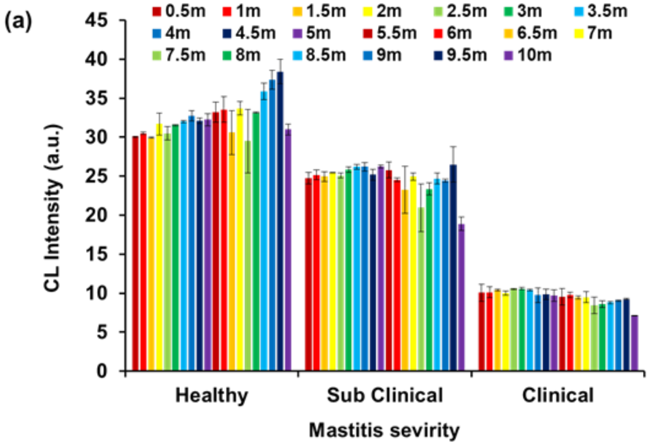


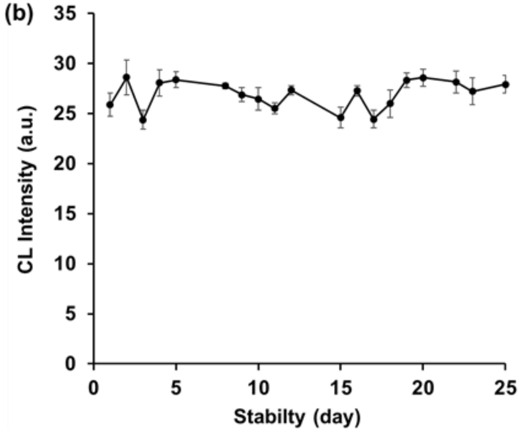
Optical Detection




(a)
Milk
Samples
(b)
>100-fold
CL signal
Enhancement
(c)
Smart phone

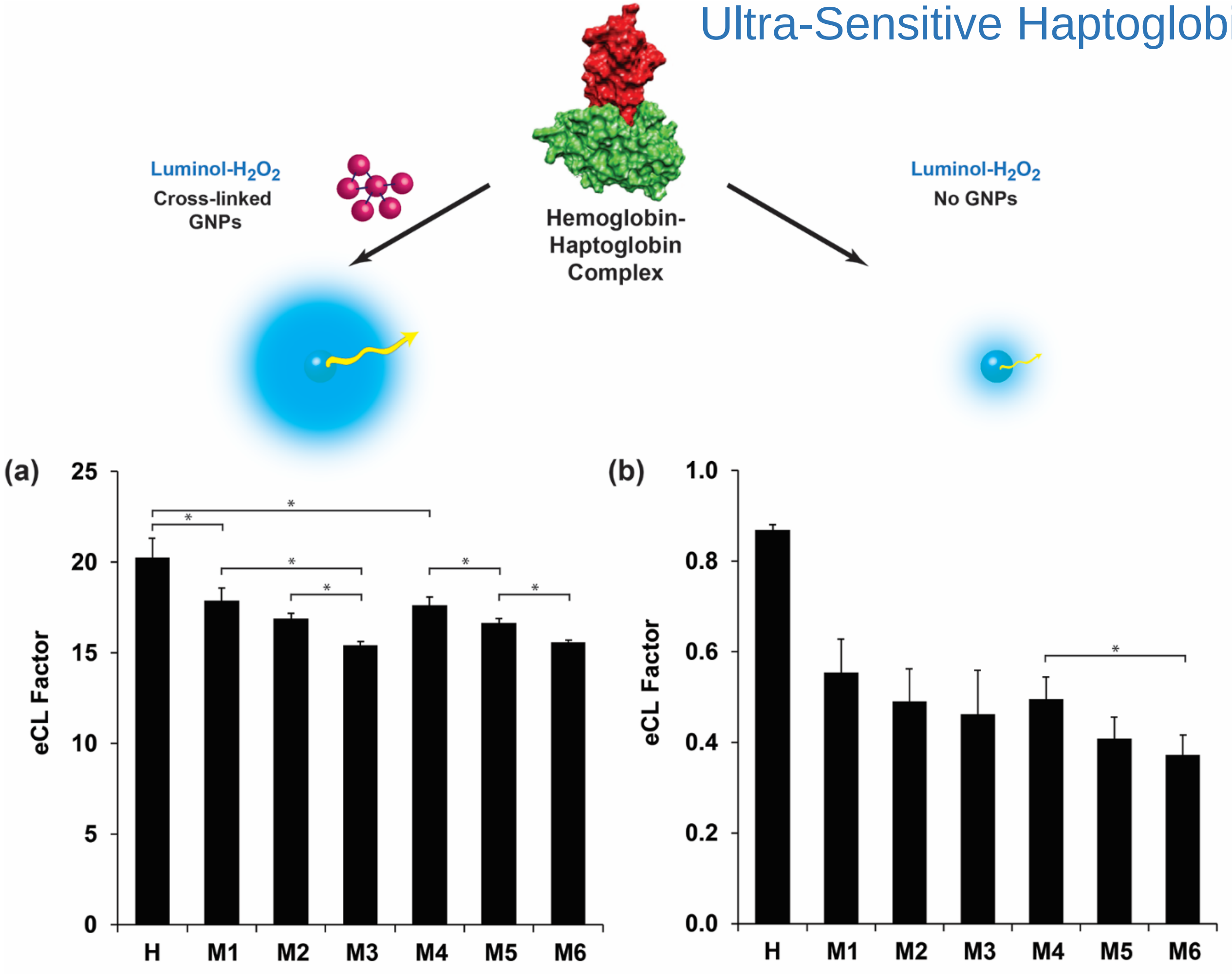

Control
0.001
0.01
0.1
1
10
µg/mL
y = -5.15ln(x) + 48.34
R² = 0.99
Nor CL (%)
Hp (µg/mL)
0
20
40
60
80
100
0.001
0.01
0.1
1
10


(a)
0.5m
1m
1.5m
2m
2.5m
3m
3.5m
4m
4.5m
5m
5.5m
6m
6.5m
7m
7.5m
8m
8.5m
9m
9.5m
10m
CL Intensity (a.u.)
0
5
10
15
20
25
30
35
40
45
Healthy
Sub Clinical
Clinical
Mastitis sevirity


(b)
CL Intensity (a.u.)
0
5
10
15
20
25
30
35
0
5
10
15
20
25
Stabilty (day)

Biomarkers for diagnosis of mastitis

Gold Nanoparticles Size Depended Enhanced Chemiluminescence for Ultra-Sensitive Haptoglobin Biomarker Detection

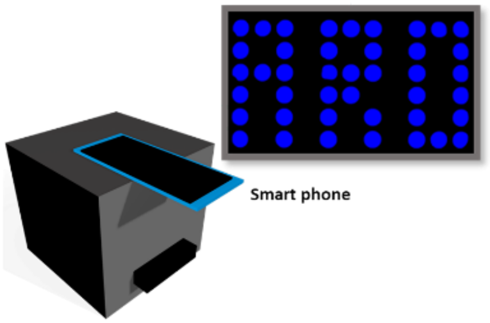


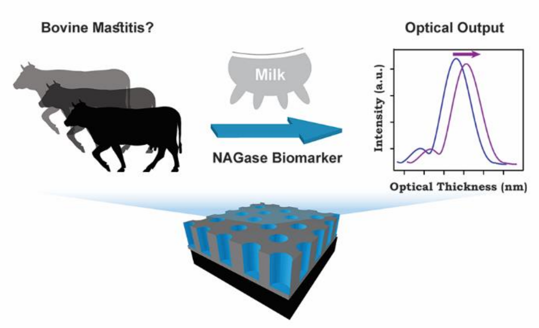
Dynamic range: 1 pg mL^{-1} to 10 μg mL^{-1}

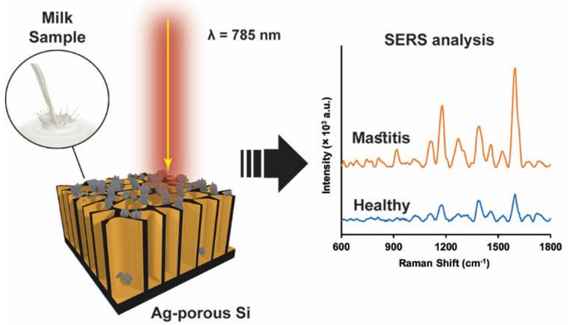
LOD 0.19 pg mL^{-1}

Sample	SCC	Pathogenic bacteria	**Haptoglobin with cross-linked GNPs (μg mL^{-1})**	**Haptoglobin ELISA (μg mL^{-1})**
H	60,000	-	0.1±0.1	0.1±0.1
M1	300,000	*E. coli*	1.4±1.2	1.2±0.01
M2	636,000	*E. coli*	3.6±1.4	3.9±1.3
M3	>1,000,000	*E. coli*	24.0±4.5	21.0±0.8
M4	337,000	*CNS*	1.6±0.9	1.7±0.3
M5	821,000	*CNS*	5.2±1.7	4.8±0.2
M6	>1,000,000	*CNS*	19.5±3.0	16.6±3.1

Bacteria Detection

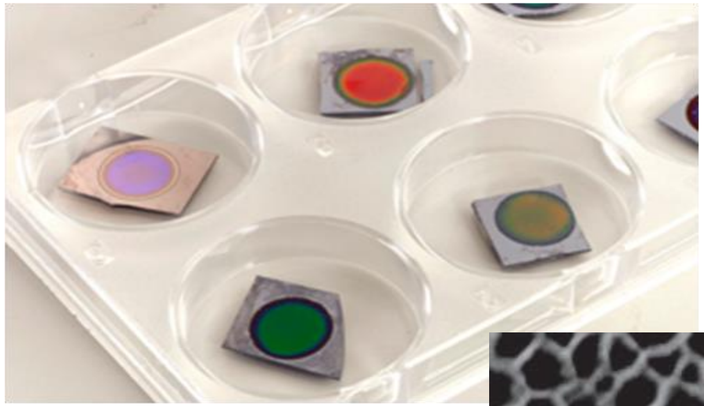


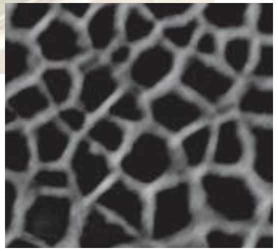




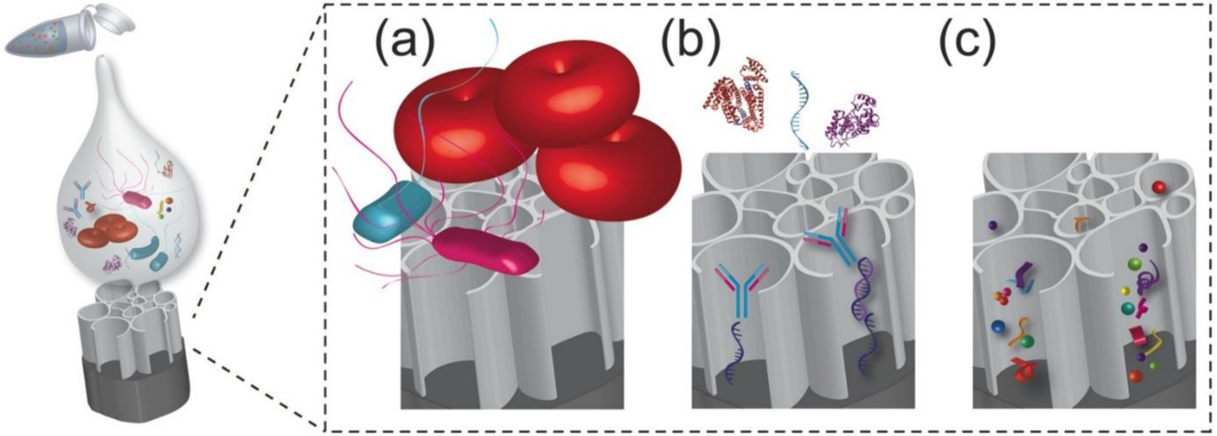
Chemiluminescence	RIFTS	SERS
LoD 5-12 CFU/mL	LoD 2 CFU/mL	LoD 2-3 CFU/mL
Overall assay ~ 60 min	Overall assay ~ 80 min	Overall assay ~ 75 min
Portable approach	Portable approach	Portable approach
0.15 $ per sample	1.0 $ per sample	0.40 $ per sample

Why use Porous Si ???





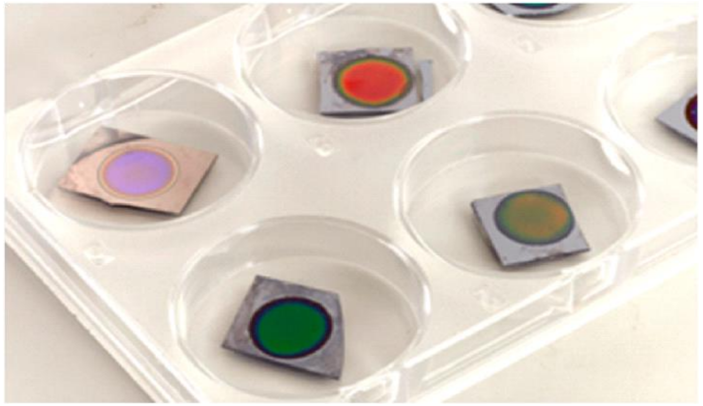
- ✓ Easy to fabricate.
- ✓ Relatively low cost material.
- ✓ Tuneable structural properties.
- ✓ Precise control of nanostructure.
- ✓ High surface area and high porous volume.
- ✓ Well known surface chemistry.
- ✓ Unique optical structures for label-free **chemical and biological sensing.**



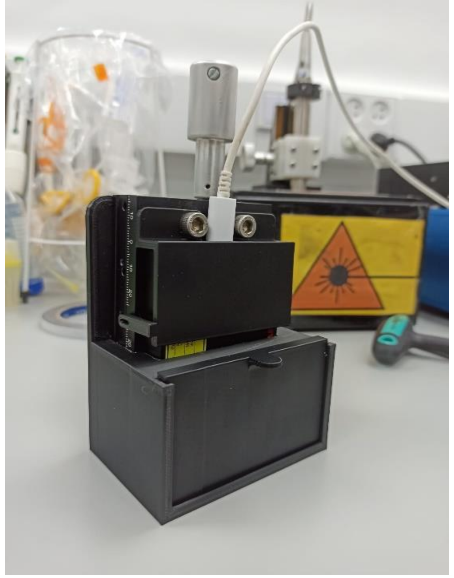
Our System

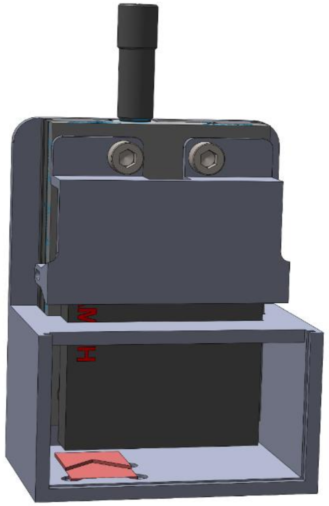
Mini-Spectrometer

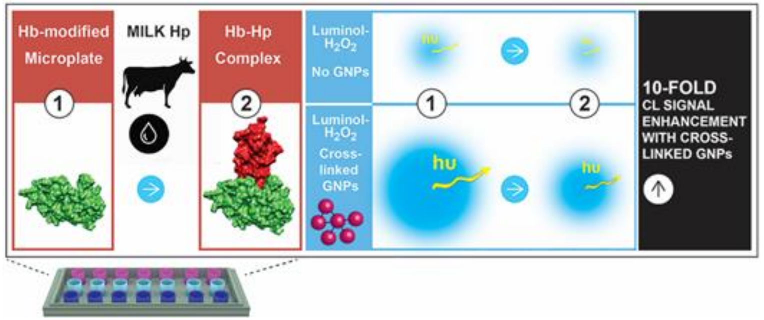
Patented Nano sensors

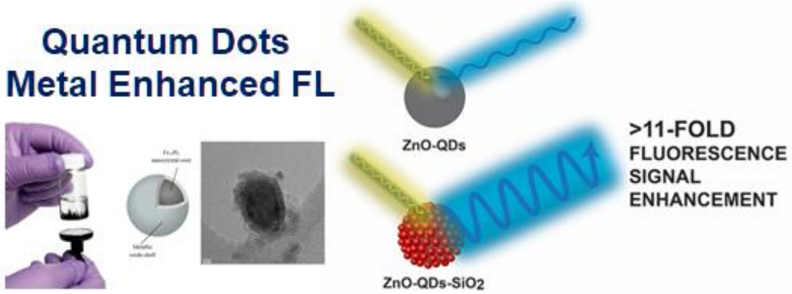


Next Challenge: Technology utilization by the farmer/grower









Acknowledgments

Collaborators:

Dr. Nanda Kumar
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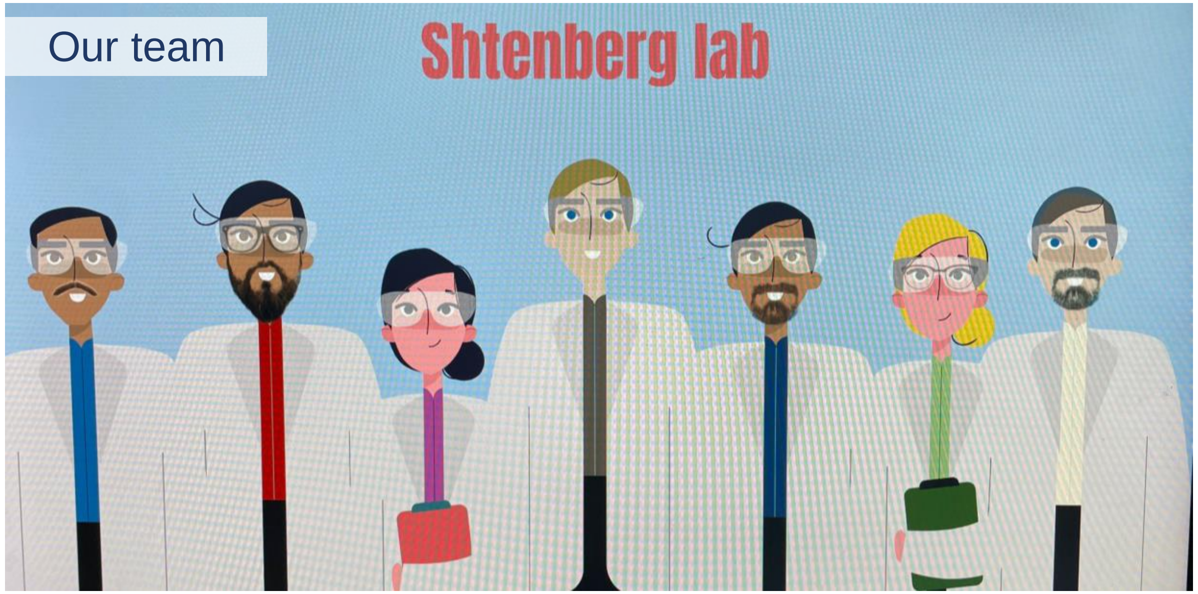
Prof. Shelley D. Minteer
Dr. Svetlana Bardenstein



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