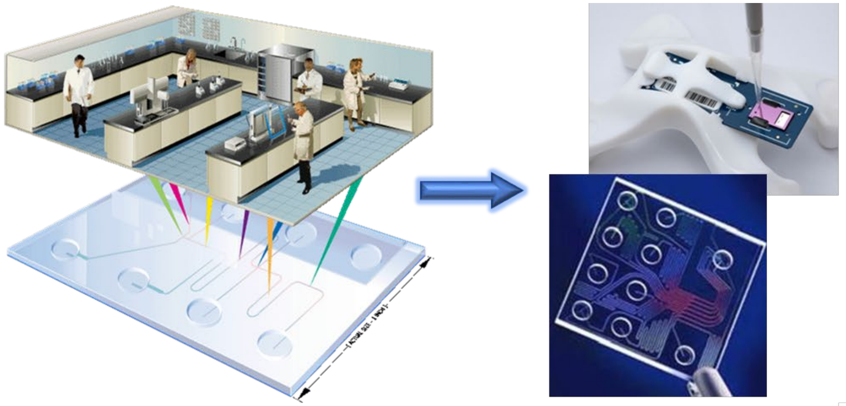
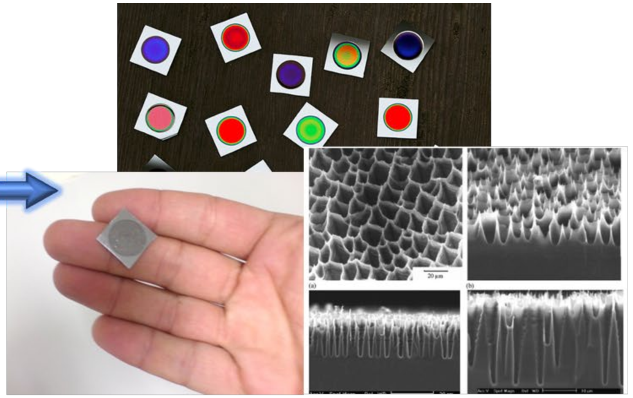
Biosensors and Nanoengineering Lab

Multiplex pathogenic bacteria detection in milk with the nanoparticle-assisted porous silicon-based SERS microarray biosensor

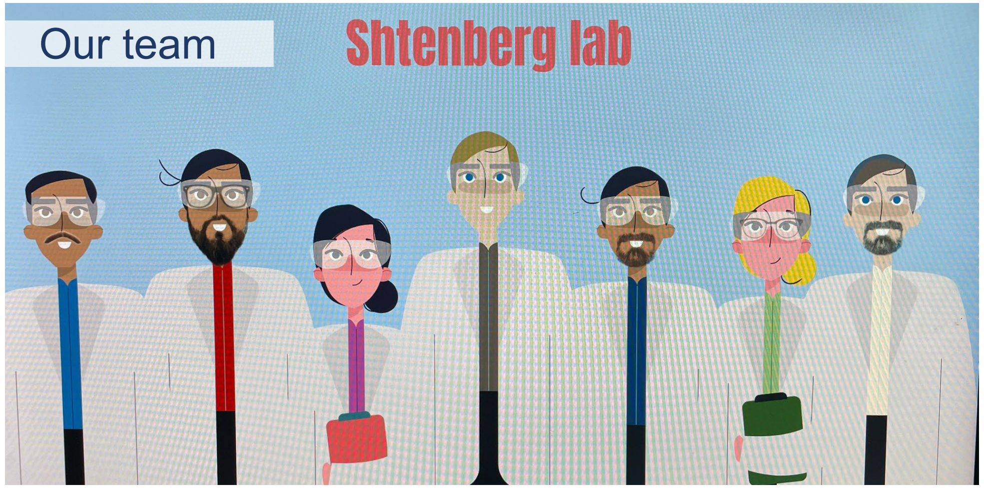


Lab-on-Chip Technology

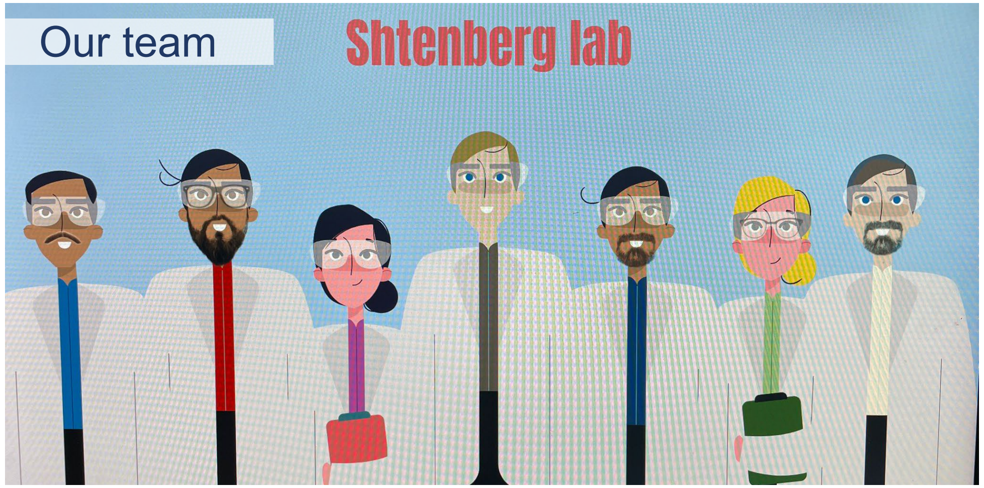


Nano-Porous Si

Divagar Muthukumar, Omer Tamari & Giorgi Shtenberg



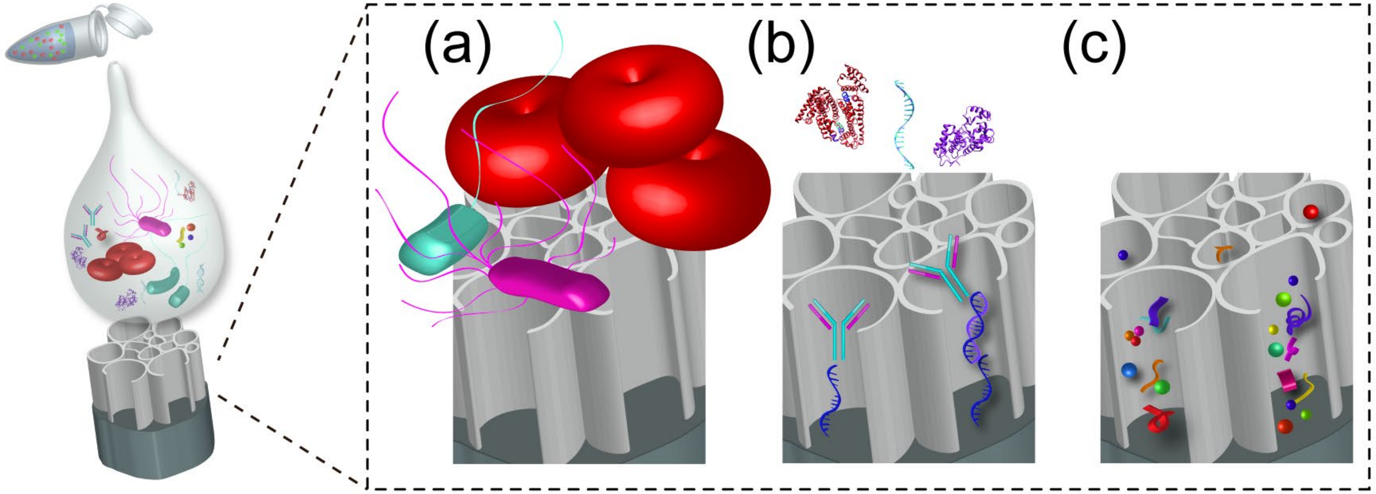
The **Biosensors and Nanoengineering lab** is focused on the development of novel label-free optical biosensors/bioassays based on nanomaterials, nanoparticles and thin-films that will transform from a laboratory-based research into a real on-site “lab-on-chip” platforms for addressing problems in fields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

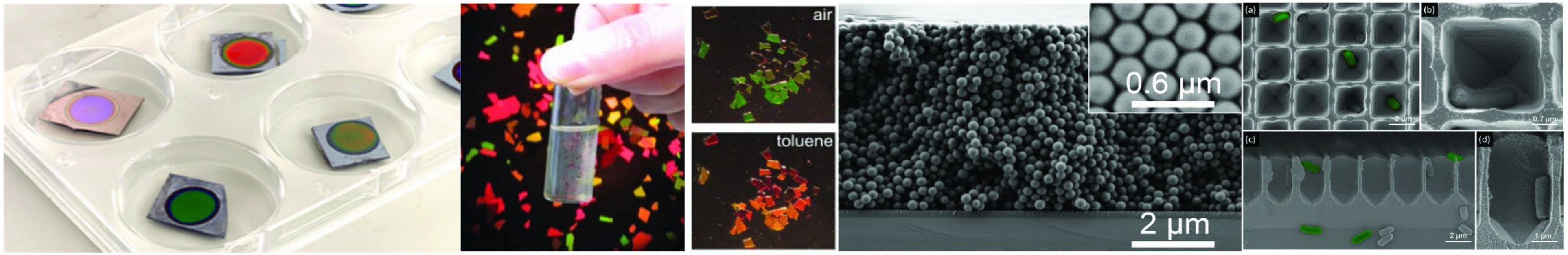
Real-Time & Label-Free Detection



High-throughput
Parallel
Rapid
Label-free
Portable

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

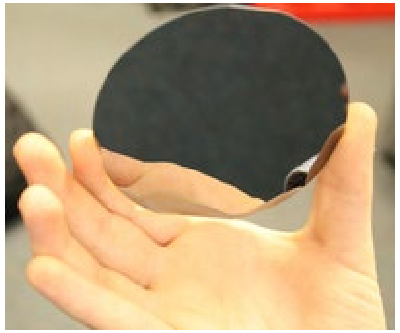
by Nanoengineered Optical Platforms



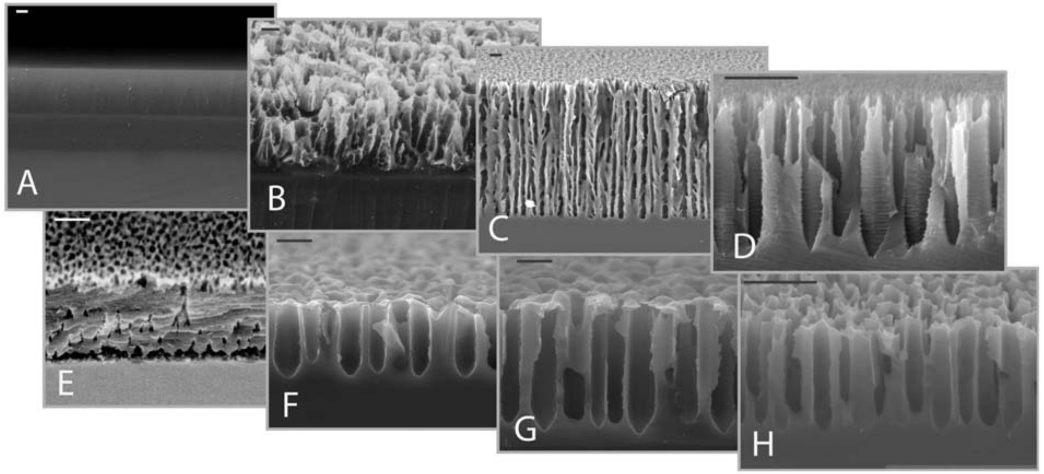
Photonic Crystals | **Optical Nanoparticles** | **Colloidal Crystals** | **Nanoarrays**

Our Chip Technology

Mirror

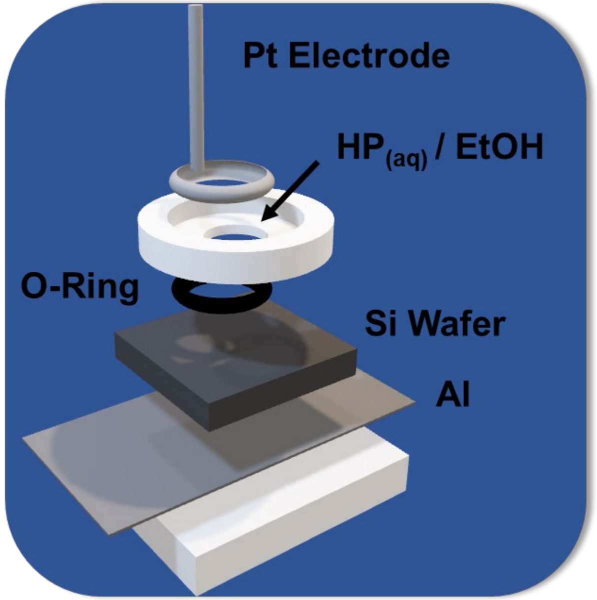


Si Wafer



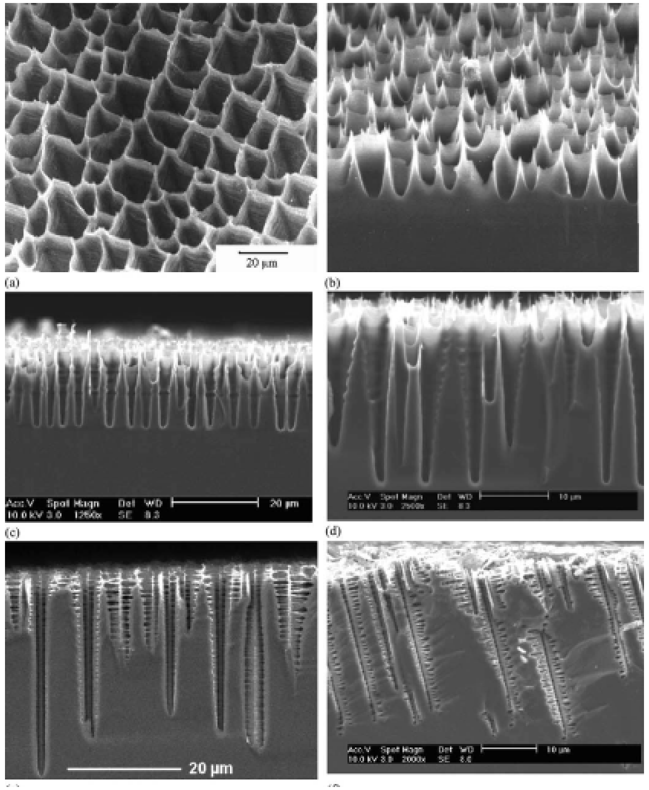
SEM images of different pore sizes and morphologies of porous Si.

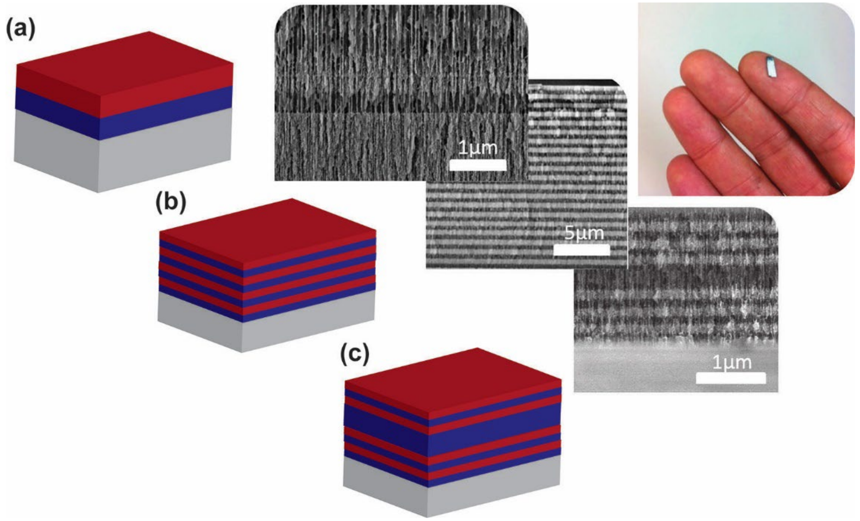
Electrochemical Etching:

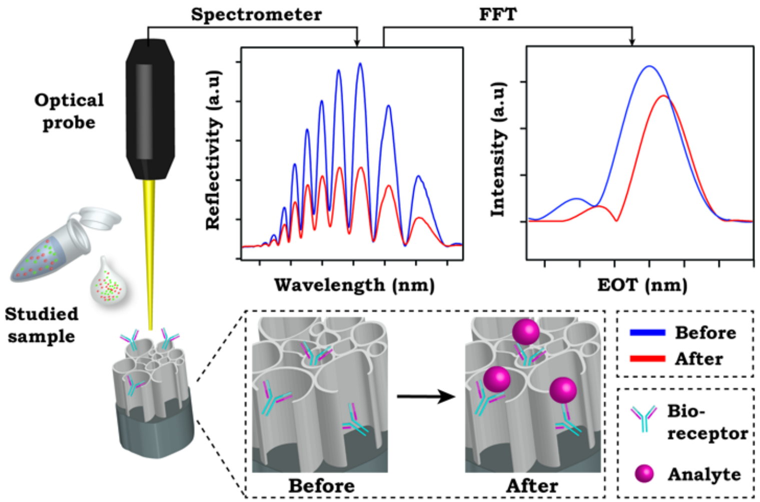


$$2Si + 6\,HF + 2\,h^+ \longrightarrow \underbrace{\equiv Si{-}H}_{\text{Porous Si Surface}} + H_2SiF_6 + 2H^+ + 1/2\,H_2$$

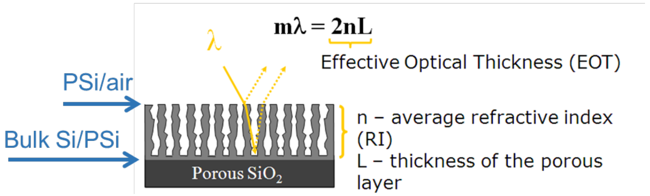
Tuning Physical Properties







Fabry-Pérot interference:



Global Burden of Foodborne Diseases

World Health Organization

May 2022

An estimated 600 million –Almost **1 in 10 people fall ill** every year from eating contaminated food and **420,000 die** as a result.

CDC
CENTERS FOR DISEASE CONTROL AND PREVENTION

Infectious diseases from contaminated food and water cause about **3,000 deaths** and 48 million non-fatal illnesses every year.

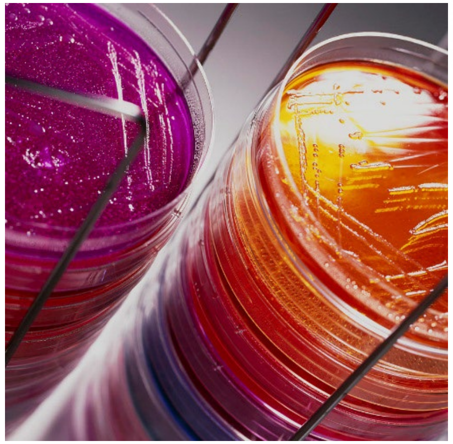
List of Selected Outbreak Investigations (2022)

- Shell Eggs – *Salmonella* Oranienburg
- Beef Products – *E. coli* O157:H7
- Frozen Strawberries – Hepatitis A
- Frozen Scallops – Hepatitis A
- Alfalfa Sprouts – *Salmonella* Reading and *Salmonella* Abony
- Flour – *E. coli* O121 and O26
- Frozen Vegetables – *Listeria monocytogenes*
- Raw Milk – *Listeria monocytogenes*
- Pistachios – *Salmonella* Montevideo
- Alfalfa Sprouts – *E. coli* O157
- Alfalfa Sprouts – *Salmonella* Muenchen and *Salmonella* Kentucky
- Organic Shake & Meal Products - *Salmonella* Virchow
- Packaged Salads – *Listeria monocytogenes*

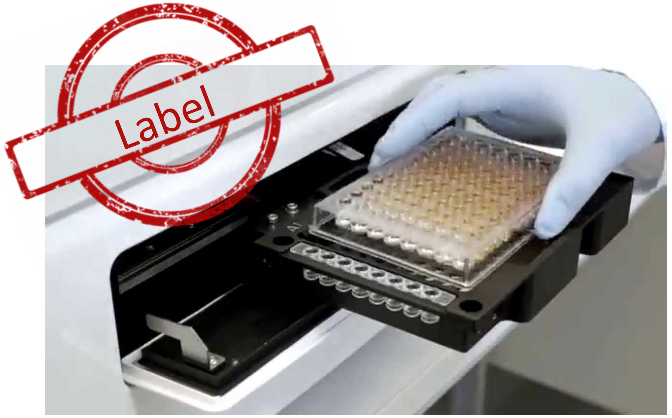
FoodNet FAST
A user-friendly data tool from FoodNet
Get started now »

25,479 Infections
5,981 Hospitalizations
170 Deaths

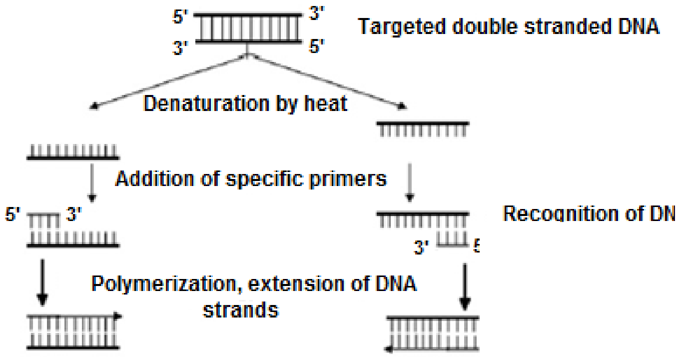
Current Bacterial Detection Methods



Culturing techniques



Enzyme-Linked Immunosorbent Assay (ELISA)



Polymerase Chain Reaction (PCR)

- ✓ Reliable
- ✓ Low limit of detection (single cell)
- ✓ Selective

- ✗ Require enrichment step
- ✗ Require lab settings and trained personnel

Rapid or Near Real-Time Analysis

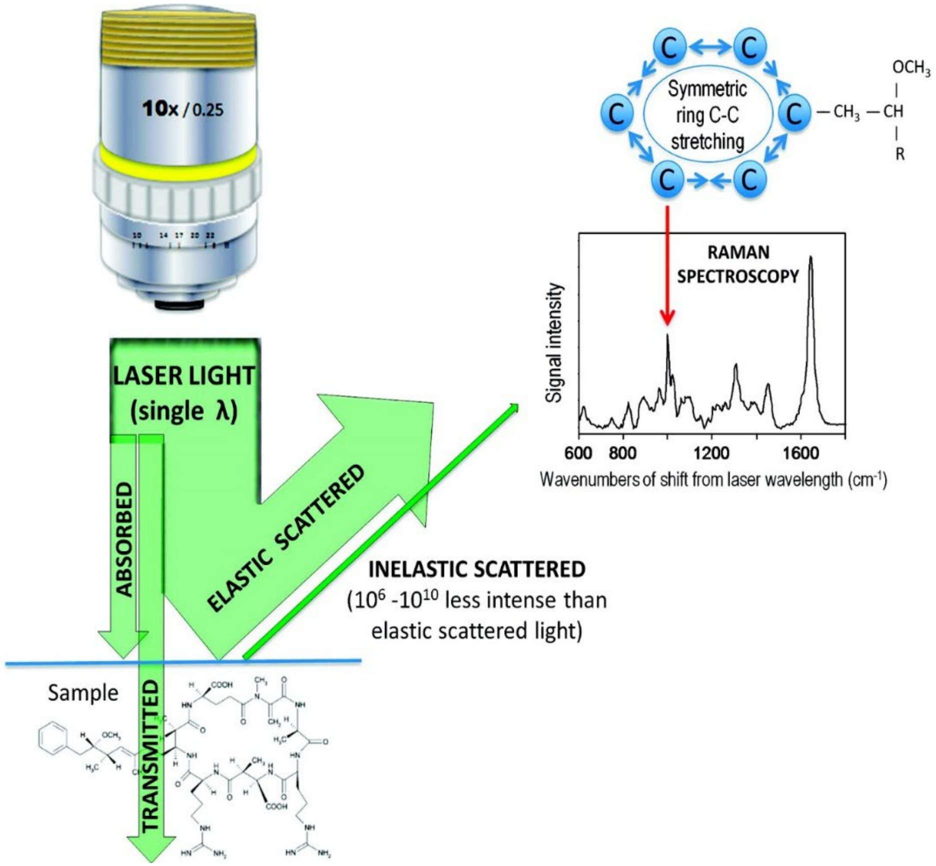
Time Line

At least 16 hr

Need: 15 min to 2 hr

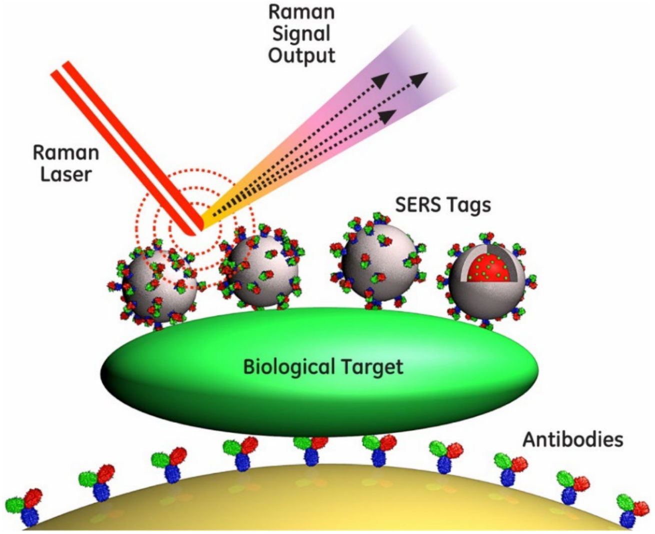
Surface Enhanced Raman Spectroscopy (SERS)

Alternative approach

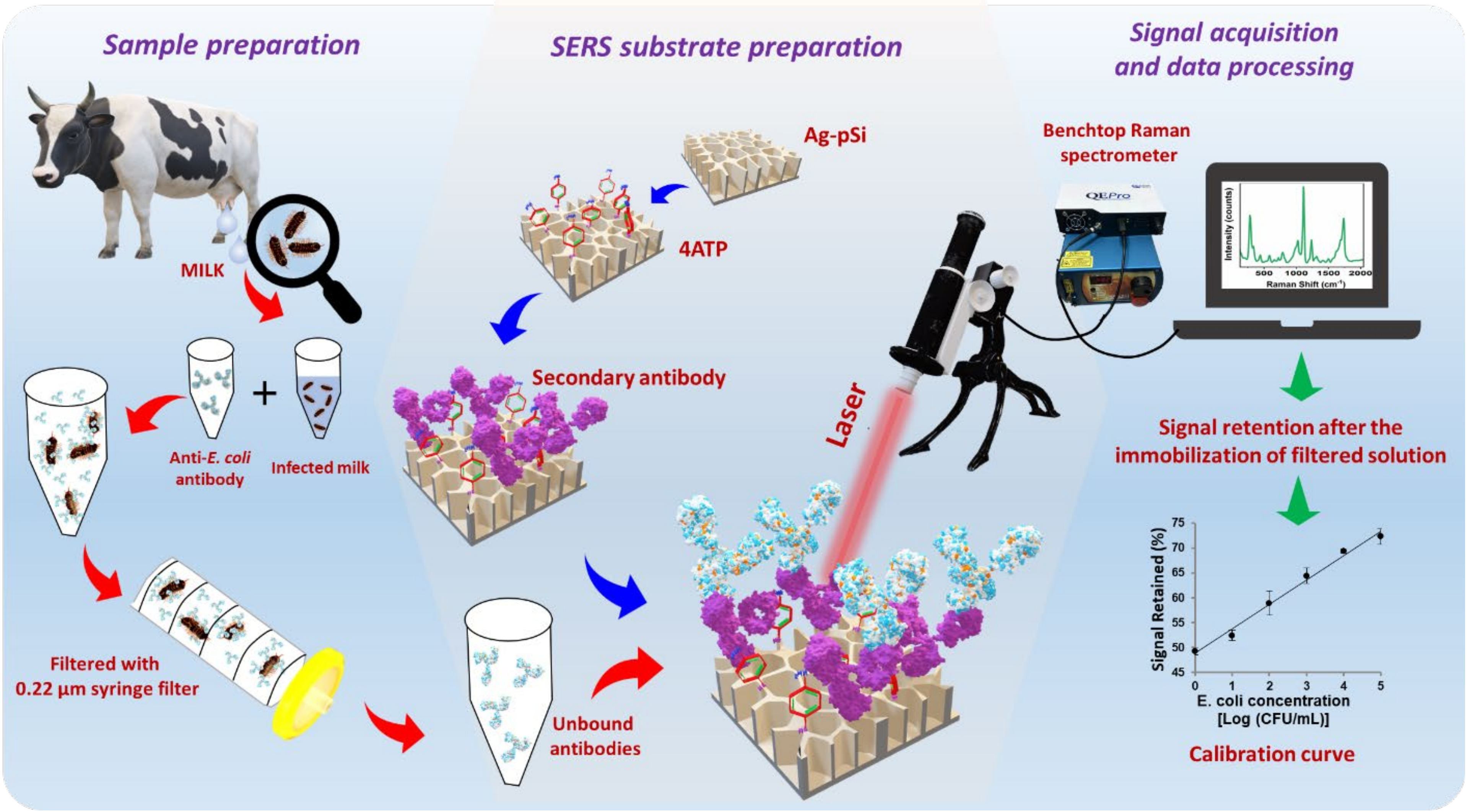


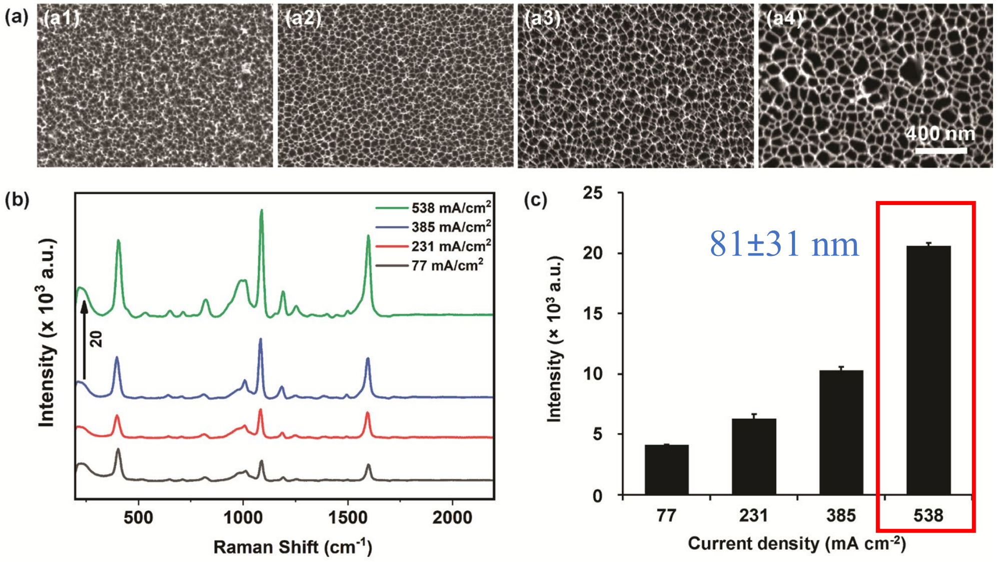
Amplification:

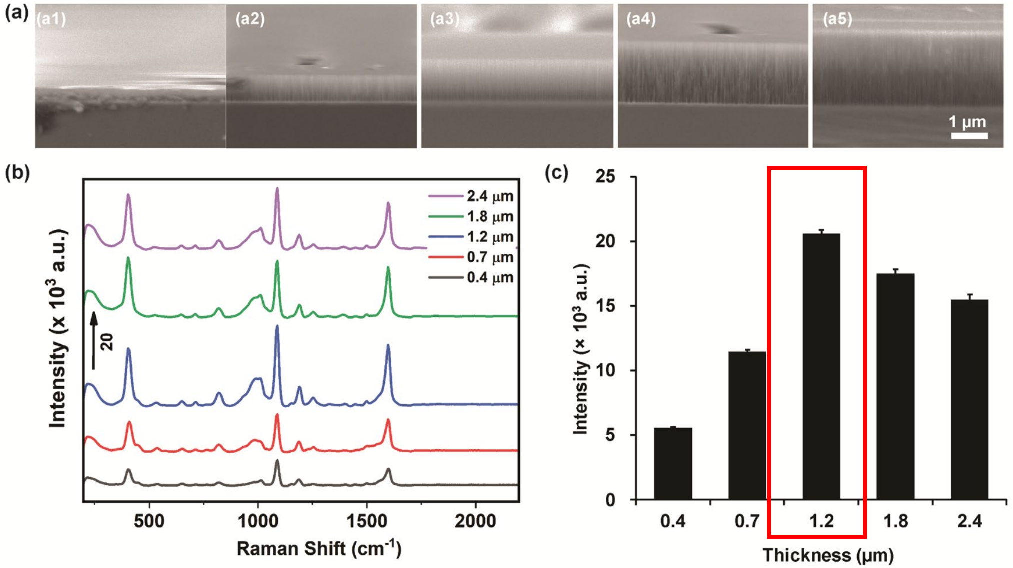
Ag NPs shape & content

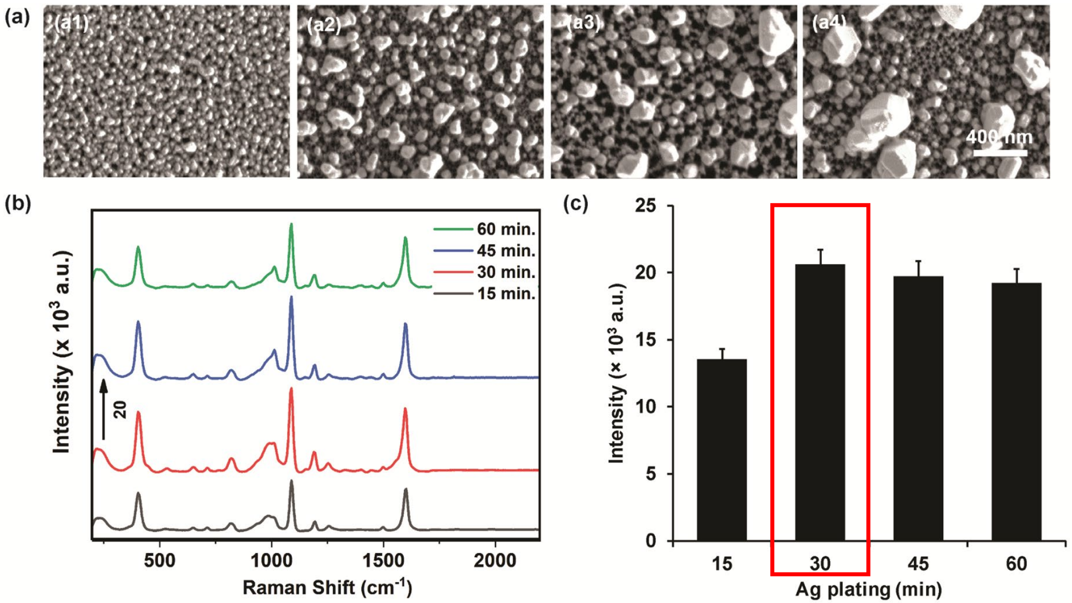


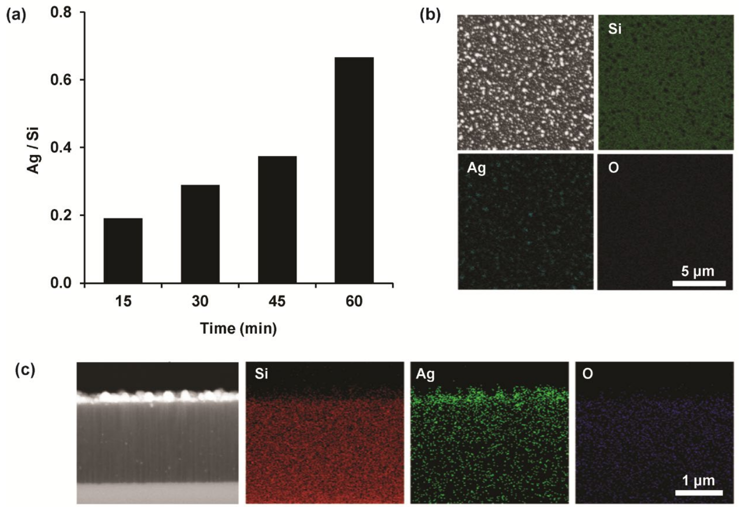
Surface Enhanced Raman Spectroscopy (SERS)

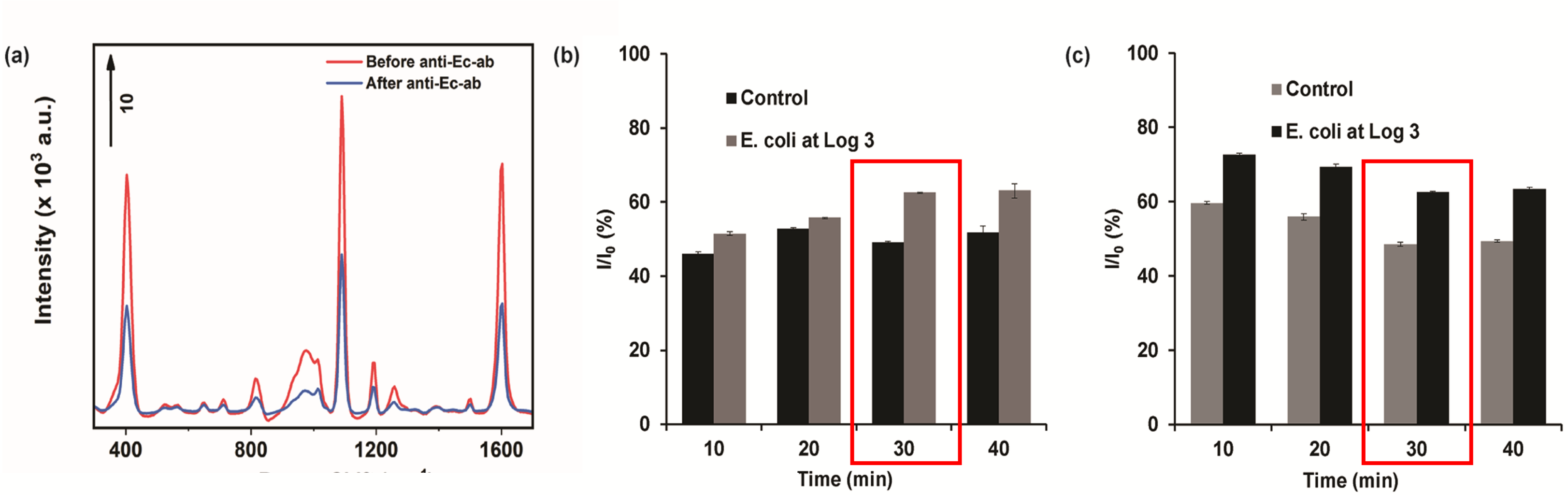


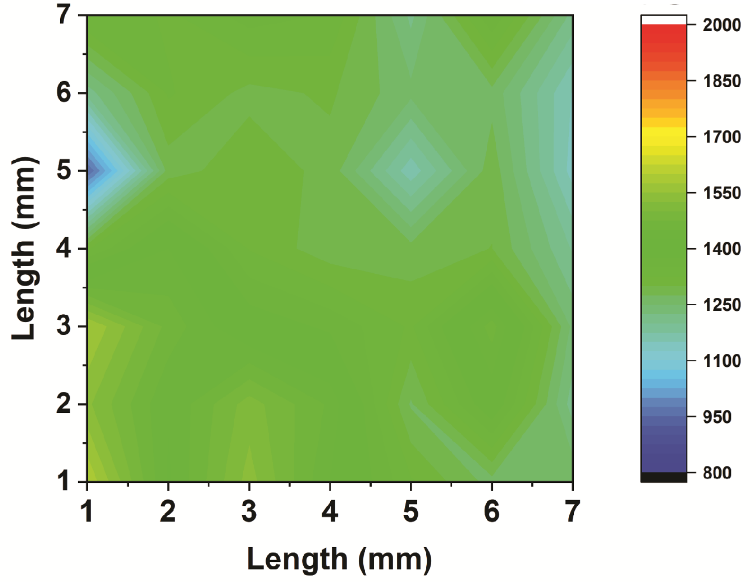

(a) (a1) (a2) (a3) (a4)
400 nm
(b)
538 mA/cm2
385 mA/cm2
231 mA/cm2
77 mA/cm2
Intensity (x 10³ a.u.)
20
Raman Shift (cm⁻¹)
(c)
81±31 nm
Intensity (x 10³ a.u.)
77 231 385 538
Current density (mA cm⁻²)

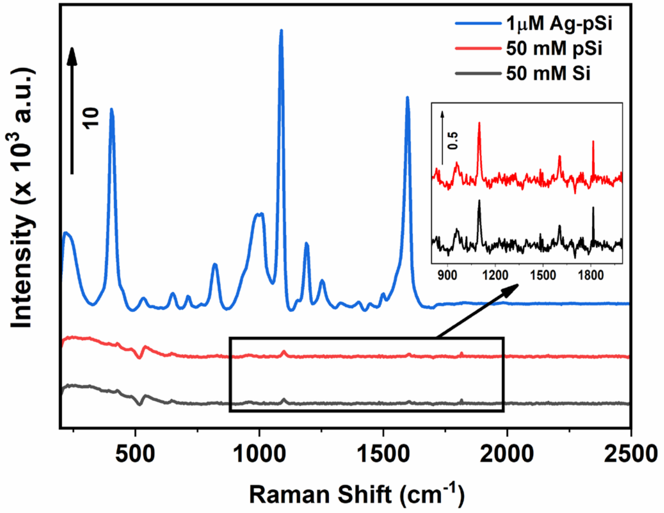

(a) (a1) (a2) (a3) (a4) (a5)
1 μm
(b)
2.4 μm
1.8 μm
1.2 μm
0.7 μm
0.4 μm
Intensity (x 10³ a.u.)
20
Raman Shift (cm⁻¹)
(c)
Intensity (x 10³ a.u.)
0.4 0.7 1.2 1.8 2.4
Thickness (μm)


(a) (a1) (a2) (a3) (a4)
400 nm
(b)
60 min.
45 min.
30 min.
15 min.
Intensity (x 10³ a.u.)
20
Raman Shift (cm⁻¹)
(c)
Intensity (× 10³ a.u.)
15 30 45 60
Ag plating (min)


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

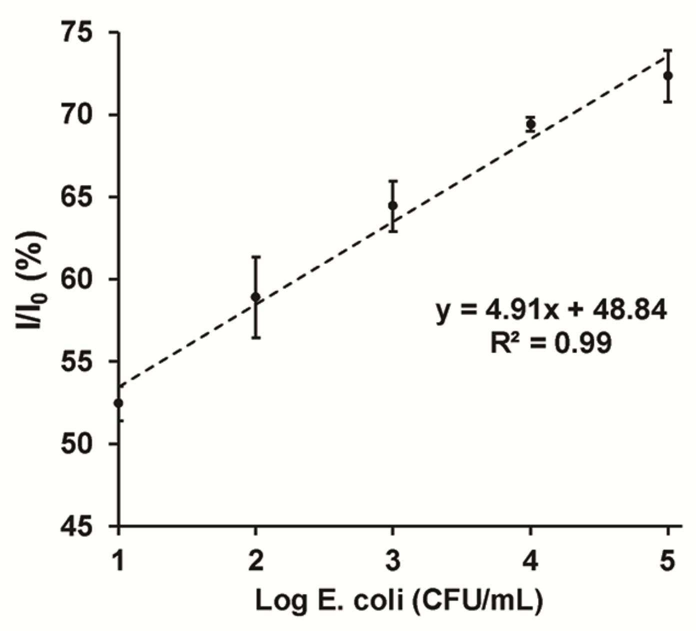




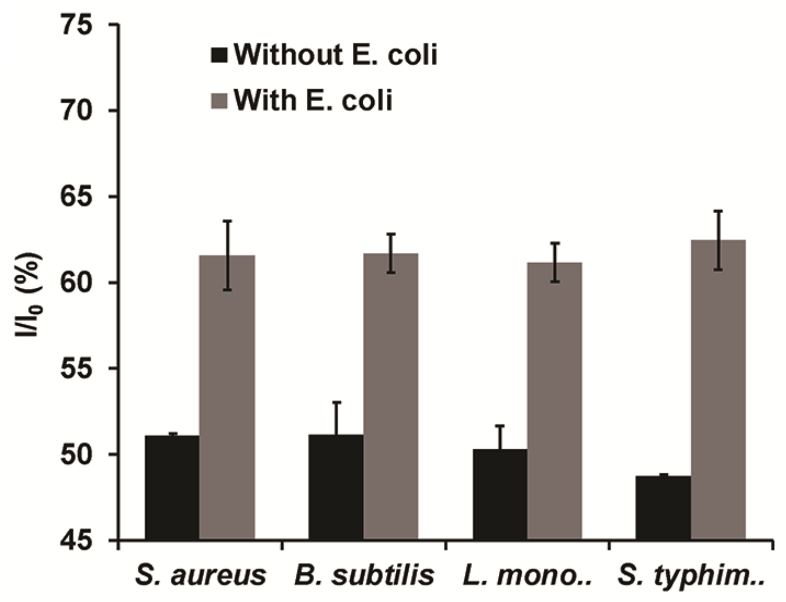


$EF_{4ATP/Ag\text{-}pSi} \sim 5.6\times10^{7}$

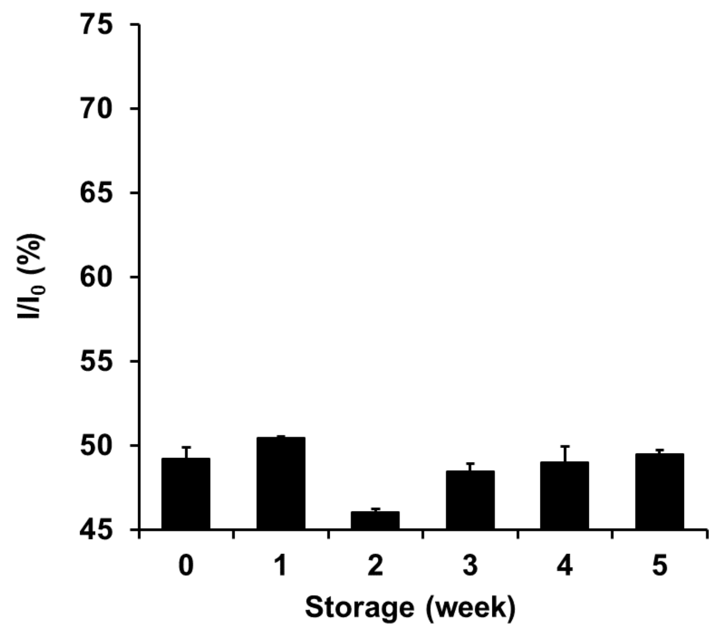
LoD 3 CFU/mL



Specificity & Selectivity

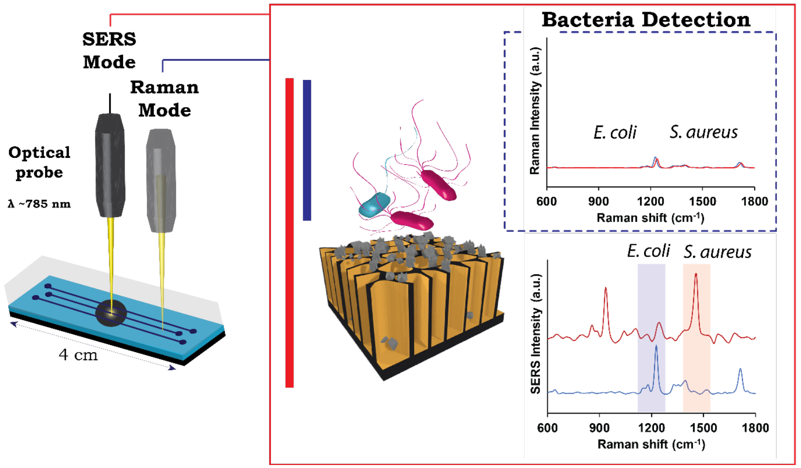


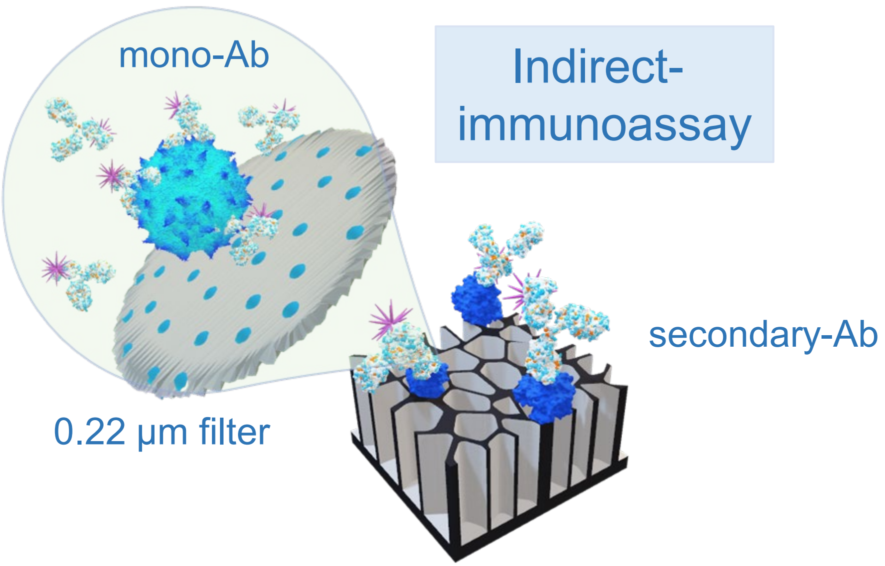
Shelf-life assessment



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

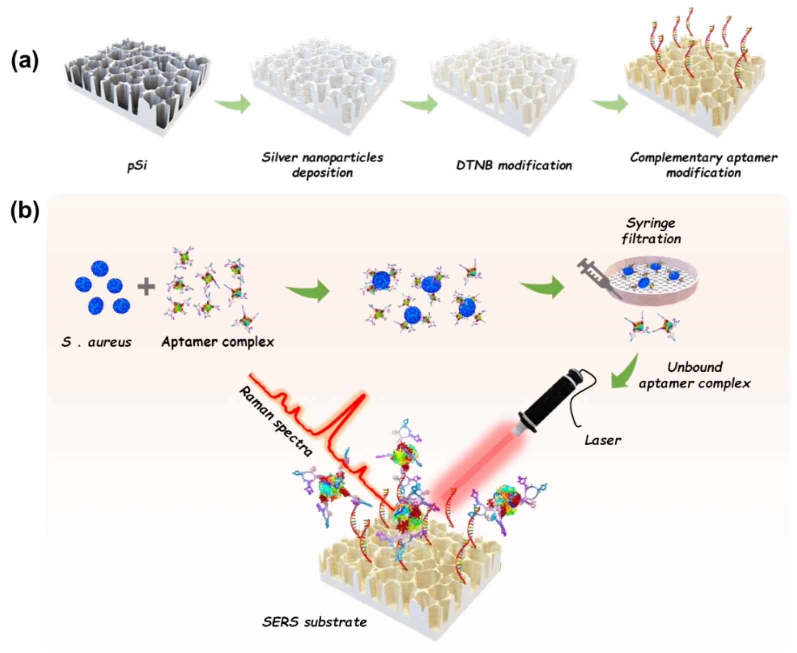




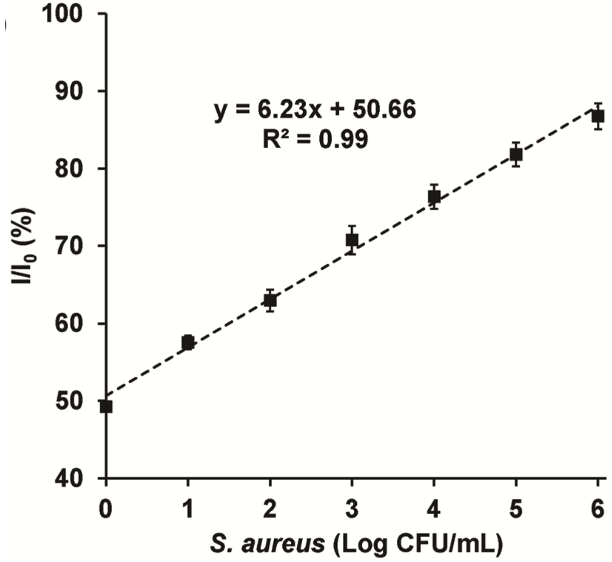
Milk sample	Status	Incubation (hr)	Plate (CFU/mL)	SERS (CFU/mL)	Recovery (%)
Commercial Bovine	Spiked E. coli	0	150	120 ± 14	80
		1	500	391 ± 187	78
		2	1,600	1,693 ± 497	106
		3	16,000	13,369 ± 1,962	84
Whole Bovine	Spiked E. coli	0	80	65 ± 4	81
		1	160	131 ± 15	82
		2	820	845 ± 168	103
		3	2,000	1,958 ± 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

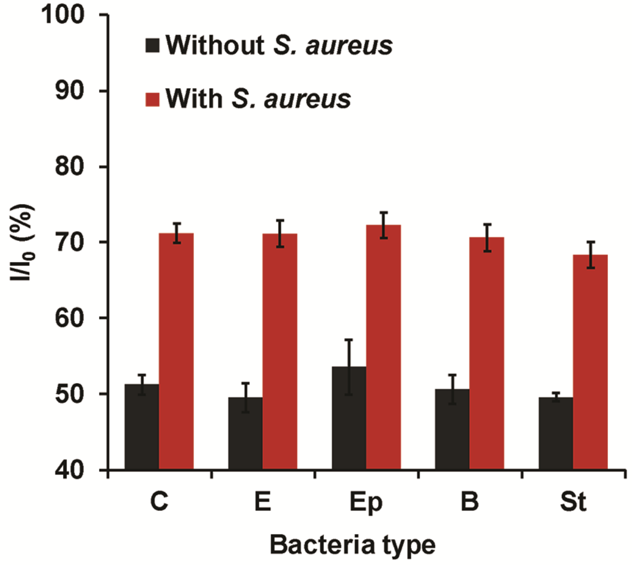
***Milk obtained by Mr. Joseph Lepar



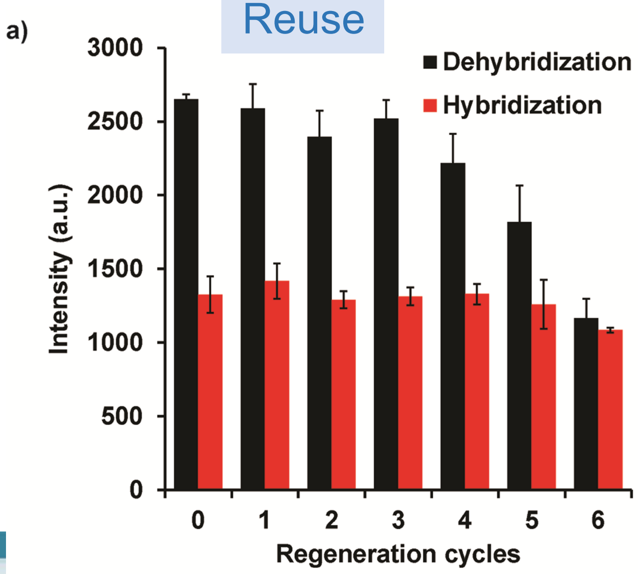
LoD 2 CFU/mL



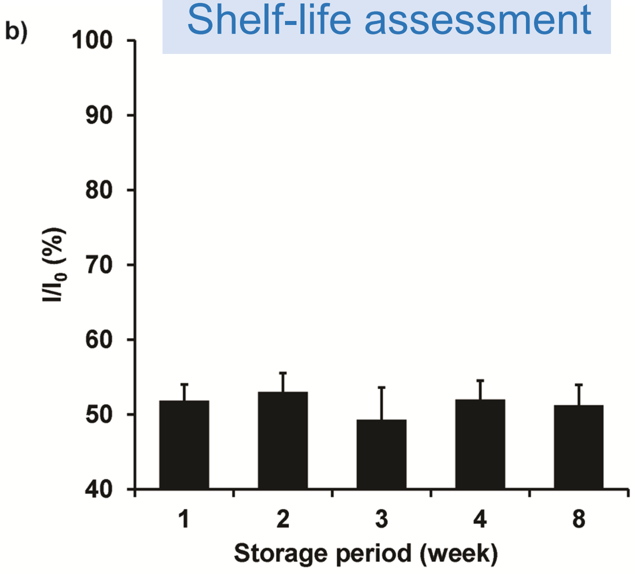
Specificity & Selectivity



Reuse



Shelf-life assessment



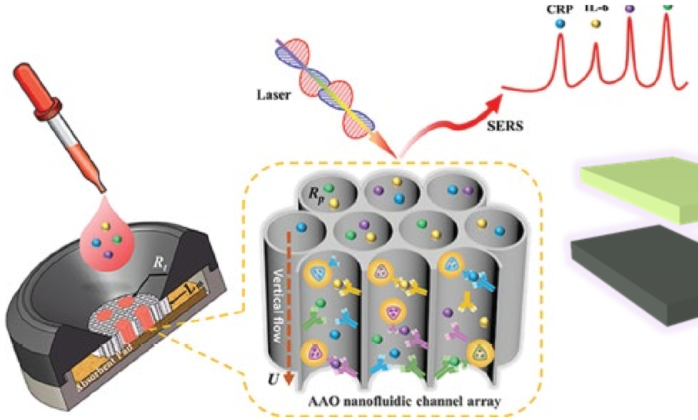
Bacteria Detection by SERS

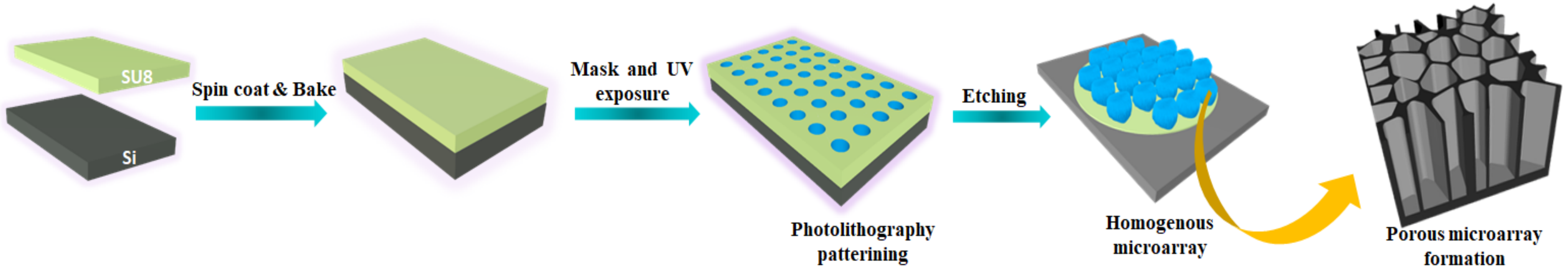
Sample	Spiked (Log CFU mL^{-1})	I/I_0 (%)	SERS (Log CFU mL^{-1})	Plate (Log CFU mL^{-1})	Recovery (%)
Fish	0	54.02±1.86	0.54±0.30	0.00±0.00	-
	2	63.01±1.26	1.98±0.20	2.14±0.08	93
	3	70.59±2.06	3.20±0.33	3.05±0.03	105
	4	78.28±1.95	4.43±0.31	4.18±0.01	106
Pasteurized Milk	0	52.76±1.84	0.34±0.29	0.00±0.00	-
	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

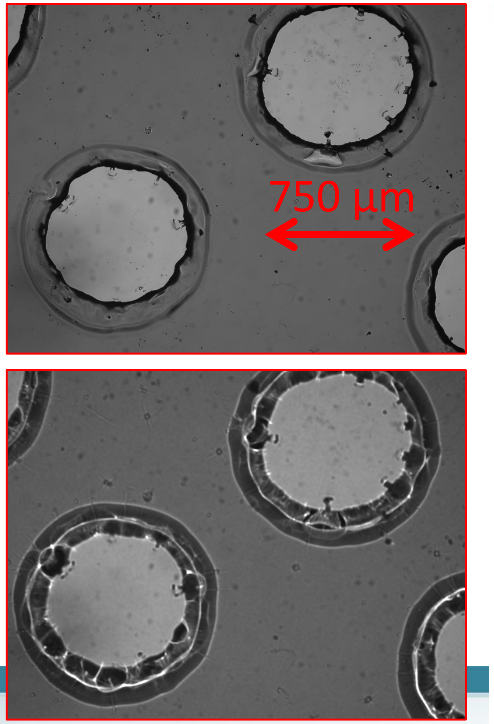
Analytical performance comparison of the developed Ag-pSi SERS substrate to other state-of-the-art techniques.

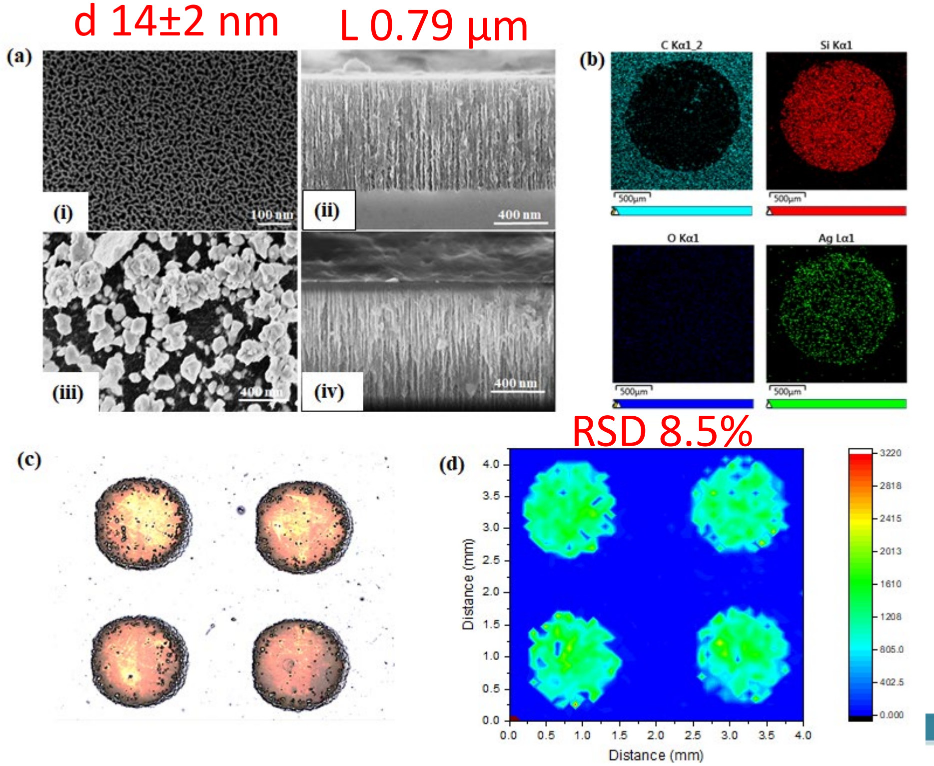
Platform	Method	Milk	Separation	LoD (CFU mL^{-1})	Detection range (CFU mL^{-1})	Total Assay duration	Ref.
Au Nanoflower	Dynamic Light Scattering	Pasteurized	Magnetic	2.7	6×10^0 - 6×10^4	>2 hr	[6]
Microelectrodes	Capacitance	Inoculated and raw milk	Centrifugation	78	$8 \times 10^1 – 3 \times 10^5$	3 hr	[7]
Carbon quantum dots	Fluorescence	Pasteurized	Magnetic	487	5×10^2 - 10^6	>40 min	[8]
Carbon dots microsphere	Fluorescence	Commercial	Magnetic	240	2.4×10^2 - 2.4×10^7	~30 min	[9]
Au nanoclusters	Colorimetry	Ultra-high temperature sterilized, pasteurized, and raw	Centrifugation	560, 500 and 490	10^2 -10^6	>4 hr	[10]
Fe_3O_4@Au	SERS	Commercial	Magnetic	10	10^1 - 10^7	>1.5 hr	[11]
Au-Ag-core-shell	SERS	Commercial	Lateral flow	69	10^2 -10^9	>2 hr	[12]
Ag-pSi	SERS	Commercial and raw	Syringe filtration	3	10^1 - 10^5	1.25 hr	This work

Multiplex SERS - Microarray

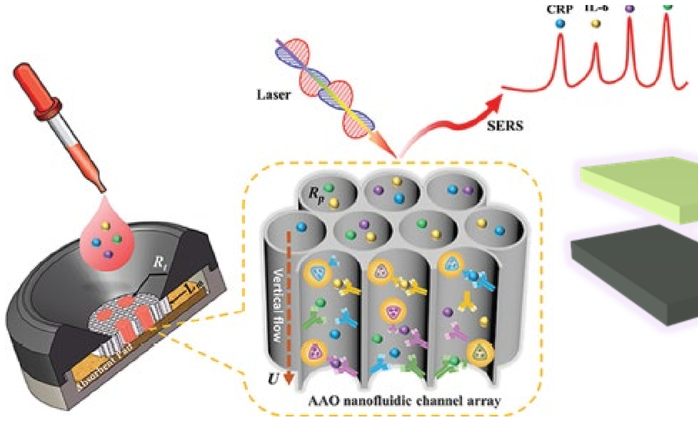


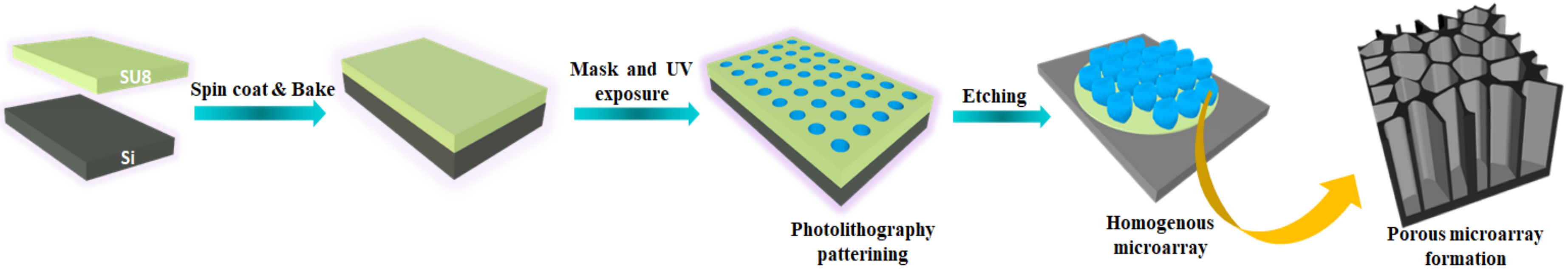


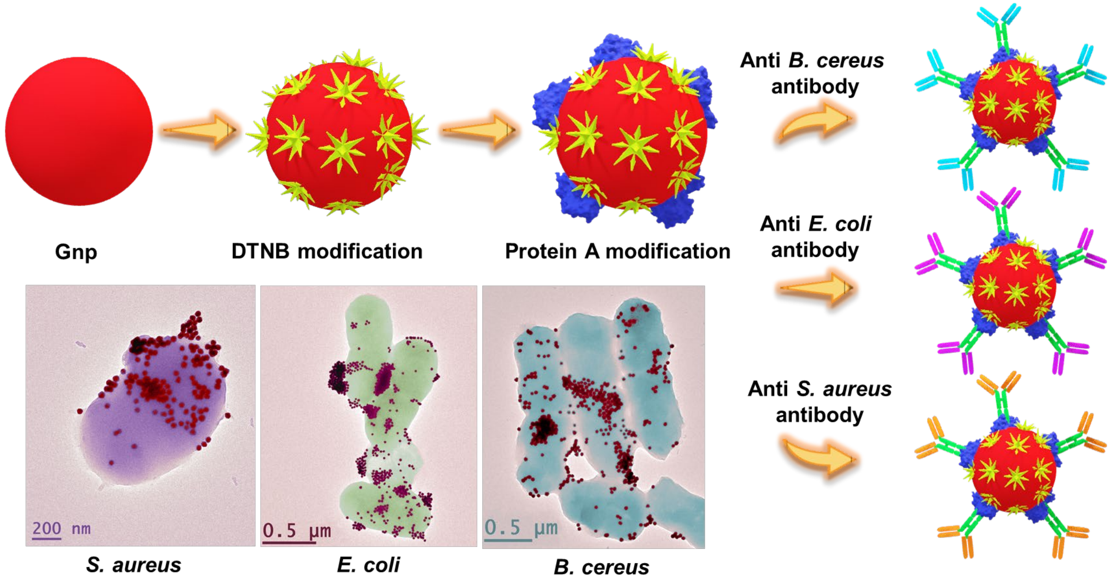


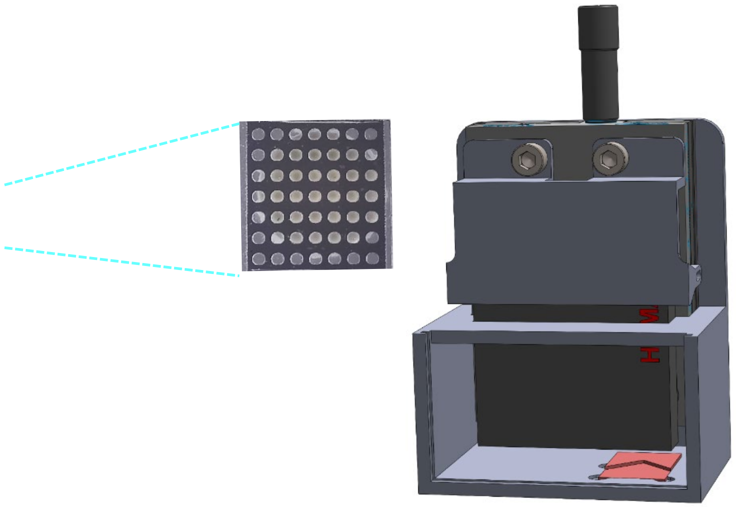


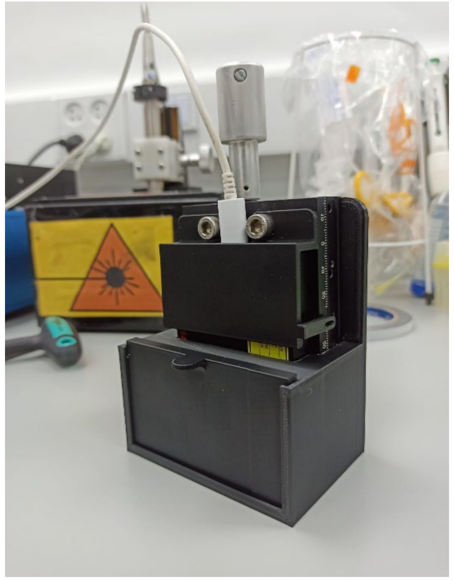
Multiplex SERS - Microarray



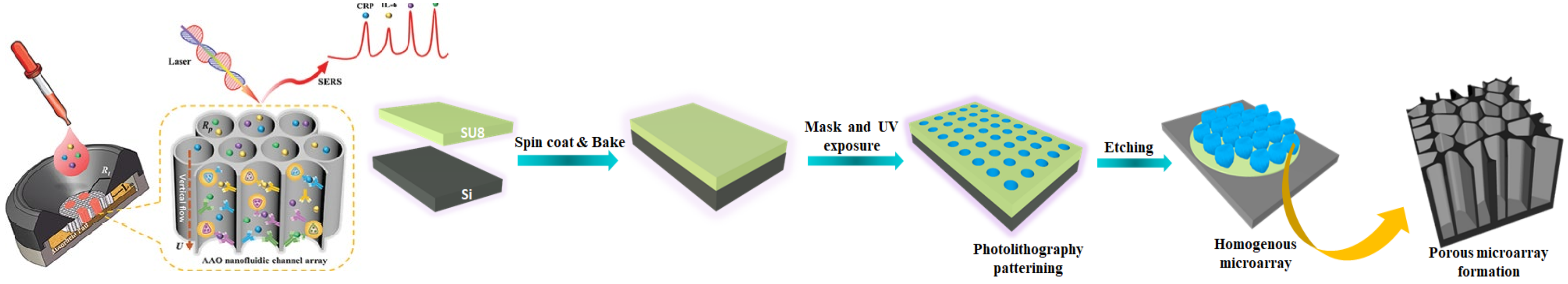


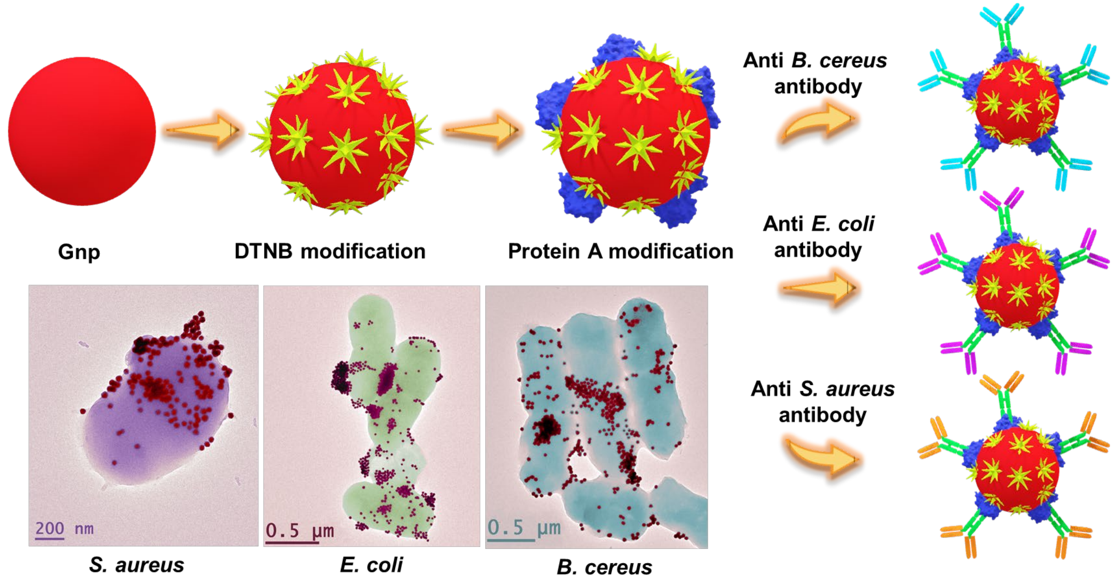






Multiplex SERS - Microarray

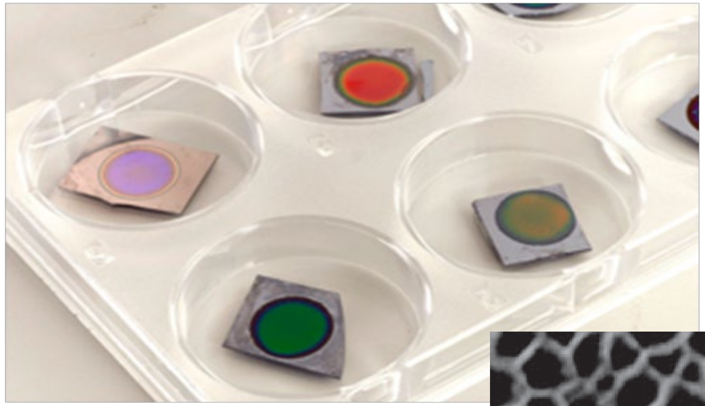


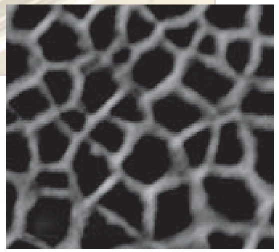


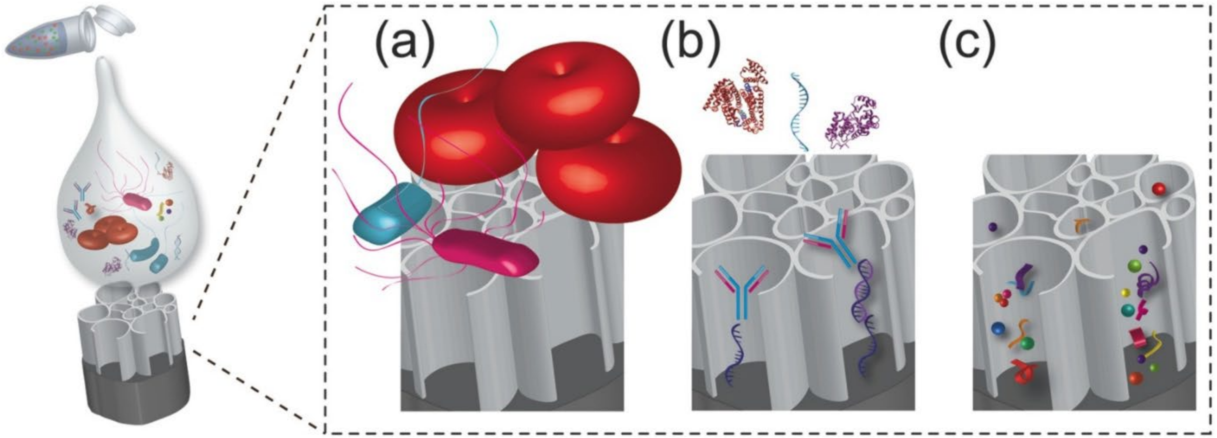
Pathogen	Spiked: Plate counting (Log CFU mL^{-1})	Measured SERS: I/Ic (%)	Measured SERS: (Log CFU mL^{-1})	Recovery (%)
Water				
B. cereus	1.9±0.1	72.5±0.9	1.7±0.1	89.3
E. coli	2.4±0.0	71.7±3.4	2.0±0.1	81.4
S. aureus	2.4±0.0	77.9±5.7	2.0±0.1	81.2
Lettuce				
B. cereus	1.9±0.1	64.8±7.2	1.7±0.0	93.2
E. 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

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.**



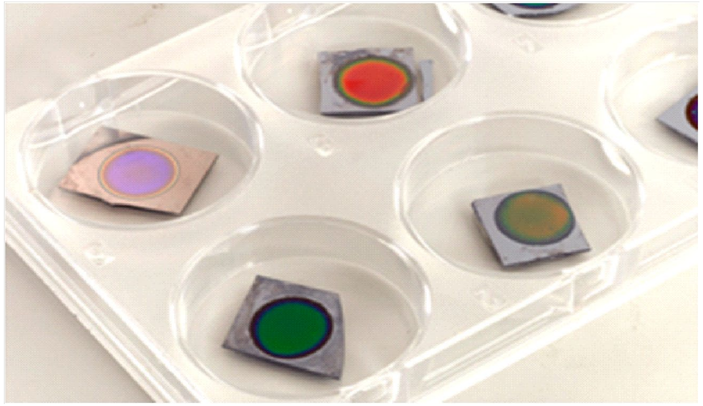




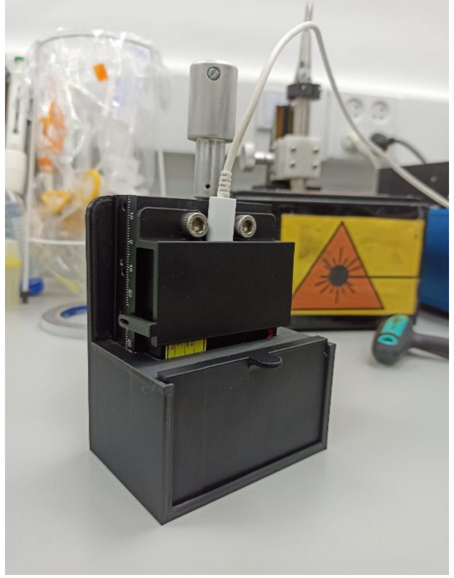
Our System

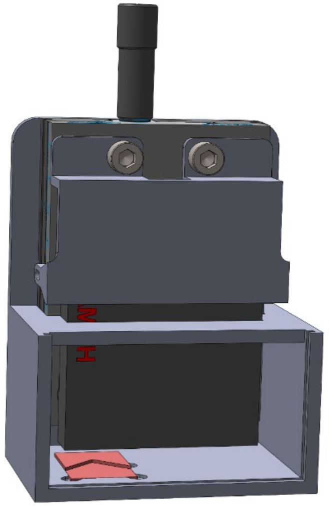
Mini-Spectrometer

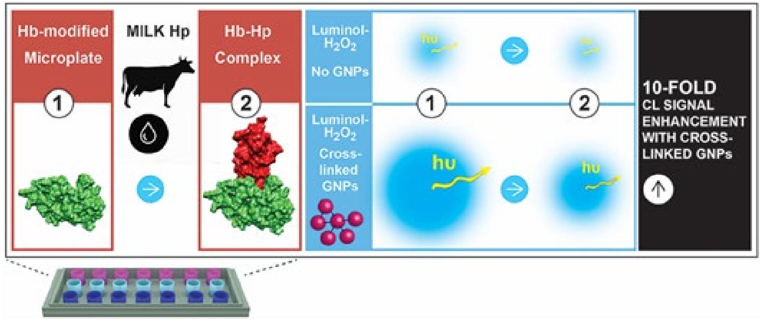
Patented Nano sensors

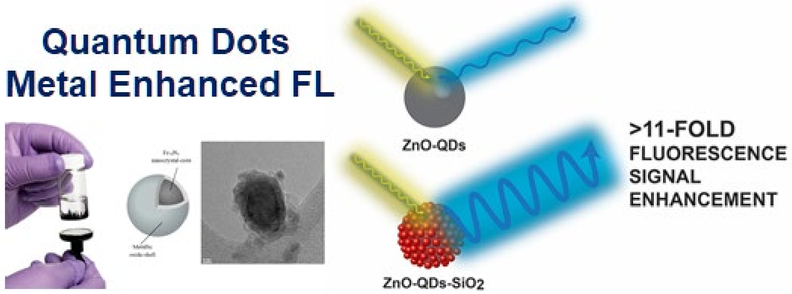


Next Challenge: Technology utilization by the farmer/grower









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Dr. Nanda Kumar
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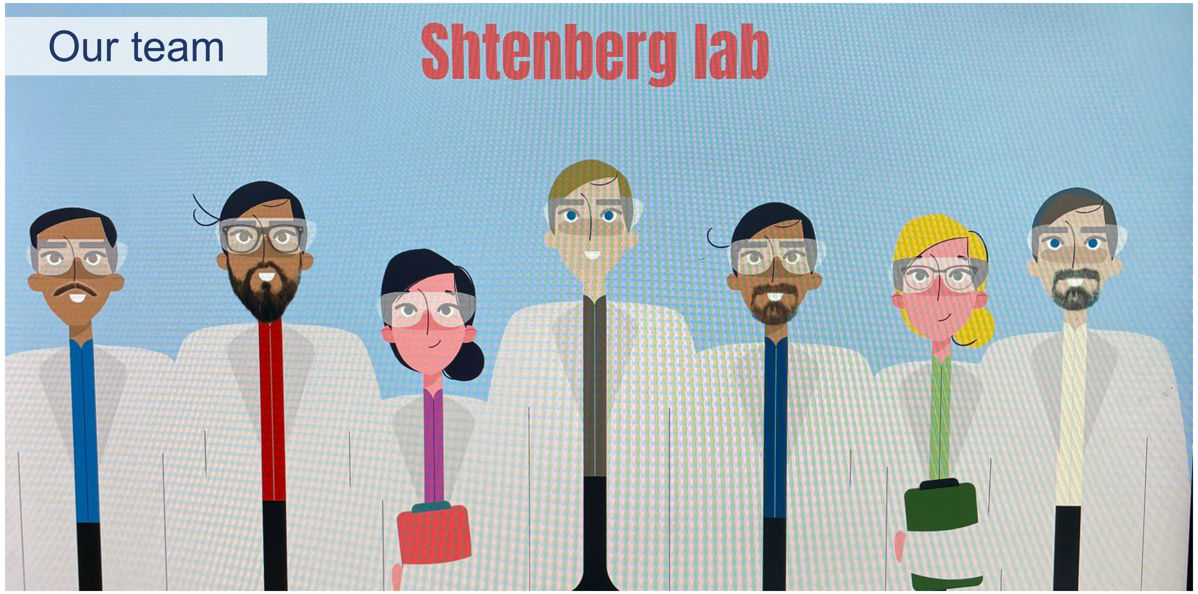

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