KKU KHON KAEN UNIVERSITY MDKKU Medical Microbiology RCEID

Medical Microbiology Seminar

Advancing SERS-Based Liquid Biopsy:

From Substrate Engineering to Clinical Differential Diagnosis

Mr. Patipan Wongboothong

2nd Year Ph.D Student of Precision Medical Microbiology, Faculty of Medicine, KKU

Advisor: Prof. Kiatichai Faksri, Ph.D

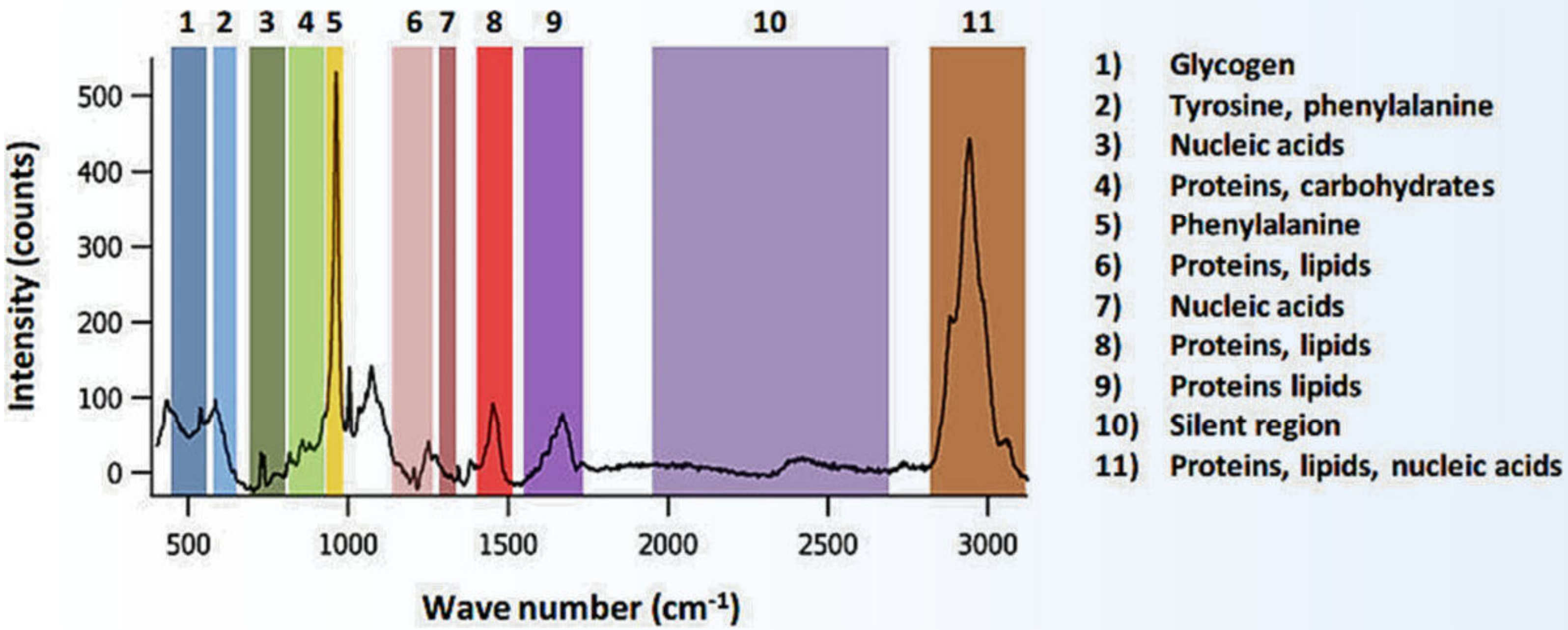
Contents

Introduction

Raman Spectroscopy

- Raman is an analytical technique that uses the vibration patterns of light to provide unique "fingerprints" for molecular identification.
- This method is non-destructive and exhibits high sensitivity.

The figure represents the different types of Raman spectroscopy



Example of a Raman spectrum of a biological sample, highlighting characteristic Raman bands and the related molecular assignment (Conforti et al., 2024)

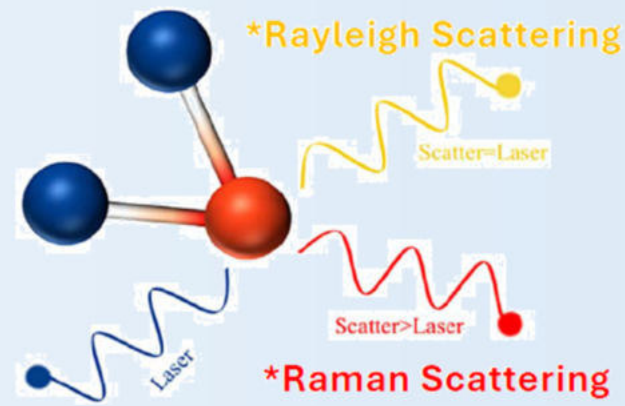


Figure represents a schematic diagram of the simple principles of the Raman scattering effect.

Conforti PM, Lazzini G, Russo P, D'Acunto M. Raman spectroscopy and AI applications in cancer grading: An overview. IEEE Access. 2024;12:54816–52. doi:10.1109/access.2024.3388841

Introduction

Surface-Enhanced Raman Spectroscopy (SERS)

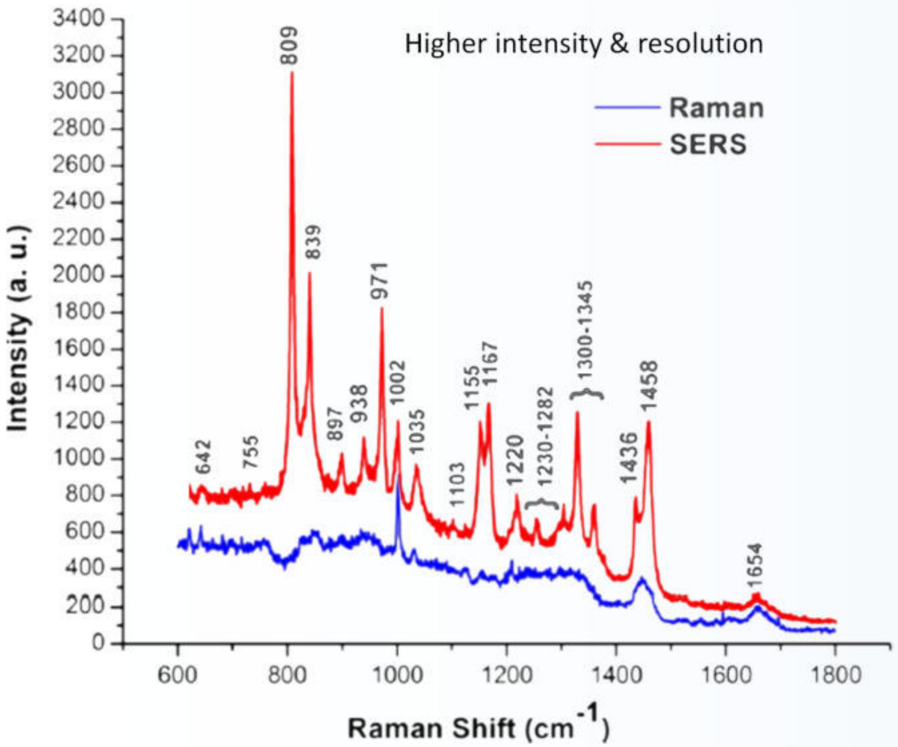


Figure represents different between SERS and Raman spectra

- SERS is a *technique* that can amplify Raman spectra, allowing for greater sensitivity in detecting low-concentration analytes.
- Can be enhanced up to 10^{10} - 10^{14} and can detect even a single molecule theoretically.

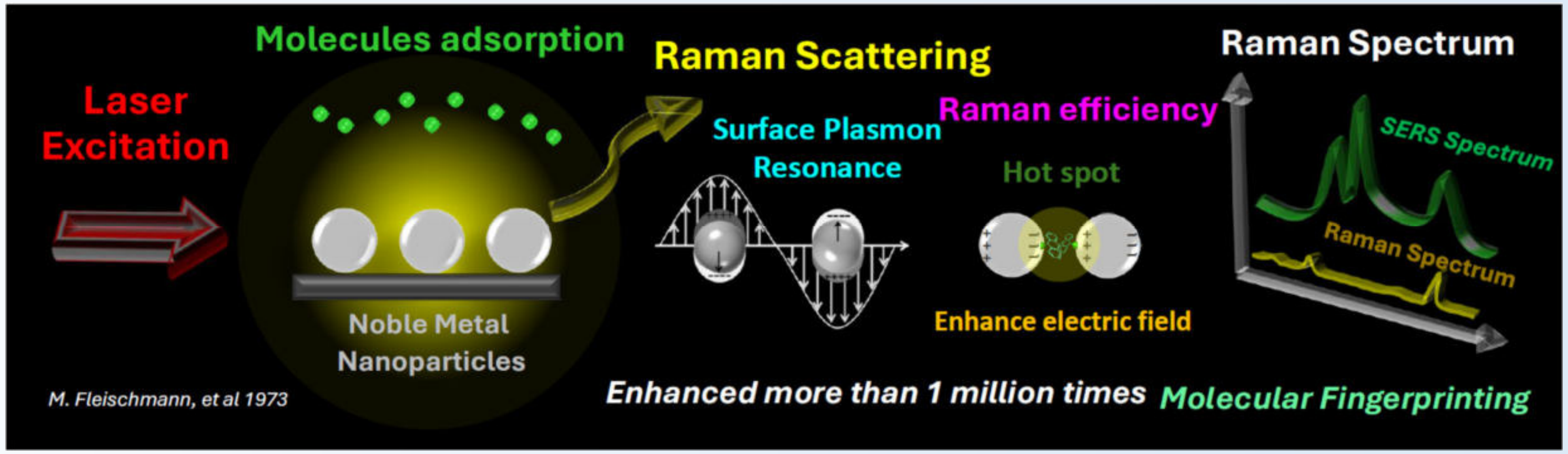
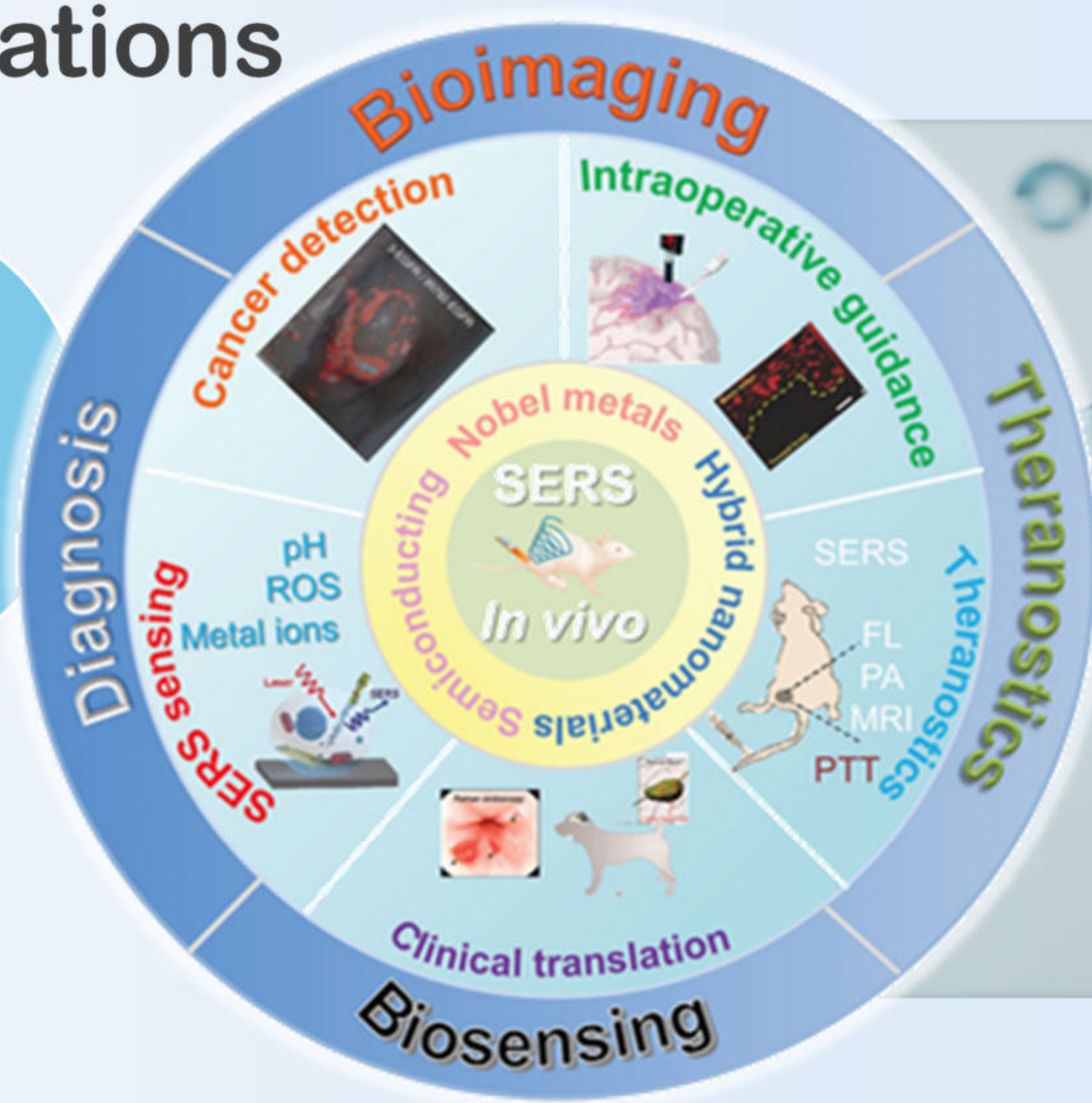


Figure represent mechanism for enhanced Raman signals from SERS (Nuntawong N., 2024)

Ong TTX, Blanch EW, Jones OAH. Surface Enhanced Raman Spectroscopy in environmental analysis, monitoring and assessment. Science of The Total Environment. 2020 Jun 10;720:137601.

SERS in Biomedical Applications

- Cancer detection
- Bacterial diagnosis
- Viral detection
- Disease diagnosis
- Metabolite detection



> ACS Omega. 2023 Aug 3;8(32):29836-29846. doi: 10.1021/acsomega.3c04910. eCollection 2023 Aug 15.

Solid-State Au Nanocone Arrays Substrate for Reliable SERS Profiling of Serum for Disease Diagnosis

Yanyan Lu [1 2], Biao Lei [1 2], Qian Zhao [1], Xiaowei Yang [1 2], Yi Wei [1], Tingting Xiao [1], Shuyi Zhu [1 2], Yu Ouyang [3], Hongwen Zhang [1 4], Weiping Cai [1 2]

Affiliations + expand

PMID: 37599935 PMCID: PMC10433333 DOI: 10.1021/acsomega.3c04910

1st paper

2nd paper

▸ Diagnostics (Basel). 2025 Mar 8;15(6):660. doi: 10.3390/diagnostics15060660

Detection of Respiratory Disease Based on Surface-Enhanced Raman Scattering and Multivariate Analysis of Human Serum

Yulia Khristoforova [1,*], Lyudmila Bratchenko [1], Vitalii Kupaev [2], Dmitry Senyushkin [3], Maria Skuratova [4], Shuang Wang [5], Petr Lebedev [6], Ivan Bratchenko [1]

Editor: Te-Chun Shen

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PMCID: PMC11940998 PMID: 40150003

1st Paper

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Affiliations + expand

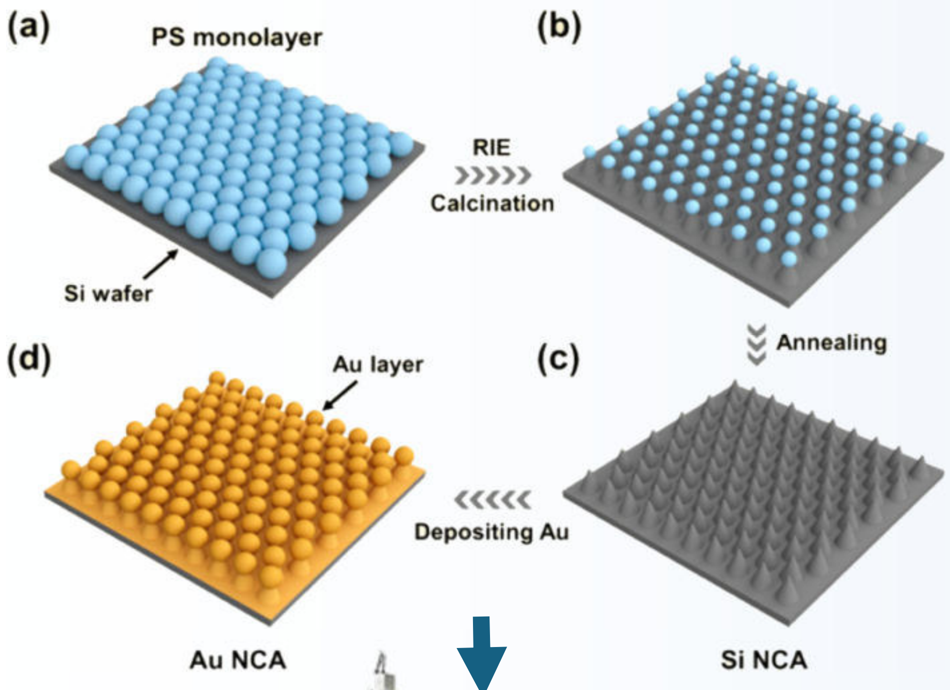
PMID: 37599935 PMCID: PMC10433333 DOI: 10.1021/acsomega.3c04910

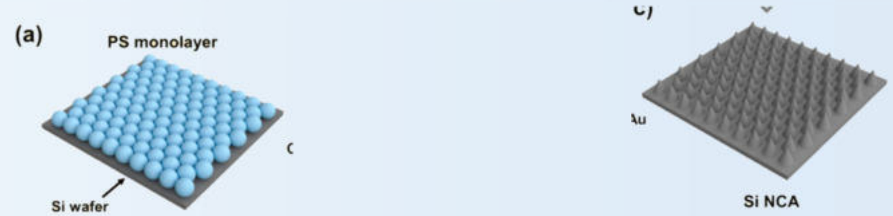
2023
Quartile 1

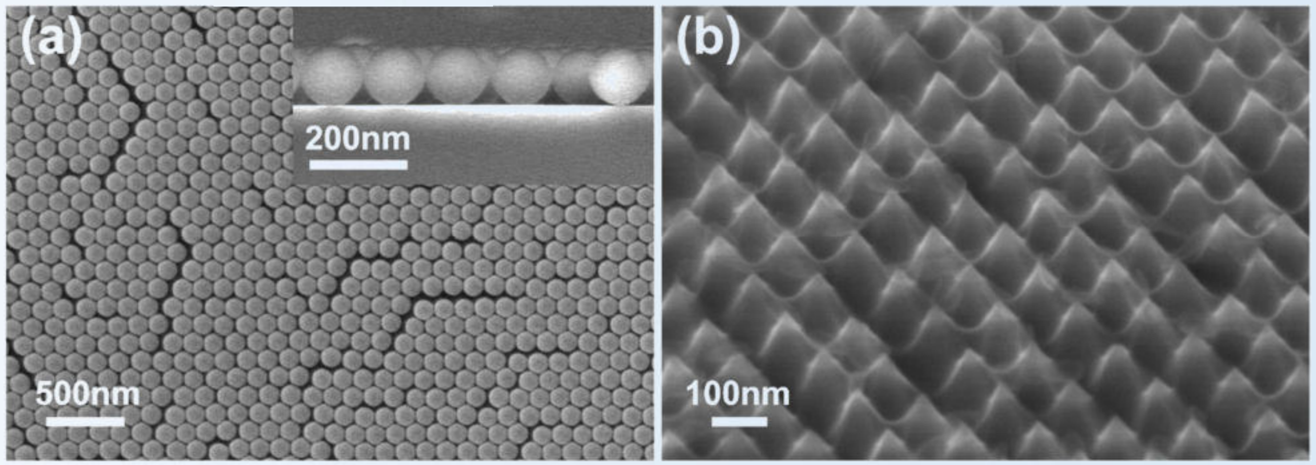
Objective: To develop a standardized detection method for obtaining highly stable and repeatable serum SERS spectra using a solid-state Au nanocone array (Au NCA) plasmonic substrate

Method & Result 1

Morphology and Structure of Au NCA







The close-packed hexagonal arrangement of Polystyrine spheres with a diameter of 120 nm

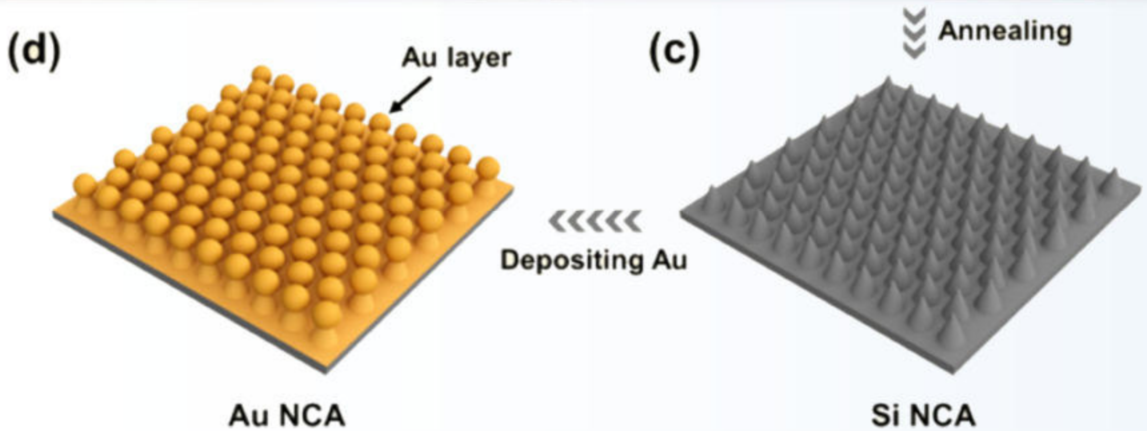
Si NCAs

FE-SEM observations during fabrication

FE-SEM: Field Emission Scanning Electron Microscope
PS: polystyrene

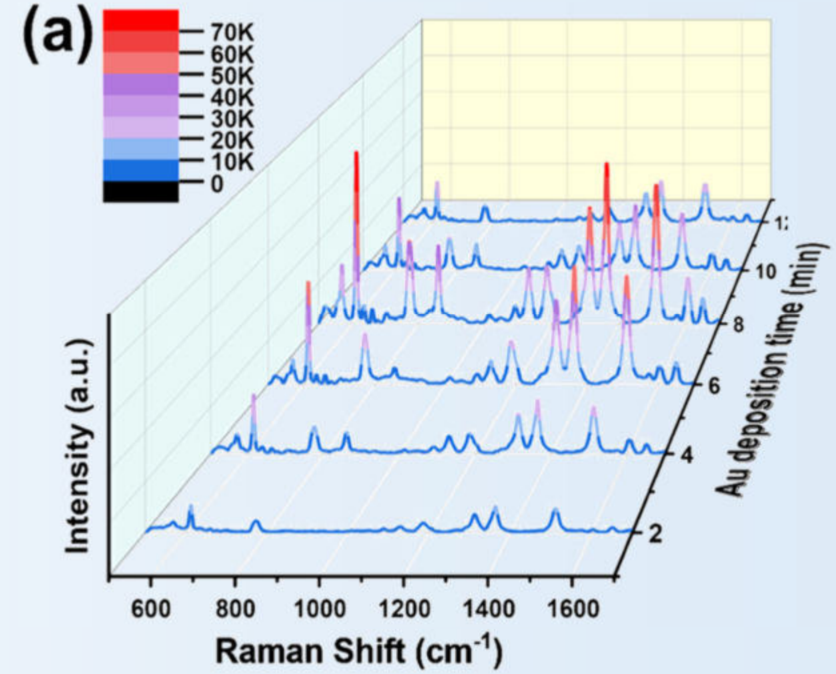
Method & Result

Reliable Au NCA Plasmonic Substrates



Varies Au deposition time (2, 4, 6, 8, 10, 12 min) with deposition rate of 12.5 nm/min

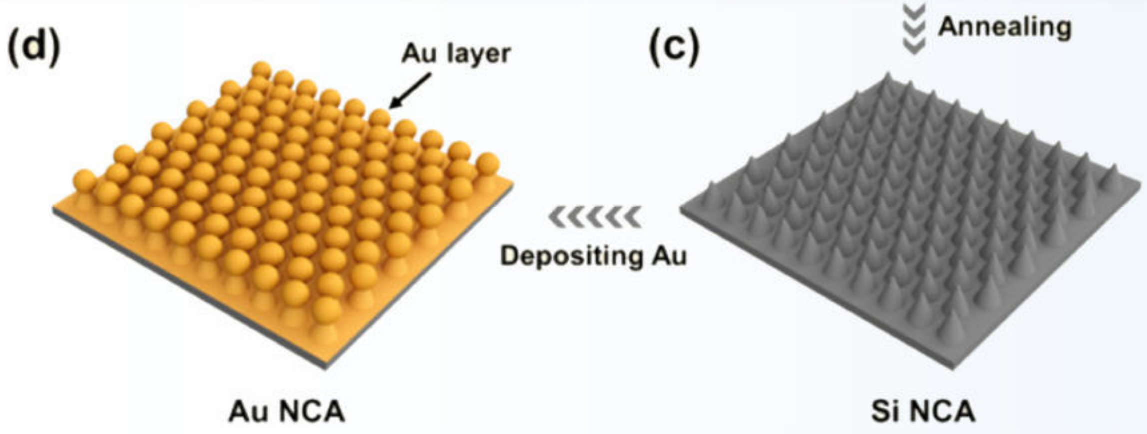
Soaked in 10^{-5} M R6G aqueous solution for 90 min



Strong signals on substrates ranging from 6 to 8 min, and the best enhancement effect was obtained with an Au deposition time of **8 min**

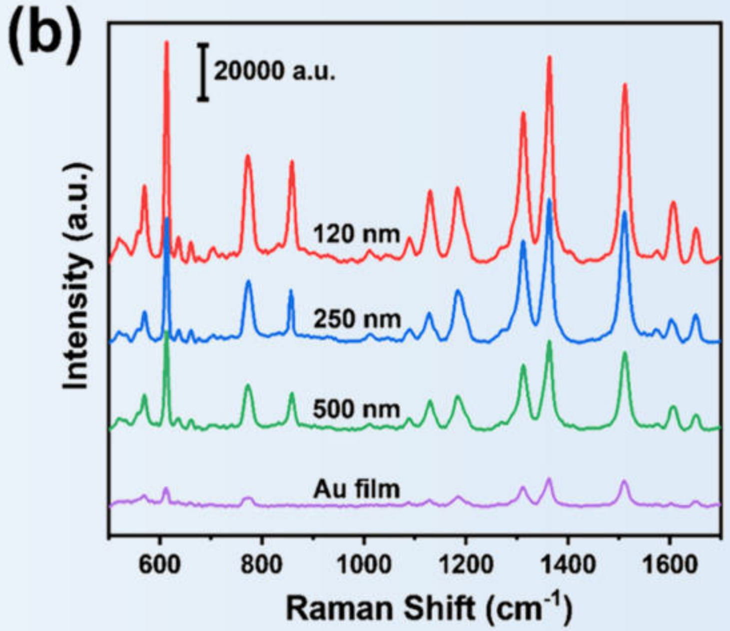
Method & Result

Reliable Au NCA Plasmonic Substrates



Varies Au colloidal sphere
(120, 250, 500 nm, gold film)

Soaked in 10^{-5} M R6G aqueous solution for 90 min

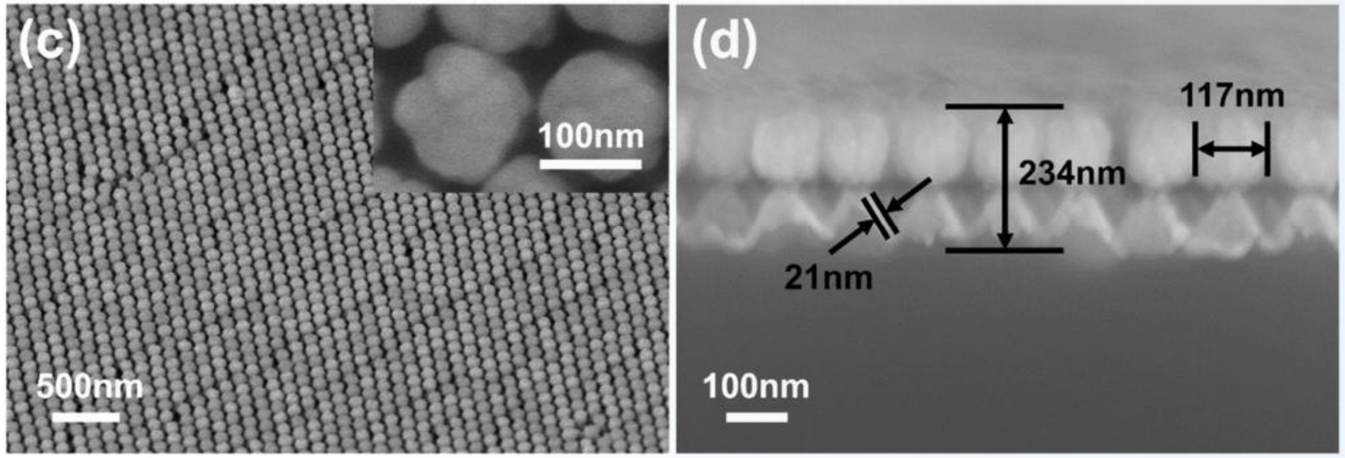


Raman signal of R6G with **120 nm** was significantly higher.

A smaller arrangement of Au particles provides a higher density of "hotspots".

Method & Result

Reliable Au NCA Plasmonic Substrates



Typical FE-SEM observations during fabrication of Au NCA plasmonic substrate

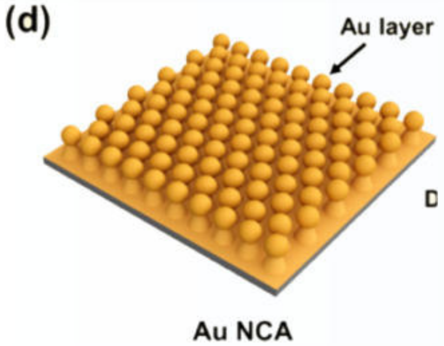
- From FE-SEM, show a uniform array of Au nanoparticles
- Size of Au NCA approximately 117 nm in diameter
- Abundant nanogaps between the Au nanoparticles
- Nearly spherical Au particles were located on top of each Si nanocone

They chose Au NCA with a period of **120 nm** and an Au deposition time of **8 min** as the preferred substrate for further research.

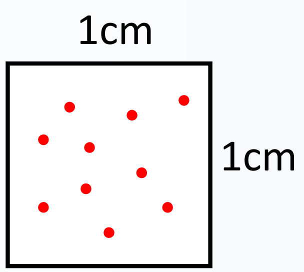
FE-SEM: Field Emission Scanning Electron Microscope

Method & Result

Reliable Au NCA Plasmonic Substrates

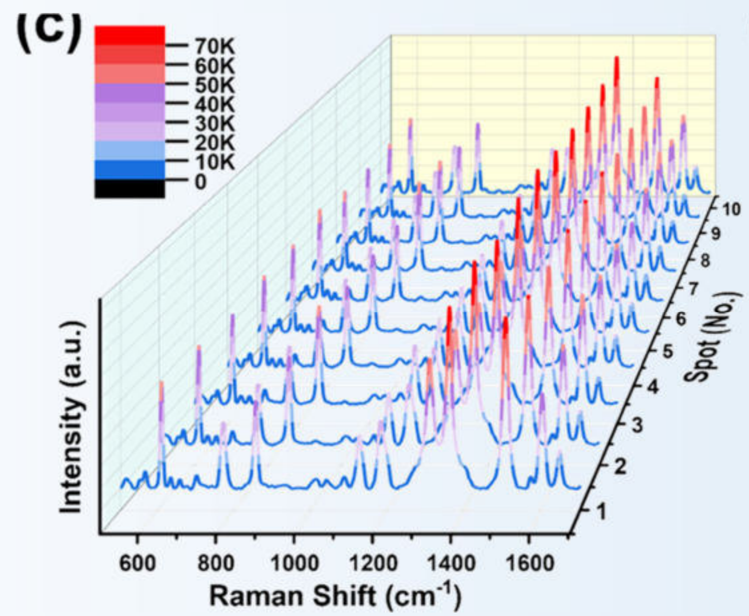


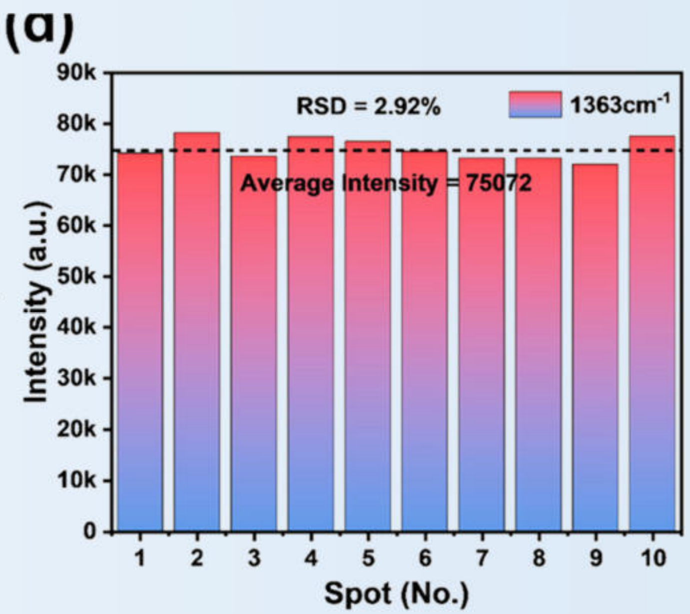
Soaked in 10^{-5} M R6G aqueous solution for 90 min



Randomly selected 10 points

Uniformity and Consistency





The relative standard deviation (RSD) of the characteristic peak intensities was small, typically less than 2.92% for the peak at 1363 cm^{-1}

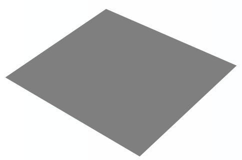
Guarantee excellent signal reproducibility of the substrate

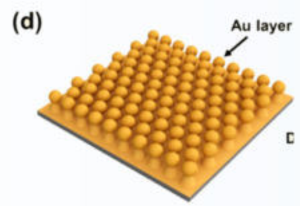
Method & Result

Quantitively evaluate the SERS enhancement effect

10 μL of 0.1 M R6G

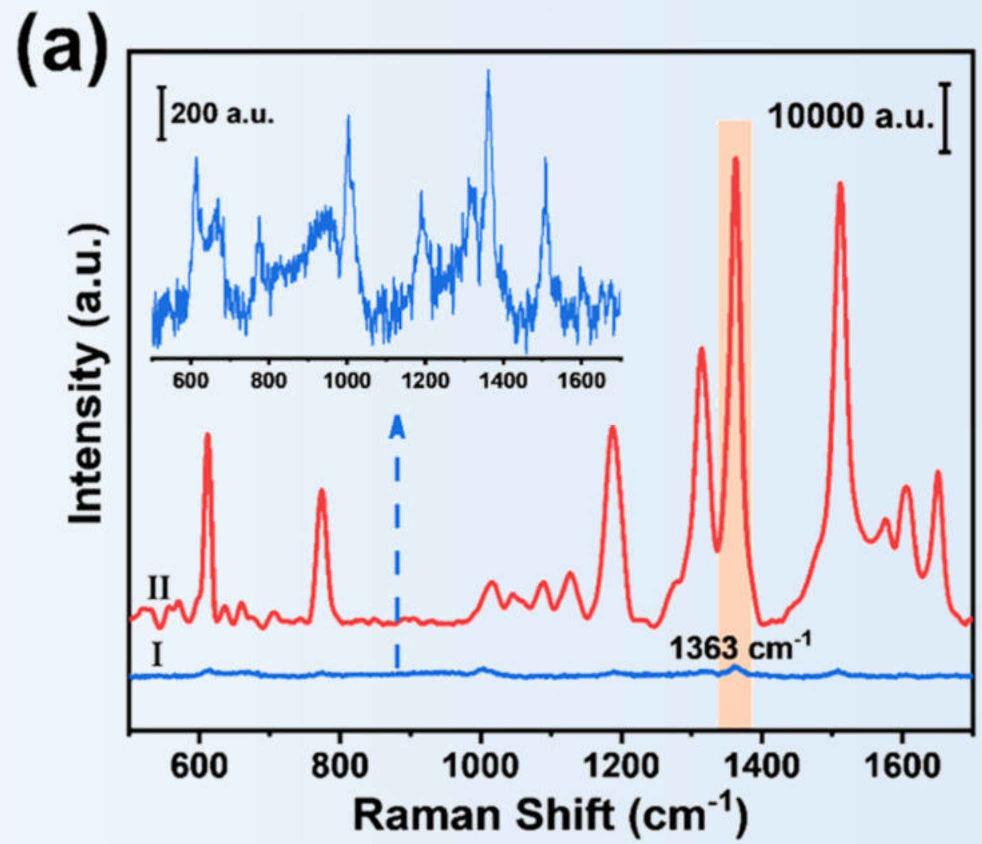
5 μL of 10^{-5} M R6G





Si wafer

Au NCA

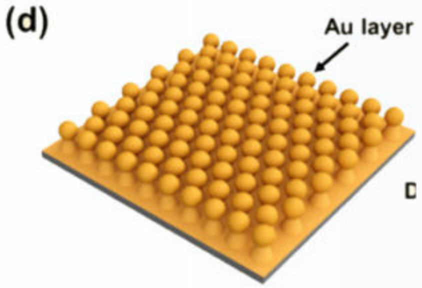


The enhancement factor (EF) estimated was up to 1.9×10^6

Au NCA has a powerful SERS enhancement up to a million times that of normal Raman

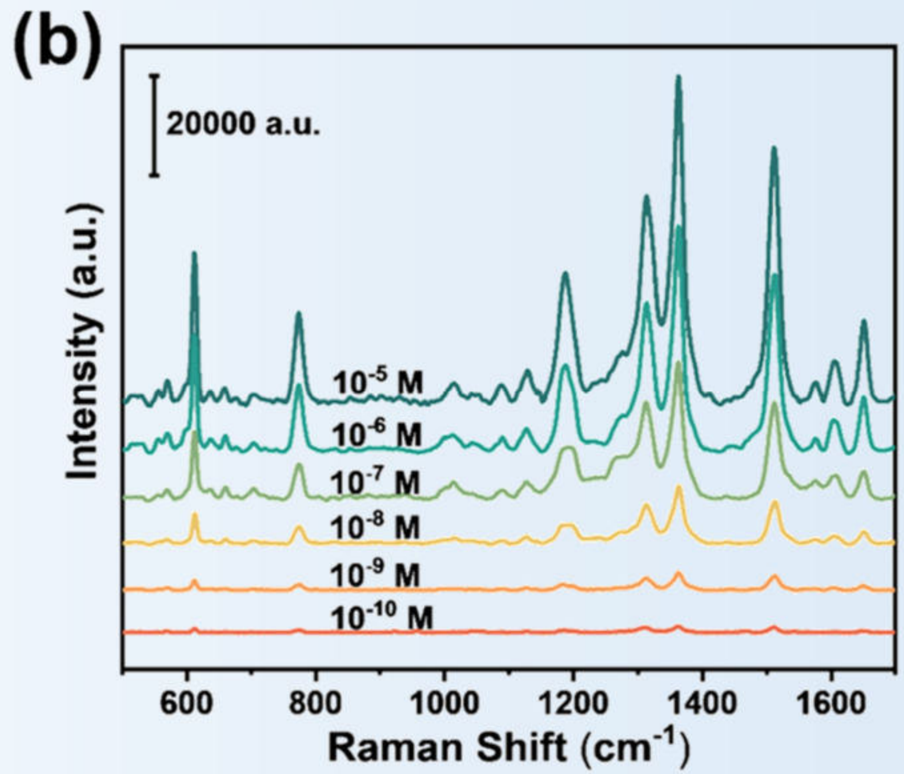
Method & Result

Quantitively evaluate the SERS enhancement effect



Au NCA

5 μL R6G with different concentrations (10^{-5}, 10^{-6}, 10^{-7}, 10^{-8}, 10^{-9}, 10^{-10} M)

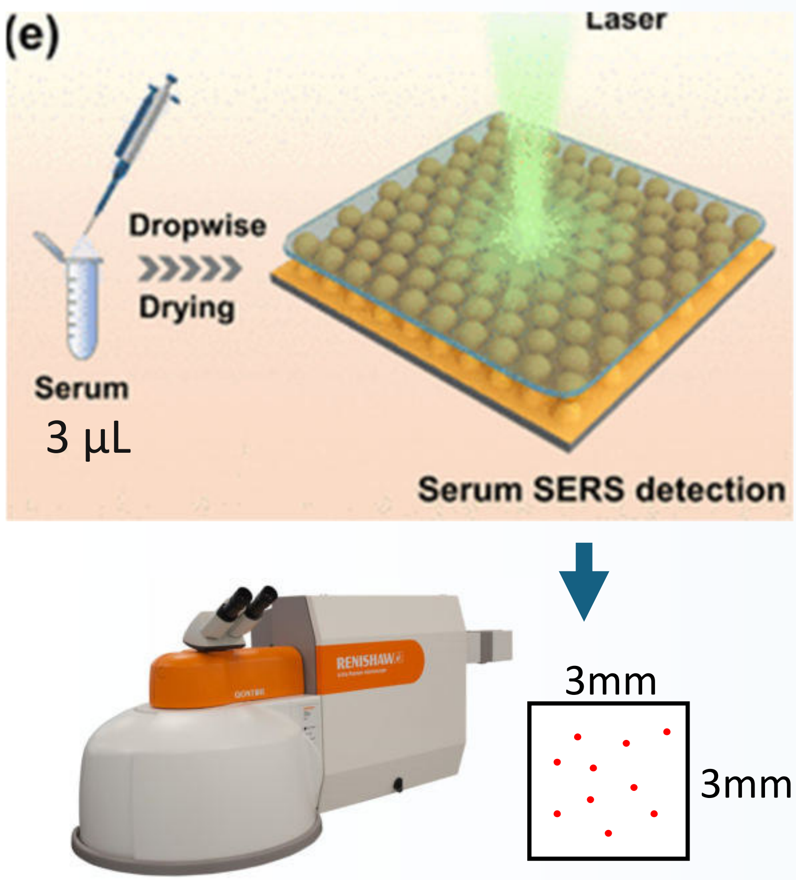


It can be found that the ratio of the substrate to the lower limit of the concentration can detect R6G to 10^{-10} M

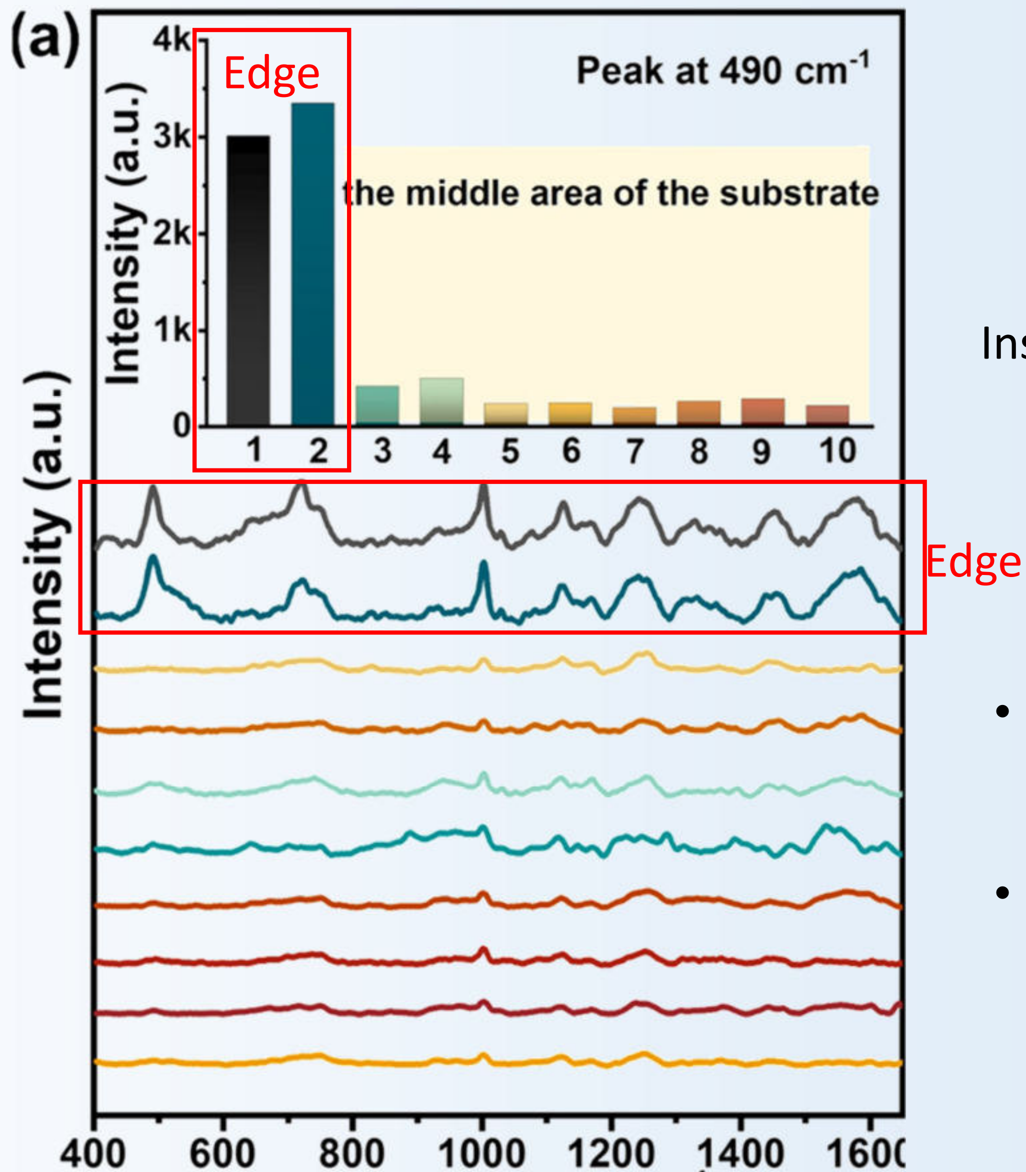
Translate to high sensitivity of the substrate (Au NCA)

Method & Result

Instability of Direct Serum SERS Analysis



Randomly selected 10 points



They found instability in the SERS spectra

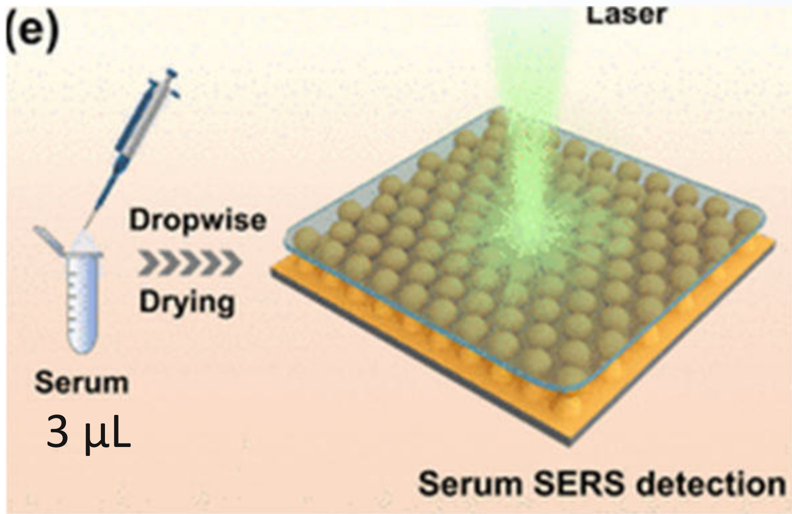
Instability happens in different areas of the substrate

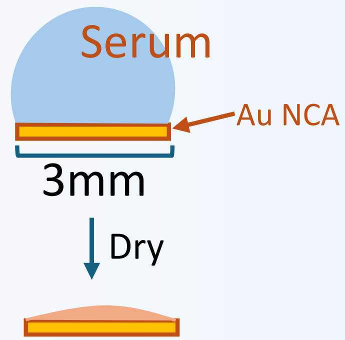
- **The central region:** weak serum SERS spectra
- **The edge region:** strong and complete SERS spectra

Why?

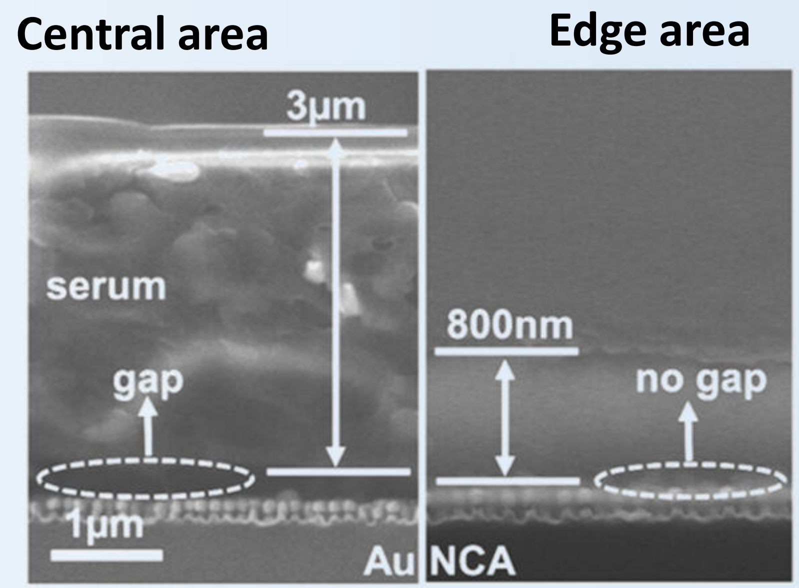
Method & Result

Instability of Direct Serum SERS Analysis





The illustration shows how the central area is thick



Cross-sectional FE-SEM image of the Au NCA plasmonic substrate after adding 3 μL of serum sample

FE-SEM

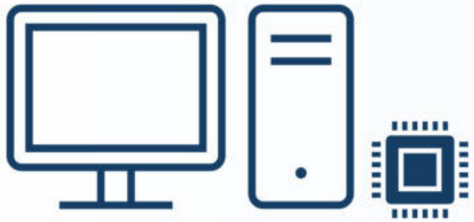
- **The central region** had an excessively thick serum layer that did not tightly cover the Au NCA and left a gap between the serum layer and the Au NCA
- **The edge region** was only a few hundred nanometers thick and closely covered the Au NCA

Instability could arise from the **high viscosity and excessive thickness** of the serum sample.

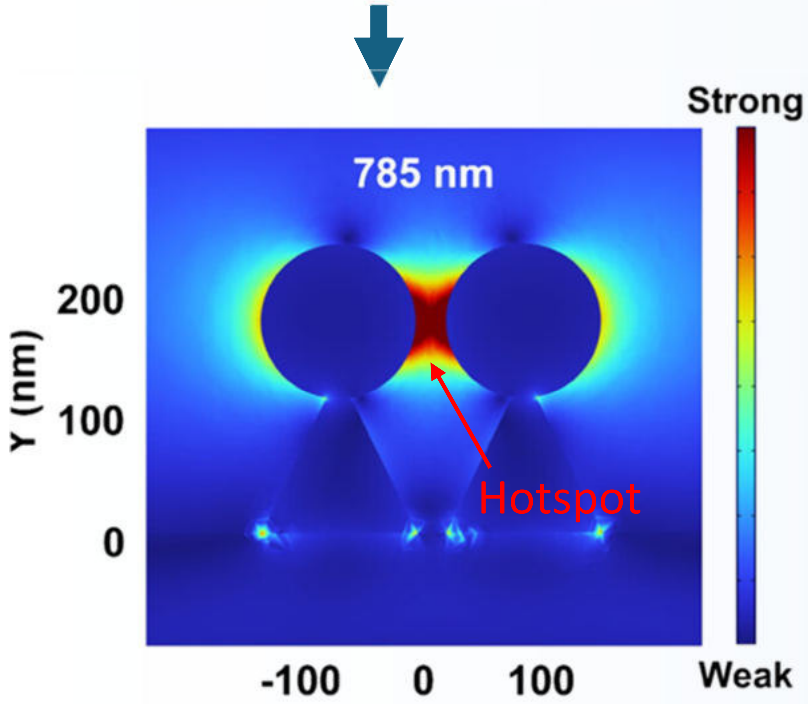
FE-SEM: Field Emission Scanning Electron Microscope

Method & Result

Instability of Direct Serum SERS Analysis



Computer simulation



The simulation diagram of the electromagnetic (EM) enhancement effect around Au NCA under 785 nm wavelength laser excitation.

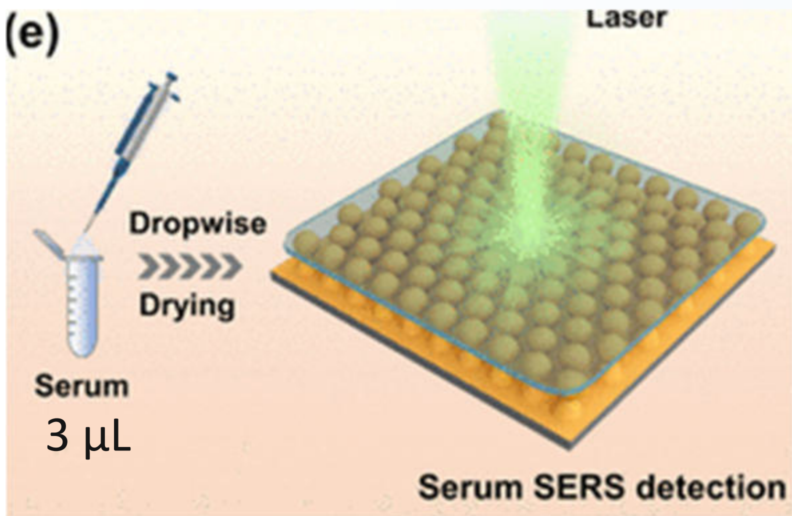
SERS enhancement is effective when the sample is in a hotspot

- Strong and stable SERS enhancement is only possible when serum molecules are homogeneously dispersed in the gaps.
- When serum thickness exceeds the electromagnetic enhancement area of the Au NCA, it is not possible to obtain a consistent and standard SERS spectrum.

To obtain strong and reliable serum SERS spectra, it is essential to dilute the serum.

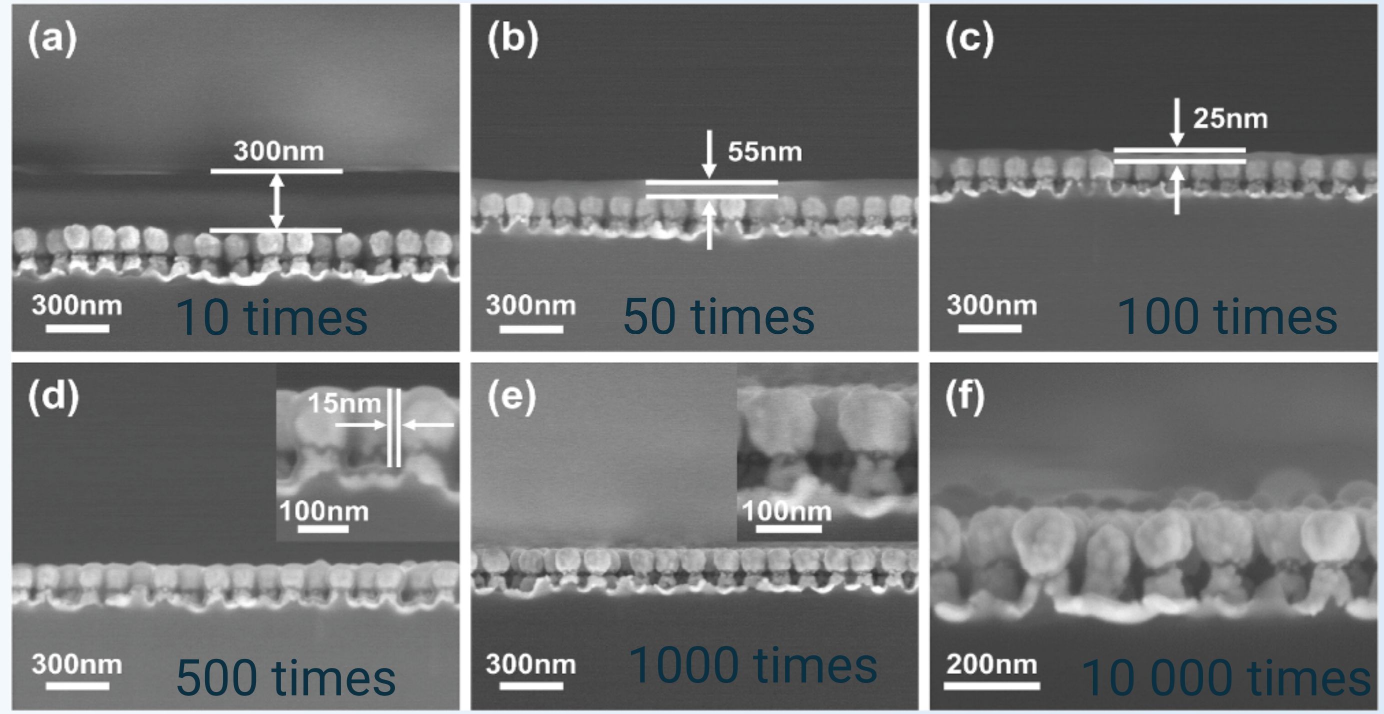
Method & Result

Concentration Optimization for High-Quality Serum SERS Spectra



Dilute serum
(10, 50, 100, 500, 1000, 10 000 times)

FE-SEM

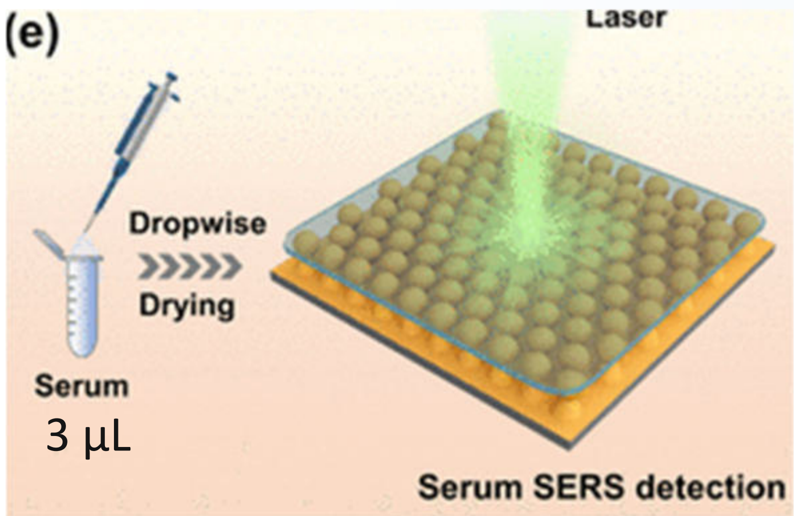


Cross-sectional FE-SEM images of serum droplets diluted

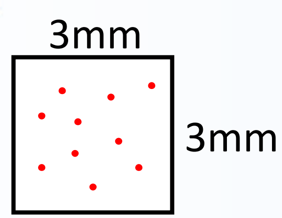
Dilute serum = decrease viscosity and thickness, improving dispersibility into the gap (hotspot)

Method & Result

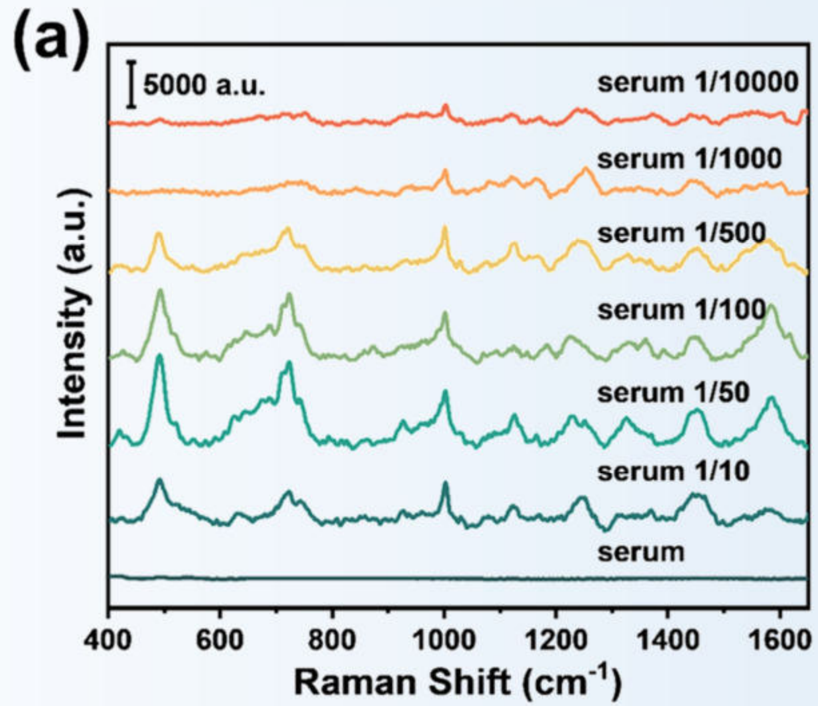
Concentration Optimization for High-Quality Serum SERS Spectra



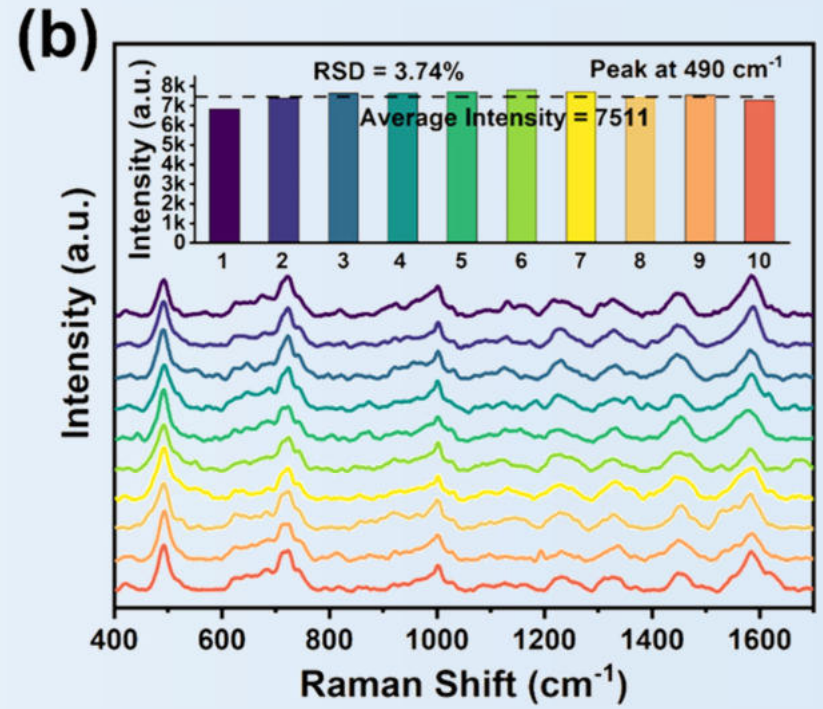
Dilute serum
(10, 50, 100, 500, 1000, 10 000 times)



Randomly selected 10 points



SERS spectra of serum samples with different dilutions obtained under 785 nm laser wavelength excitation.



SERS spectra of serum diluted 50 times

The serum dilution factor is 50–500 times, is stable and reproducible with a suitable thickness of the serum layer

The most comprehensive and highest value that indicates the optimal sample is a 50 times dilution factor.

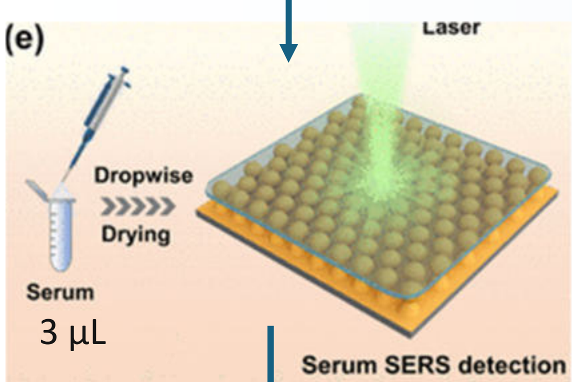
Method & Result

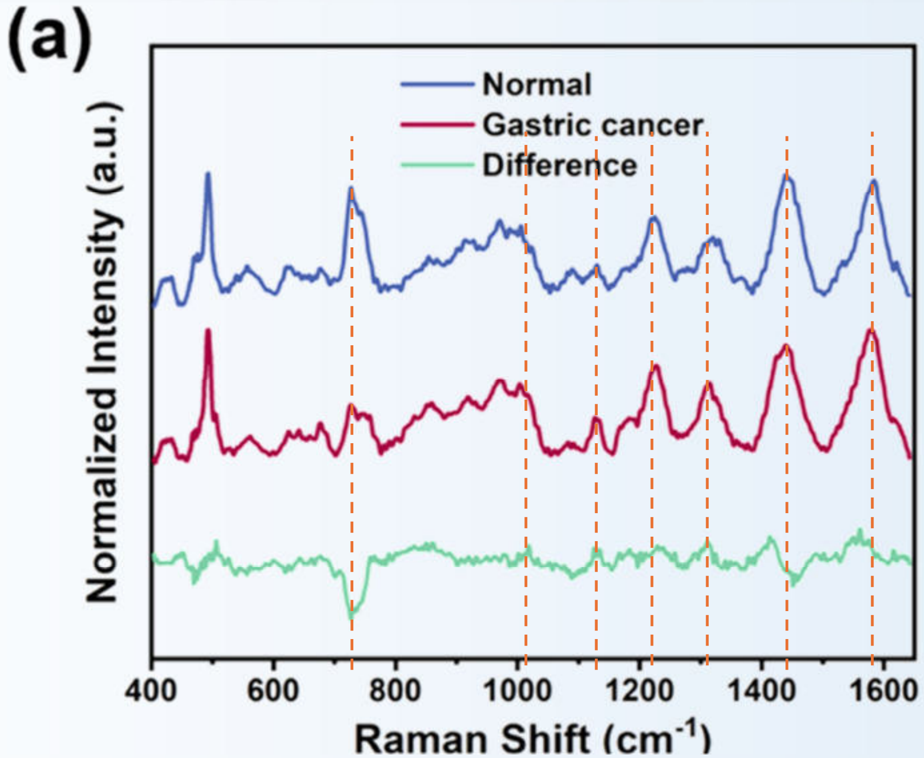
Verify the practical utility in disease diagnosis

30 healthy volunteers

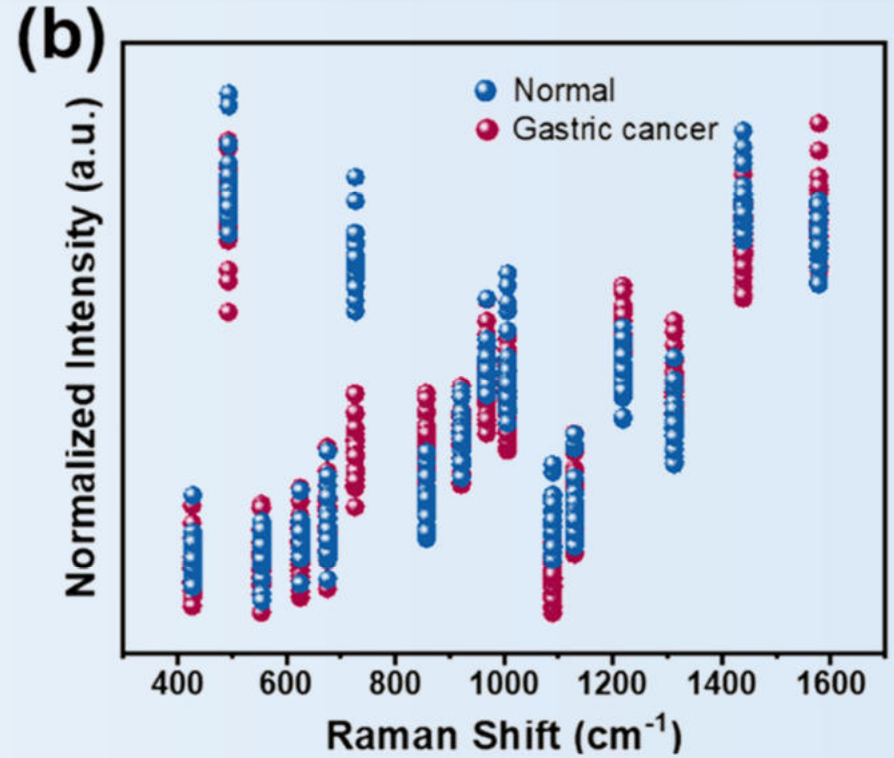
30 gastric cancer patients

Dilute 50 times





Differences in serum SERS spectra between normal individuals and gastric cancer patients



Scatter diagram of different bands of serum SERS spectra between normal individuals and gastric cancer patients

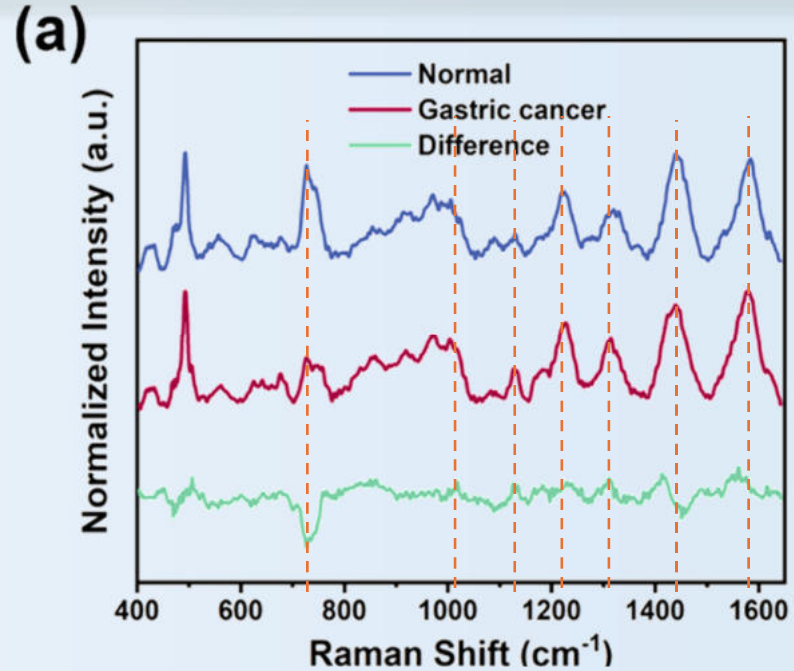
- The two curves have certain similarities in shape, but the SERS intensities of many peaks vary significantly.
- Noticeable differences emerge between the normal serum and gastric cancer serum at multiple peak positions, such as 726, 1006, 1129, 1218, 1312, 1439, and 1578 cm^{-1}

Method & Result

Verify the practical utility in disease diagnosis

Reduce when compared to normal

Raman shift (cm^{-1})	vibrational mode	assignment
427	\	cholesterol and cholesterol ester
490	C deformation to vibration	isoleucine
554	S–S stretching vibration	tryptophan
625	δ (C–S)	tyrosine
726	C–H bending vibration, C–S anti conformation	nucleic acid
857	C–H indole ring vibration	phosphatidic acid
921	\	glycogen and lactic acid
968	C–N deformation vibration	nucleic acid
1006	ring breathing vibration	tryptophan, histidine, and phenylalanine
1089	phospholipid C–C stretching vibration	nucleic acid and lipid
1129	C–C stretching vibration	protein, phospholipid, and saccharides
1218	ring breathing vibration	phenylalanine
1312	ring symmetric stretching methylene CH_2 bending vibration	tryptophan and lipid
1439	CH_2 bending or scissoring	protein and phospholipid
1578	\	tyrosine, tryptophan, and phenylalanine



Higher levels of nucleic acids and lower levels of certain proteins in the serum of gastric cancer patients indicate the process of carcinogenesis.

As gastric cancer cells undergo metabolic and proliferative activities, large quantities of proteins are shed, causing a relatively higher concentration of nucleic acids in the serum.

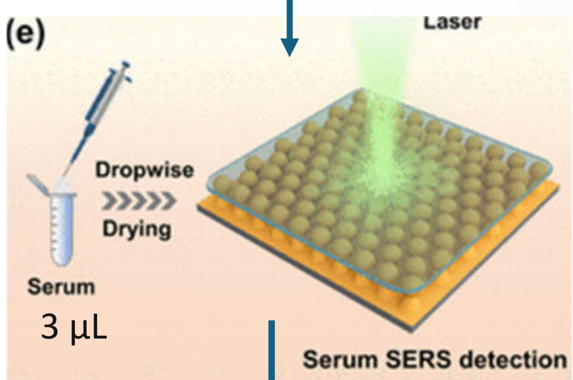
Method & Result

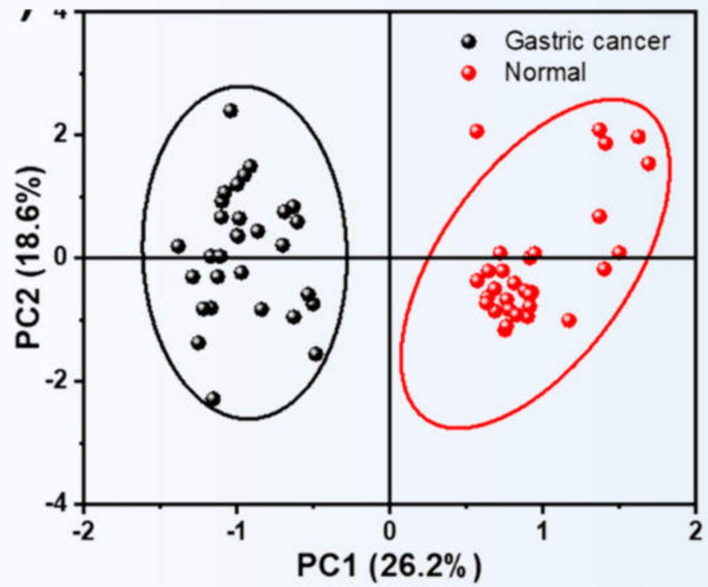
Verify the practical utility in disease diagnosis

30 healthy volunteers

30 gastric cancer patients

Dilute 50 times



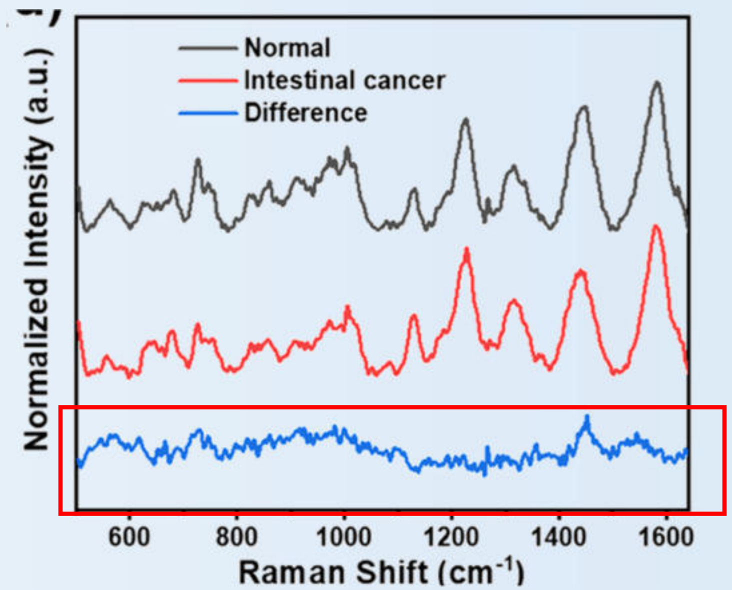


the SERS spectra of serum from both groups exhibited distinct clustering

Effectively differentiate between two groups using the PCA

30 healthy volunteers

20 Intestinal cancer patients



Show the difference between the SERS spectra of normal individuals and intestinal cancer patients

Conclusion 1st paper

A high-activity Au nanocone array (Au NCA) plasmonic substrate with microstructures was successfully prepared and has high sensitivity with reliability, reproducibility, and uniformity.

The suitable dilution rate is 50 for the serum sample.

It can be applied for disease detection, in particular, cancer, in this example.

2nd Paper

▸ Diagnostics (Basel). 2025 Mar 8;15(6):660. doi: 10.3390/diagnostics15060660

Detection of Respiratory Disease Based on Surface-Enhanced Raman Scattering and Multivariate Analysis of Human Serum

Yulia Khristoforova [1,*], Lyudmila Bratchenko [1], Vitalii Kupaev [2], Dmitry Senyushkin [3], Maria Skuratova [4], Shuang Wang [5], Petr Lebedev [6], Ivan Bratchenko [1]

Editor: Te-Chun Shen

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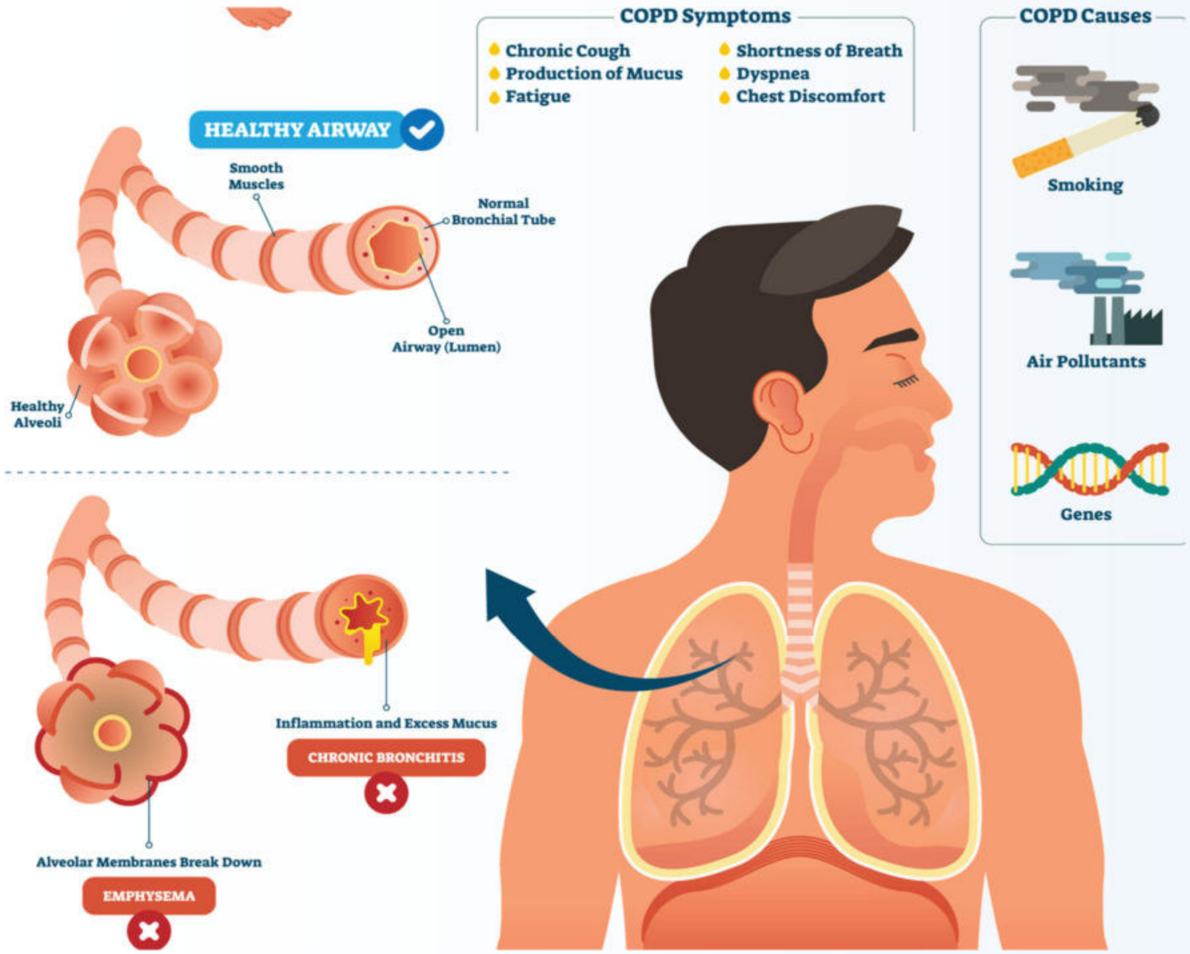
PMCID: PMC11940998 PMID: 40150003

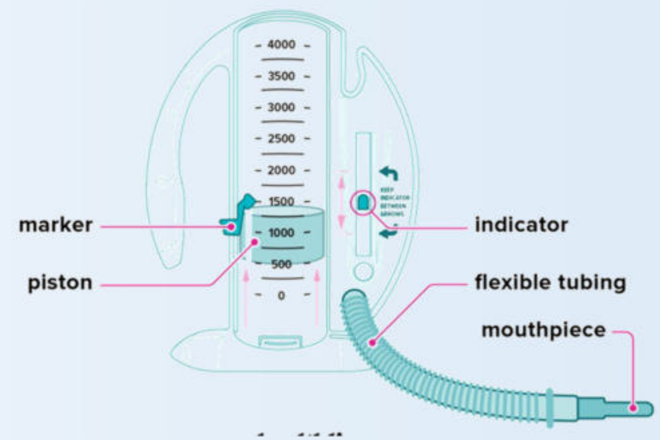
2025
Quartile 1

Objective: To evaluate the efficacy of SERS of human serum combined with multivariate analysis to detect respiratory diseases and differentiate between specific respiratory conditions.

Chronic Obstructive Pulmonary Disease (COPD)



- Chronic obstructive pulmonary disease (COPD) is the fourth leading cause of death worldwide, causing 3.5 million deaths in 2021 (WHO), approximately 5% of all global deaths.
- COPD isn’t curable.
- The most common symptoms of COPD are difficulty breathing, chronic cough and fatigue.



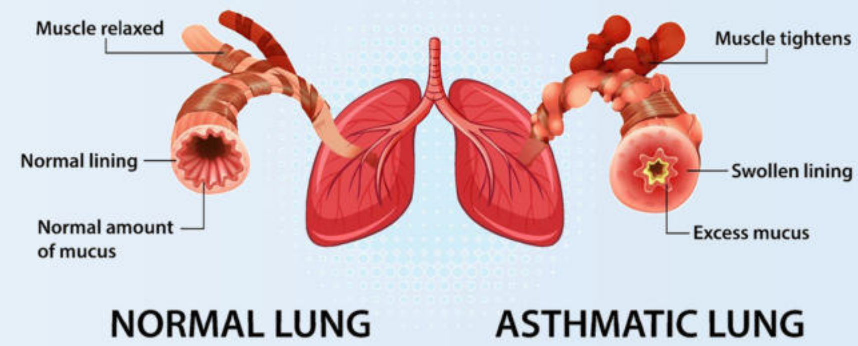
Primary use spirometry for diagnose COPD

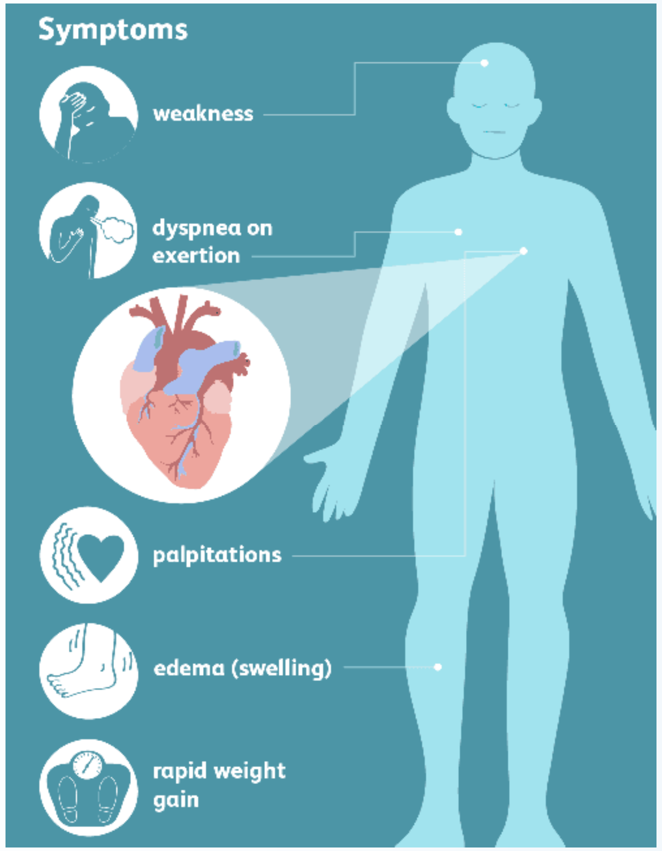
- **Chronic Bronchitis**: Inflammation and narrowing of the airways with mucus hypersecretion.
- **Emphysema**: Destruction of the alveoli (air sacs), leading to lost elasticity and trapped air.

Problem: low sensitivity for 'early' pathological changes and is difficult to interpret, especially in 'older' patients due to natural lung aging.

Bronchial Asthma (BA)

- A chronic inflammation of the airways. unlike COPD, this obstruction is often reversible.
- Shortness of breath, tightness of chest, Wheezing, Excessive coughing
- In older adults, Asthma can look almost identical to COPD. This leads to the Asthma-COPD Overlap**,** making it very hard which treatment the patient needs.



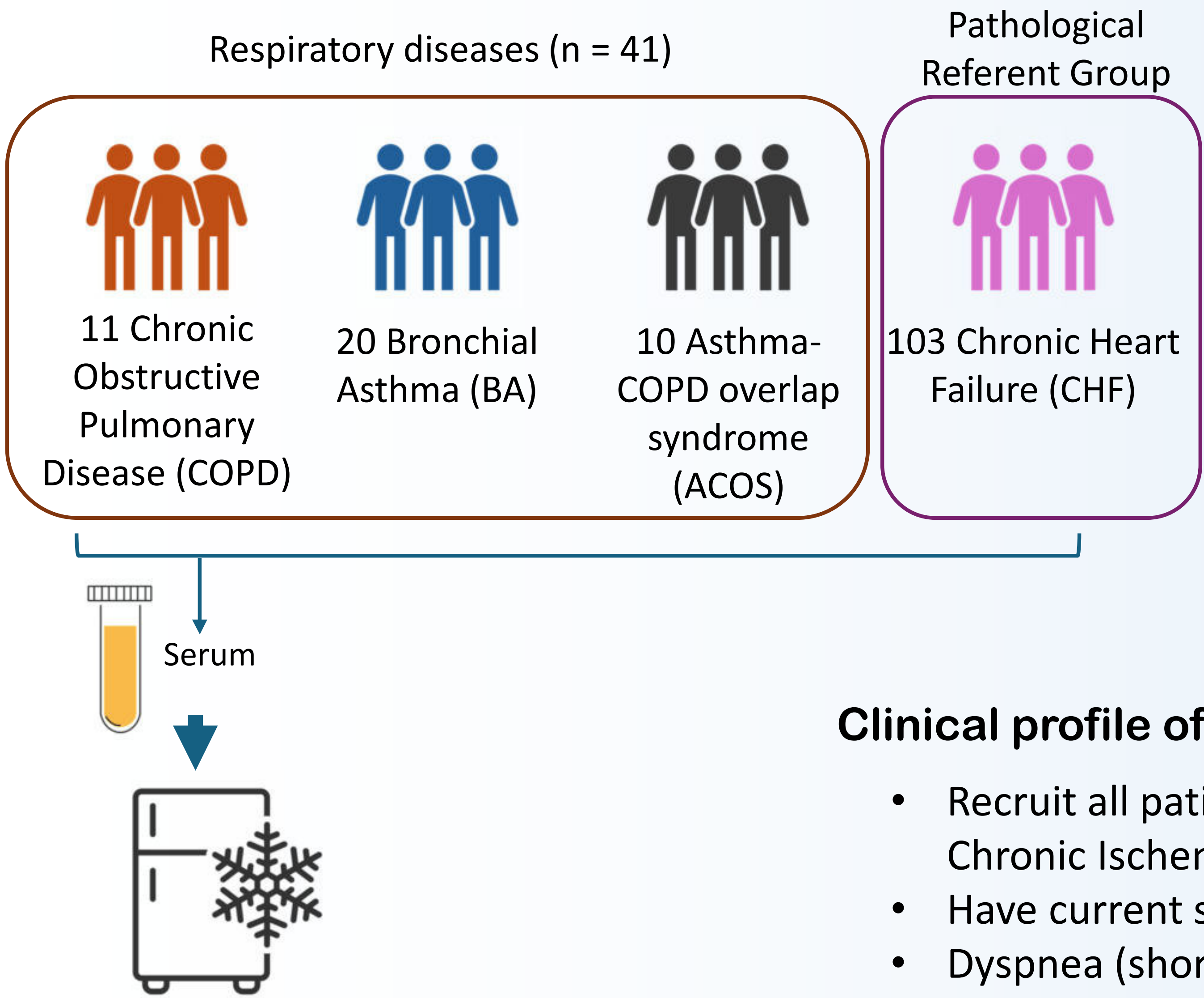


Chronic Heart Failure (CHF)

- A chronic condition where the heart muscle is too weak or stiff to pump blood effectively to meet the body's needs.
- Symptom similar to COPD: dyspnea/shortness of breath and fatigue

Method & Result

Study Population



BA: Bronchial Asthma
COPD: Chronic Obstructive Pulmonary Disease
ACOS: Asthma-COPD Overlap Syndrome

Table 1. Summary of analyzed patient groups.

Group of Subjects	Number of Patients	Mean Age (Min–Max)	Total Number of Spectra
Respiratory diseases (COPD + BA + COPD&BA)	41 (21 male, 20 female)	61 (39–74)	143
Chronic heart failure (CHF)	103 (76 male, 27 female)	65 (43–74)	309

- All patients with COPD corresponded to moderate and severe disease
- CHF was caused by chronic ischemic heart disease in all 103

Clinical profile of Chronic Heart Failure (CHF)

- Recruit all patient who developed heart failure due to Chronic Ischemic Heart Disease (clogged arteries)
- Have current symptoms of heart failure.
- Dyspnea (shortness of breath) and fatigue (the same clinical symptom as COPD and BA)

Method & Result

Study Population

Table 2. Summary of patients with respiratory diseases.

	BA, n = 20	COPD, n = 11	COPD&BA, n = 10	p-Value		
		Mean ± SD		$p_{BA-COPD}$	$p_{BA-ACOS}$	$p_{COPD-ACOS}$
Smoker's index (packs/years)	0.032 ± 1.66	14.46 ± 16.63	27.38 ± 12.33	0.001	0.001	0.012
Body mass index	28.37 ± 4.96	26.77 ± 3.72	29.20 ± 5.70	0.146	0.409	0.057
Experience of the BA, year	13.73 ± 8.77	–	9.15 ± 8.81	–	0.070	–
Experience of the COPD, year	–	5.50 ± 5.05	7.31 ± 4.75	–	–	0.289
IGS, µg/day	301.75 ± 258.98	102.50 ± 216.63	326.15 ± 261.71	0.002	0.729	0.008
The number of exacerbations per year	1.55 ± 0.75	1.69 ± 1.08	2.31 ± 2.06	0.728	0.273	0.525
ACT, scores	16.82 ± 5.71	–	13.15 ± 4.58	–	0.047	–
CAT, scores	–	20.47 ± 8.06	22.75 ± 5.40	–	–	0.494
FEV_1 (%)	77.40 ± 20.45	53.55 ± 28.06	53.48 ± 15.24	0.006	0.002	0.956
FVC (%)	79.17 ± 20.69	74.66 ± 34.76	65.53 ± 15.26	0.632	0.051	0.505
FEV_1/FVC	0.79 ± 0.09	0.62 ± 0.15	0.64 ± 0.13	0.001	0.005	0.720

IGS—inhaled glucocorticosteroids; ACT—Asthma Control Test; CAT—COPD Assessment Test; FEV_1—forced expiratory volume in one second; FVC—forced vital capacity.

- Bronchial **Asthma and COPD** groups clinically differences in smoking history, IGS, and lung function (FEV_1)
- Significant in IGS (Steroid drug) could potentially influence SERS spectra through the presence of drug metabolites in blood, complicating the chemical fingerprinting.

BA: Bronchial Asthma
COPD: Chronic Obstructive Pulmonary Disease
ACOS: Asthma-COPD Overlap Syndrome

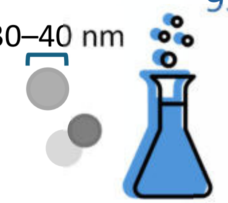
Method

SERS Fabrication

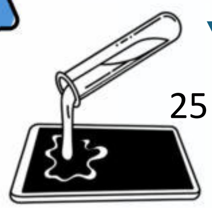
Silver nitrate + Sodium citrate
($AgNO_3$) ($Na_3C_6H_5O_7$)

95˚C 30 min

30–40 nm



Ag colloid solution



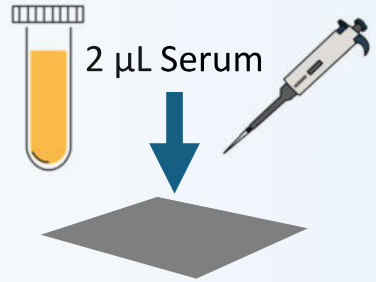
25 mL

Aluminum foil

Dry Room temp.

Particle size around 200 nm

SERS Fabrication



2 μL Serum

Dry Room temp. 30 min

Raman spectroscopy
(785 nm laser excitation)
EnSpectr software

Serum SERS spectra

Preprocessing

Smoothing → Baseline correction → Normalization

- Smoothing (Savitzky–Golay filter)
- Baseline correction (polynomial)
- Normalization (standard normal variate method (SNV))

Multivariate analysis (PLS-DA)

1. Respiratory diseases (COPD + BA+ COPD&BA) VS Pathological referent group (CHF)
2. COPD VS BA

Method & Result

SERS Serum Spectra

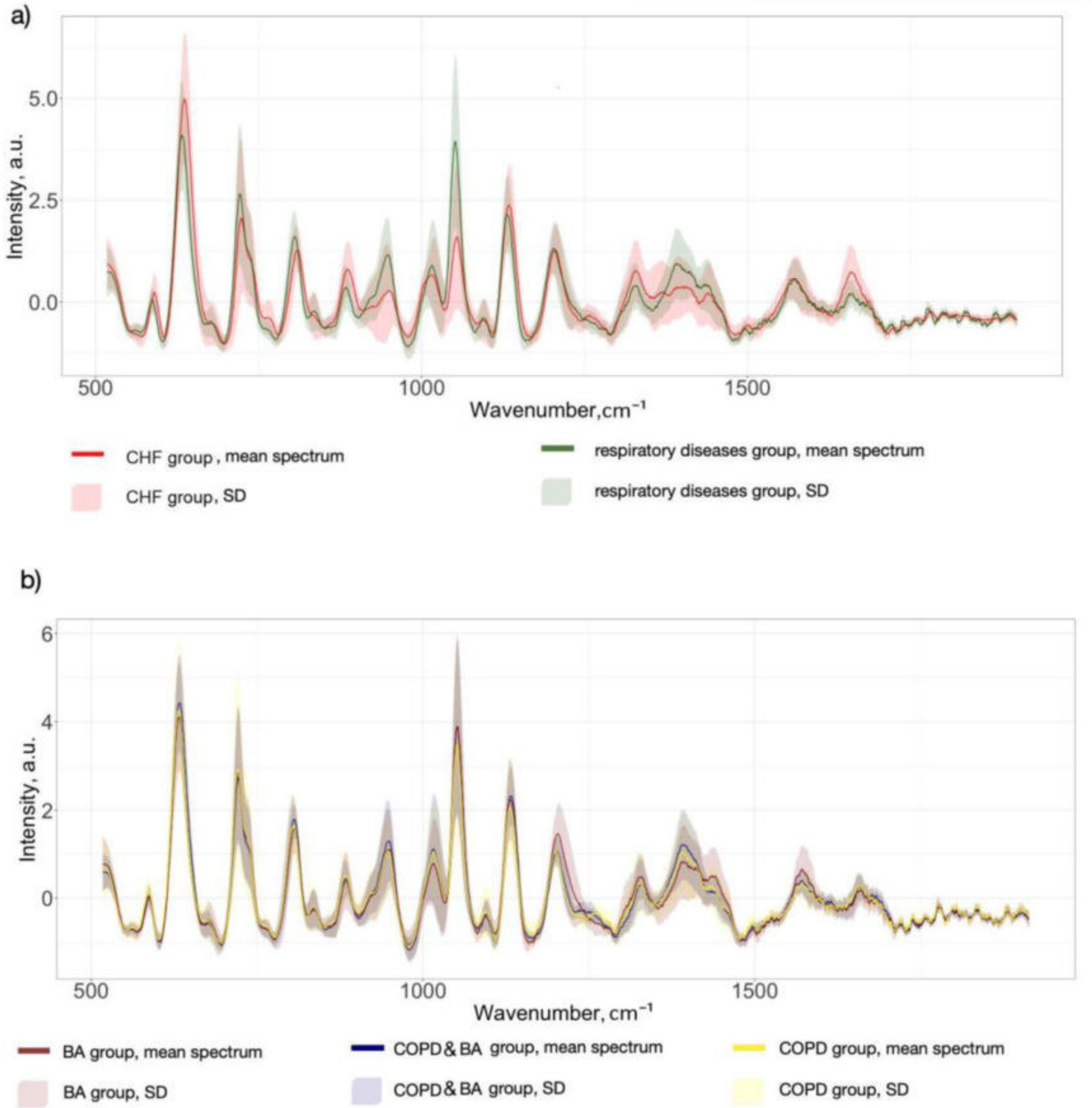


Figure 2. The mean spectra with standard deviation (SD) of human serum of patients with different pathologies: (a) respiratory diseases vs. CHF; (b) different types of respiratory diseases: COPD, BA, and COPD&BA.

Biochemical Assignments of SERS Peaks

Wavelength (cm^{-1})	Primary Assignment	Biological Category
587	Phosphatidylinositol	Lipid
638	Uric Acid	Metabolite (Oxidative Stress)
724	Adenine, Coenzyme A, DNA/RNA	Nucleic Acid
805	L-Serine, Glutathione	Protein/Amino Acid
890	Tryptophan, Glutathione	Protein/Amino Acid
947	C-C stretching (Proteins)	Protein
1008	Phenylalanine	Amino Acid
1051	Glycogen	Carbohydrate (Energy)
1132	D-Mannose	Carbohydrate
1207	Tryptophan, Phenylalanine	Amino Acid
1329	CH_2 Torsion mode	Lipid
1390	C-N, C-H groups	Lipid/Protein
1442	CH_2/CH_3 deformations	Lipid/Protein
1568	DNA/RNA bases	Nucleic Acid
1657	Amide I	Protein

Method & Result

SERS Serum Spectra

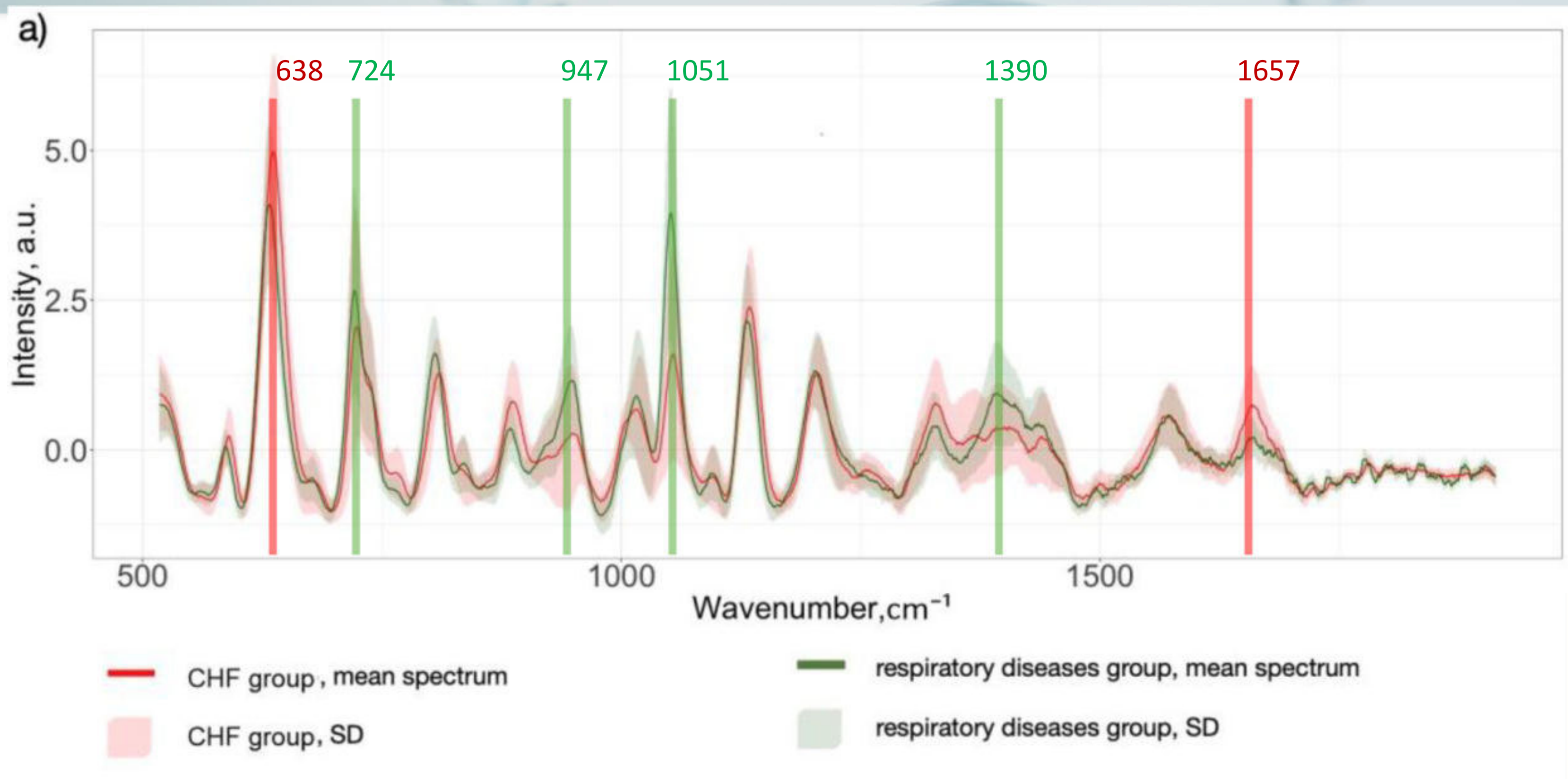


Figure 2. The mean spectra with standard deviation (SD) of human serum of patients with different pathologies: respiratory diseases vs. CHF

The highlighted spectral differences were observed between the mean spectra of the respiratory disease group and CHF.

Respiratory diseases group, the SERS signal intensities at 724, 947, 1051, and 1390 cm^{-1} were higher, while the peaks at 638 and 1657 cm^{-1} showed a decrease.

Method & Result

SERS Serum Spectra

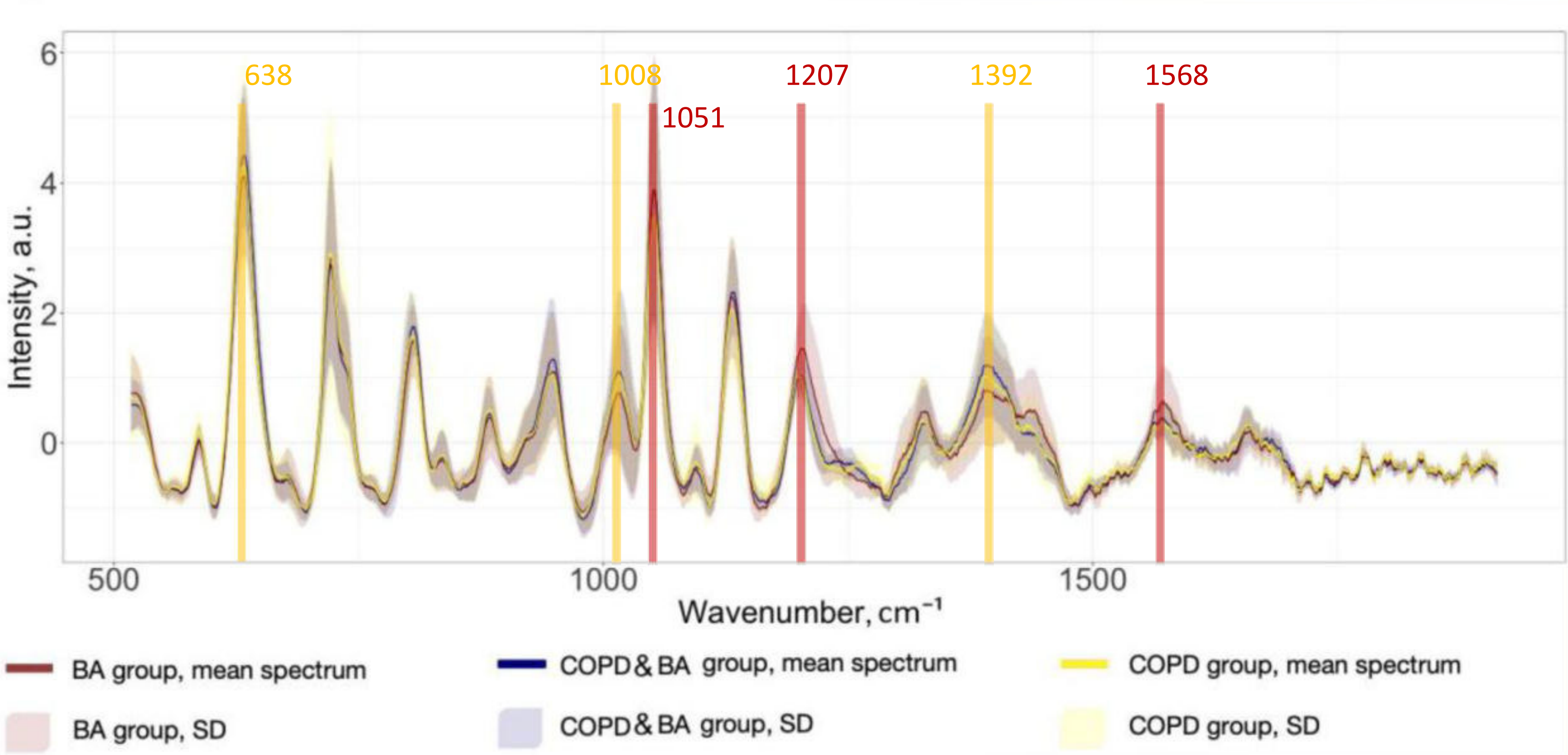


Figure 2. The mean spectra with standard deviation (SD) of human serum of patients with different pathologies: different types of respiratory diseases: COPD, BA, and COPD&BA.

Spectral differences between COPD and BA cases were observed at several bands

COPD: higher intensities at the 638, 1008, and 1392 cm^{-1}

BA: higher intensities at the 1051, 1207, and 1568 cm^{-1}

The serum composition does not fundamentally change in different diseases; the composition ratios are changed.

To identify significant spectral differences between groups of patients and use them for differentiation, multivariate statistical methods should be applied.

BA: Bronchial Asthma
COPD: Chronic Obstructive Pulmonary Disease

Method & Result

Multivariate Analysis

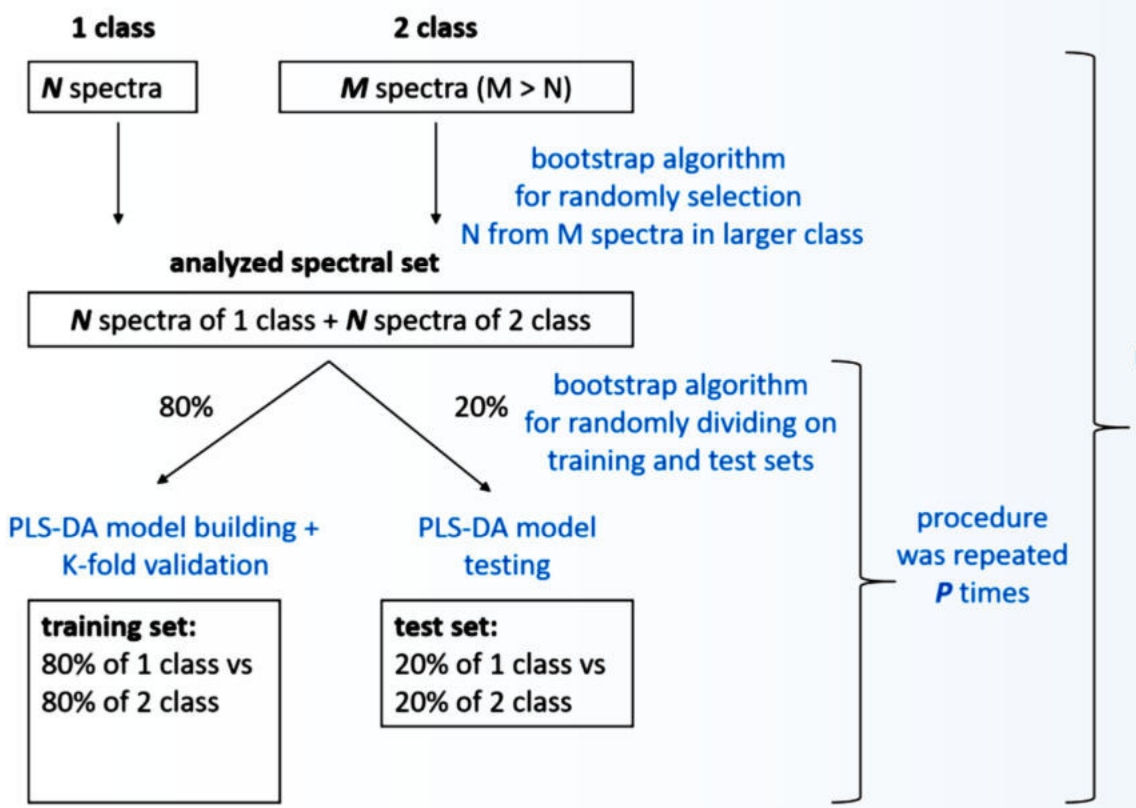
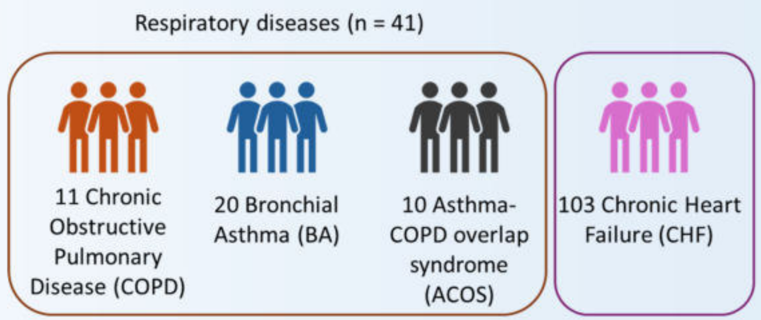


Figure 1. Scheme of PLS-DA model building procedure.



Model I use 81 patients (41 vs 41)
Model II use 22 patients (11 vs 11)

Variable	Definition	Model I (Resp vs CHF)	Model II (COPD vs BA)
T	Times specific patients were selected (Outer Loop)	5	3
P	Times data was split 80/20 (Inner Loop)	5	3
Total	$T \times P$	**25**	**9**

BA: Bronchial Asthma
COPD: Chronic Obstructive Pulmonary Disease
CHF: Chronic Heart Failure

Method & Result

PLS-DA Classification Models

Table 3. Classification results.

Classification Models		Specificity Mean (Min–Max)	Sensitivity Mean (Min–Max)	Accuracy Mean (Min–Max)	ROC AUC Mean (Min–Max)
Model I Respiratory diseases (COPD + BA+ COPD&BA) vs. CHF (pathological referent group)	Training set	0.95 (0.92–1.0)	0.94 (0.91–0.99)	0.95 (0.94–0.98)	0.97 (0.96–1.0)
	Test set	0.97 (0.86–1.0)	0.85 (0.70–1.0)	0.92 (0.82–1)	0.96 (0.85–1.0)
Model II COPD vs. BA	Training set	0.92 (0.86–1.0)	0.86 (0.75–0.92)	0.89 (0.85–0.96)	0.93 (0.78–0.99)
	Test set	0.57 (0.17–1.0)	0.64 (0.0–1.0)	0.61 (0.1–1.0)	0.72 (0.53–1.0)

From the table, Model I get high accuracy, specificity, sensitivity, and reliability (AUC). This confirms SERS can effectively screen for lung disease against CHF.

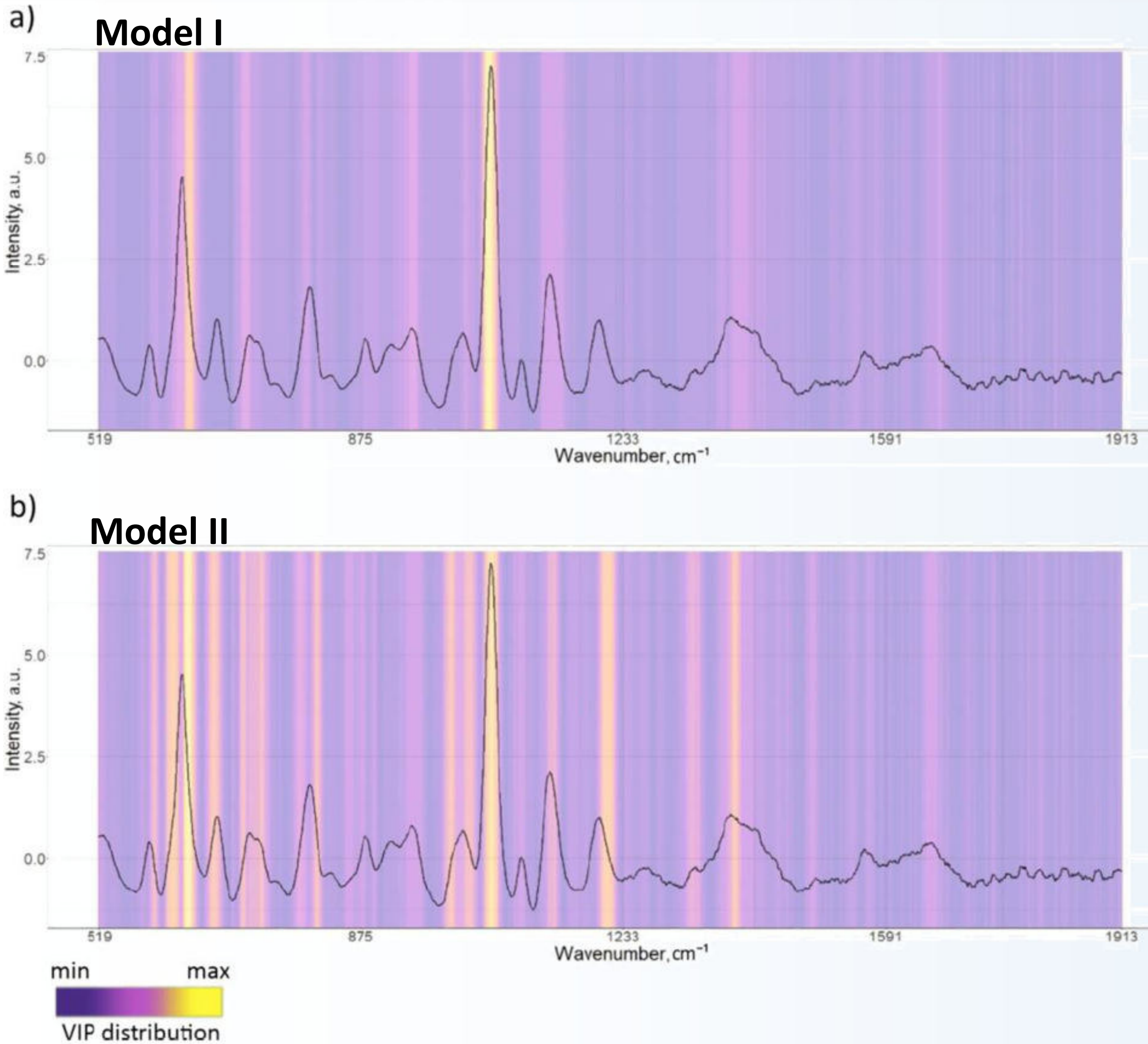
However, in Model II has the huge drop (0.89 → 0.61). This can translate The model is good at training data but failed on new data (Overfit). And AUC of 0.72 is considered fair. It suggests the chemical differences between COPD and Asthma are too weak to be reliable.

AUC Range	Classification Level
0.90 - 1.00	Excellent
0.80 - 0.90	Good
0.70 - 0.80	Fair
0.60 - 0.70	Poor
0.50 - 0.60	Failure

(Irawan, 2018)

BA: Bronchial Asthma
COPD: Chronic Obstructive Pulmonary Disease
CHF: Chronic Heart Failure

Method & Result



Distinct vertical yellow bands at 638 (Uric acid) and 1051 (Glycogen) cm^{-1}
Confirmed marker that can classify those COPD and BA with Chronic Heart Failure

Multiple important peaks that is the key for classification.

The bands may represent subtle differences in the training set, but because the test accuracy is only 0.61, these aren't disease markers.

These peaks might just be random variations between patients (e.g., diet, medication) rather than the disease itself.

Figure 3. VIPs' distribution for constructed PLS-DA models: (a) "respiratory diseases vs. CHF"; (b) COPD vs. BA.

Conclusion 2nd paper

PLS-DA achieved a high accuracy of **0.92** for screening respiratory diseases (COPD and Asthma) against other conditions with similar symptoms, specifically Chronic Heart Failure (CHF) by significant differences in **uric acid** (in CHF) and **glycogen** (in respiratory disease) levels.

The model attempting to distinguish COPD from Asthma failed (accuracy 0.61) because these two diseases share very similar serum metabolic profiles and pathogenetic mechanisms.

Criticism

	Strong points	Weak points
1st Paper	• Protocol standardization • Substrate engineering	• Lack of detailed validation
2nd Paper	• Good statistical validation • Choose clinical relevance of control group (CHF)	• Inferior substrate stability • Small sample size • Lack of biochemical verification

Acknowledgement

Advisor

Prof. Dr. Kiatichai Faksri

Thank you

for your attention