# Advanced Urinary Metabolomics for Noninvasive Cancer Screening via SERS-Based Sensing Platforms


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# Outline

1. **Background of Metabolomics for Cancer Diagnosis**

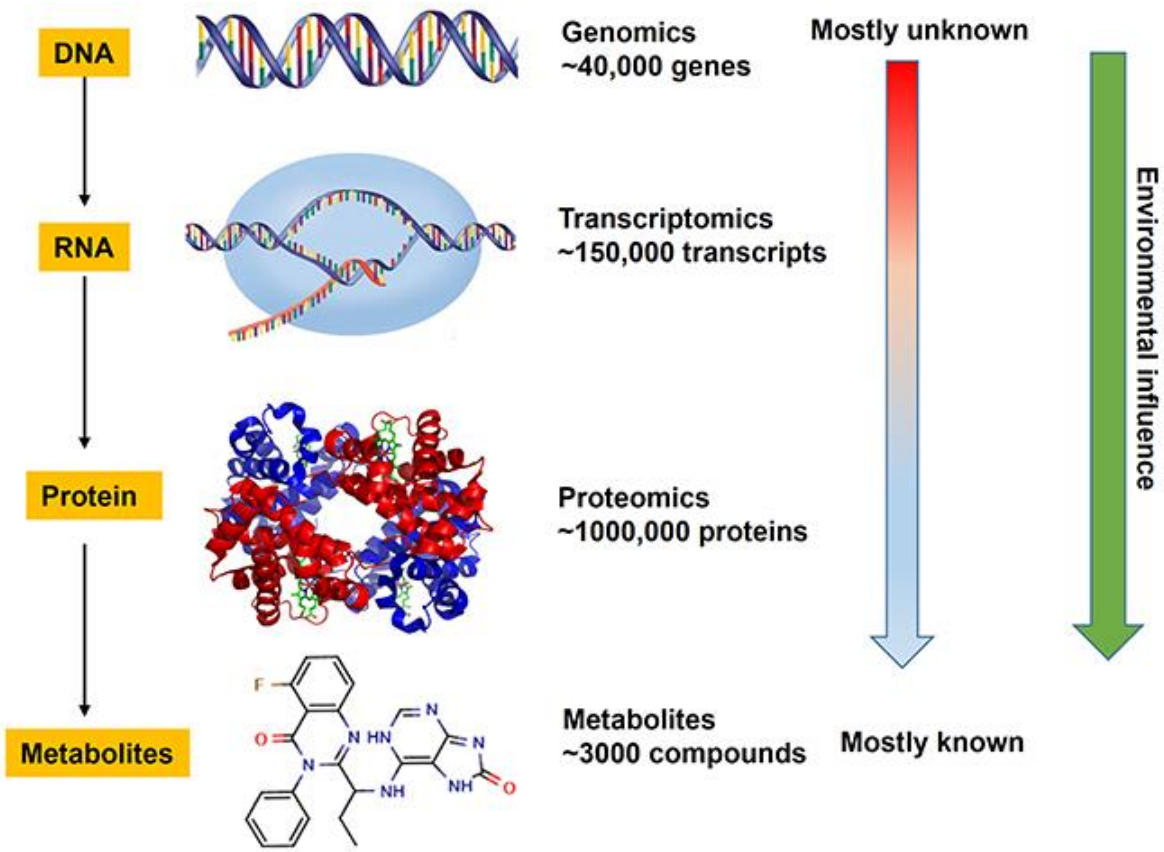
2. **Part 1.** Fingerprinting analysis of urinary metabolites using surface-carbonized silver nanowires on a filter membrane

3. **Part 2.** Enhancing the identification of pancreatic cancer through surface-enhanced Raman scattering of electro-chemically separated urine components

4. **Conclusion**

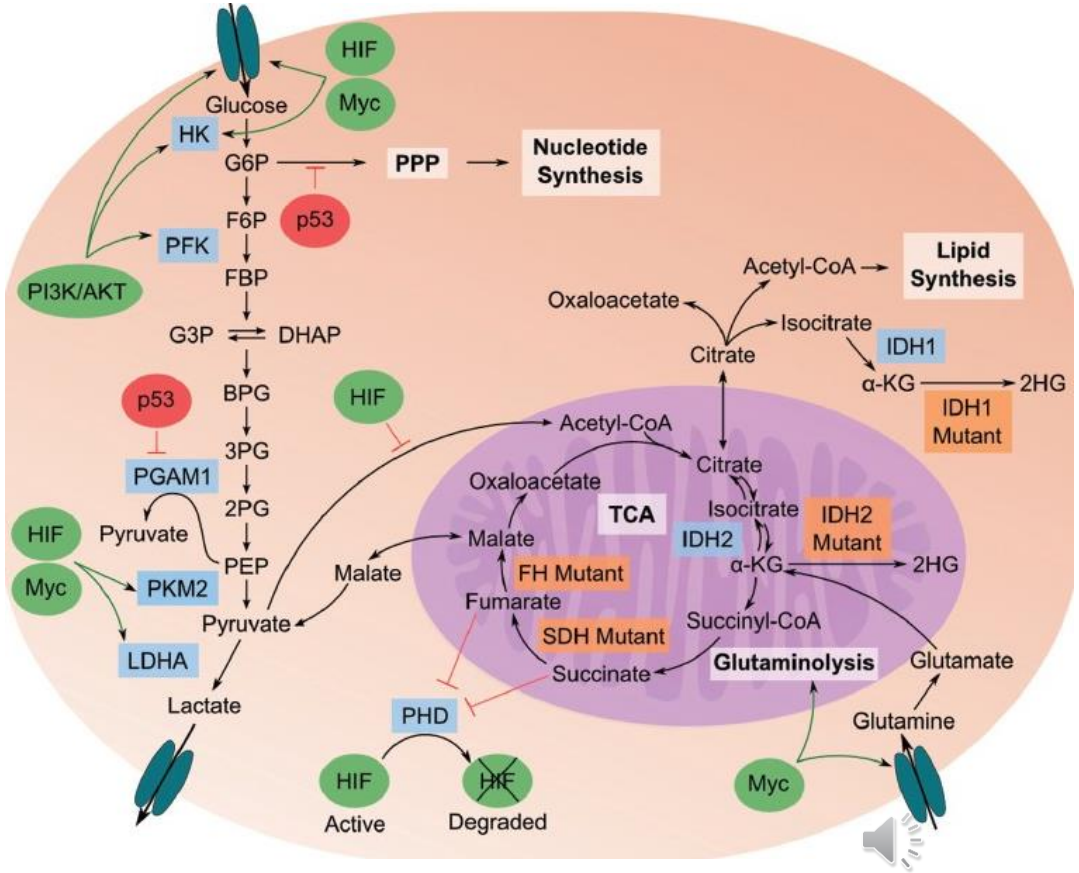
# Metabolomics for Cancer Diagnosis

## Advantages of metabolomics over other omics




*Oncotarget. 2017; **8**:115774-115786 [Ref 1]*

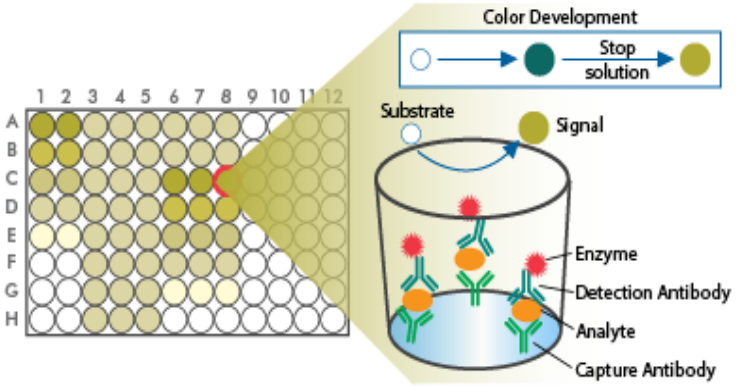
## Tumor metabolome




*Journal of Carcinogenesis. 2013, **12** (9): 9 [Ref 2]*

# Conventional metabolite measurement techniques

## ELISA
**Enzyme-Linked Immunosorbent Assay**




## LC/GC-MS
**Liquid Chromatography / Gas Chromatography-Mass Spectrometry**

## NMR
**Nuclear Magnetic Resonance**


- **Disadvantages:**
  - Relatively **long measurement time**
  - **Expensive equipment**
  - **Artifacts** due to ionization and other factors

analytical chemistry

**NMR-Guided Mass Spectrometry for Absolute Quantitation of Human Blood Metabolites**

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View Author Information

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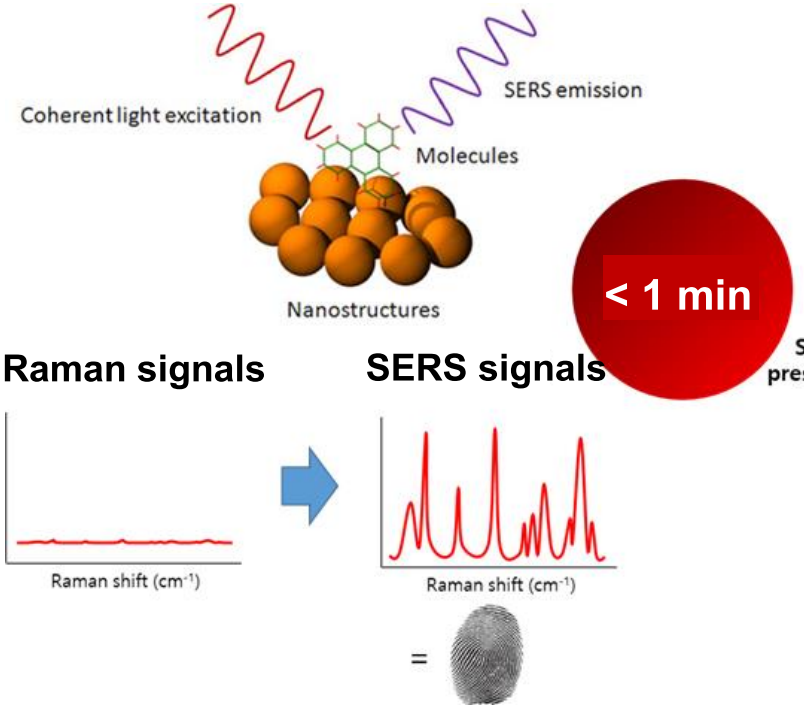
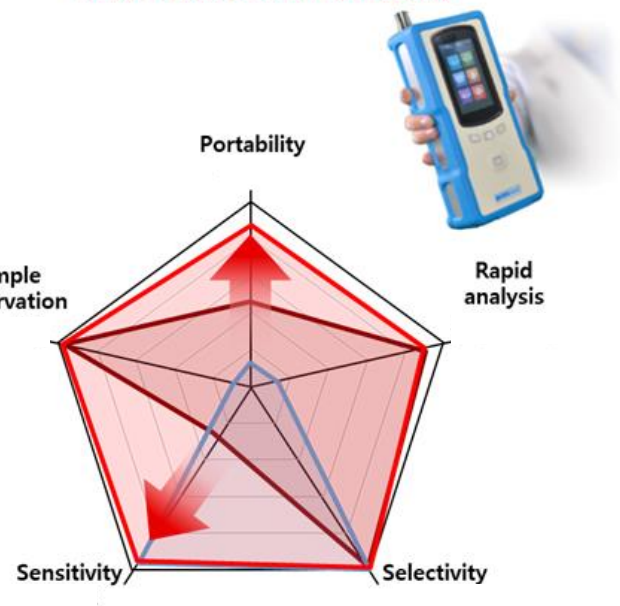
# SERS-based metabolite analysis for cancer diagnosis

## SERS-based Metabolite Analysis

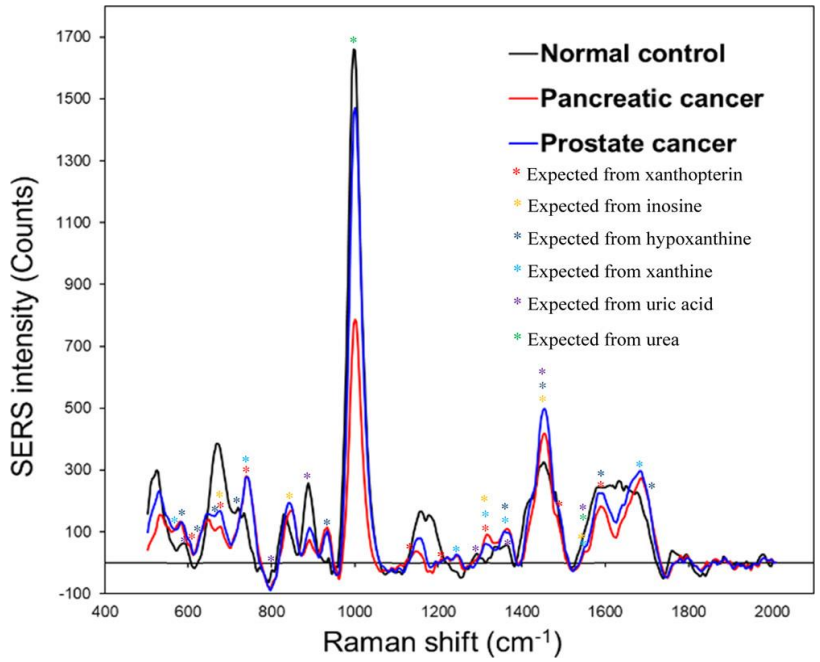
- Label-Free Fingerprinting




Surface-enhanced Raman spectroscopy




## Spectral Overlap Issues in body fluids




- **SERS** enables **rapid, sensitive, and non-destructive detection of molecular fingerprints using portable, plasmon-enhanced systems**.
- But, body fluids contain numerous components → **complex, overlapping spectra**
- Low signals of disease-related metabolite → need for a platform that enables **discrimination of metabolite signals**

# PART 1.

## Fingerprinting analysis of urinary metabolites using surface-carbonized silver nanowires on a filter membrane

# Clinical Characteristics

| Group | Number | Age | Gender (M:F) | CA 19-9 (U/mL) | | PSA (ng/mL) | |
|---|---|---|---|---|---|---|---|
| | | | | >37 | ≤37 | >2.5 (>4.0) | ≤2.5 (≤4.0) |
| Normal Control | 56 | 55.2±11.3 | 36:20 | 56 | 0 | 36 (36) | 0 (0) |
| Pancreatic Cancer | 40 | 70.5±6.5 | 19:21 | 25 | 15 | – | |
| Prostate Cancer | 36 | 66.4±6.4 | 36:0 | – | | 35 (26) | 1 (9) |
| Total | 132 | 64.0±11.1 | 91:41 | | | | |

* Stage number of pancreatic cancer patients.

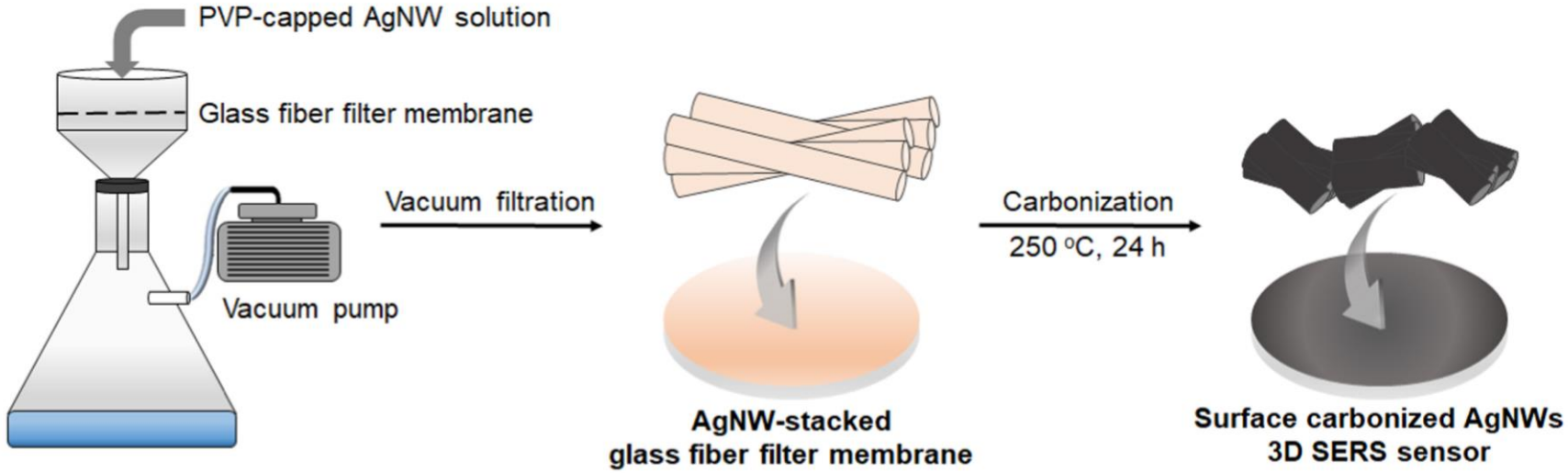
0 stage (n=5); 1A stage (n=6); 1B stage (n=11); 2A stage (n=1); 2B stage (n=10); 3 stage (n=7).

* Stage number of prostate cancer patients.

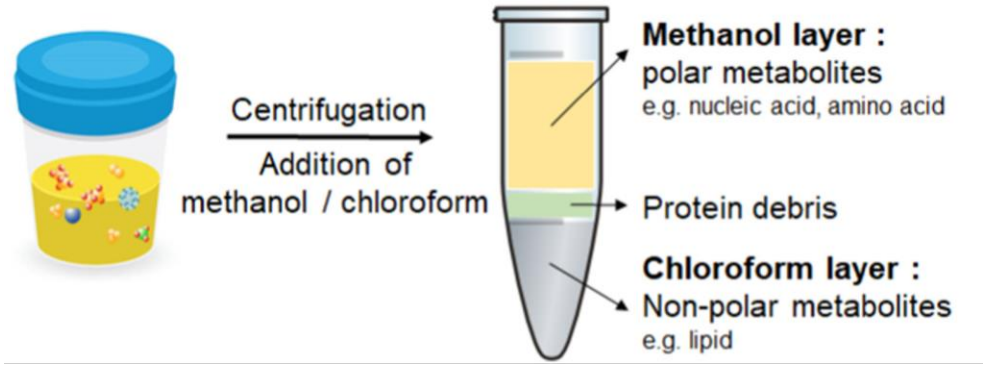
2 stage (n=23) and 3A stage (n=13).

# Fabrication of Surface-Carbonized 3D SERS Sensor

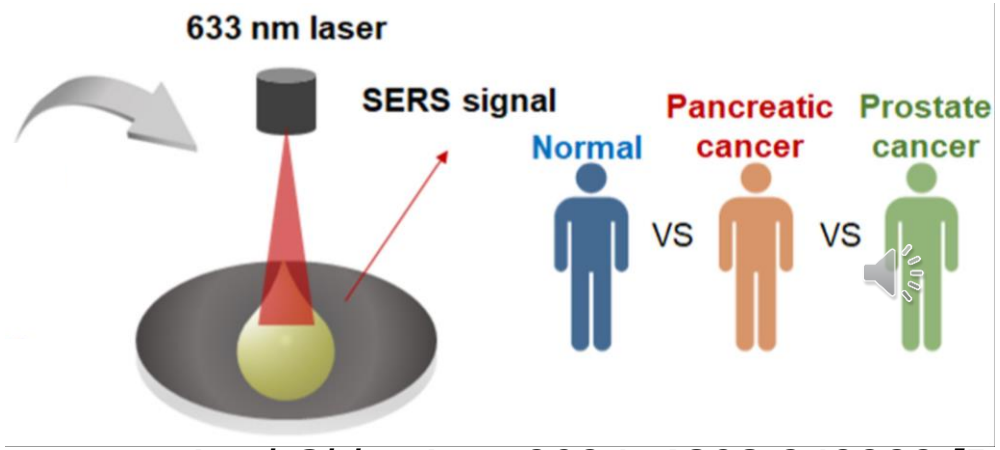
## Fabrication of 3D SERS Sensor




## Urine metabolites Separation according to polarity



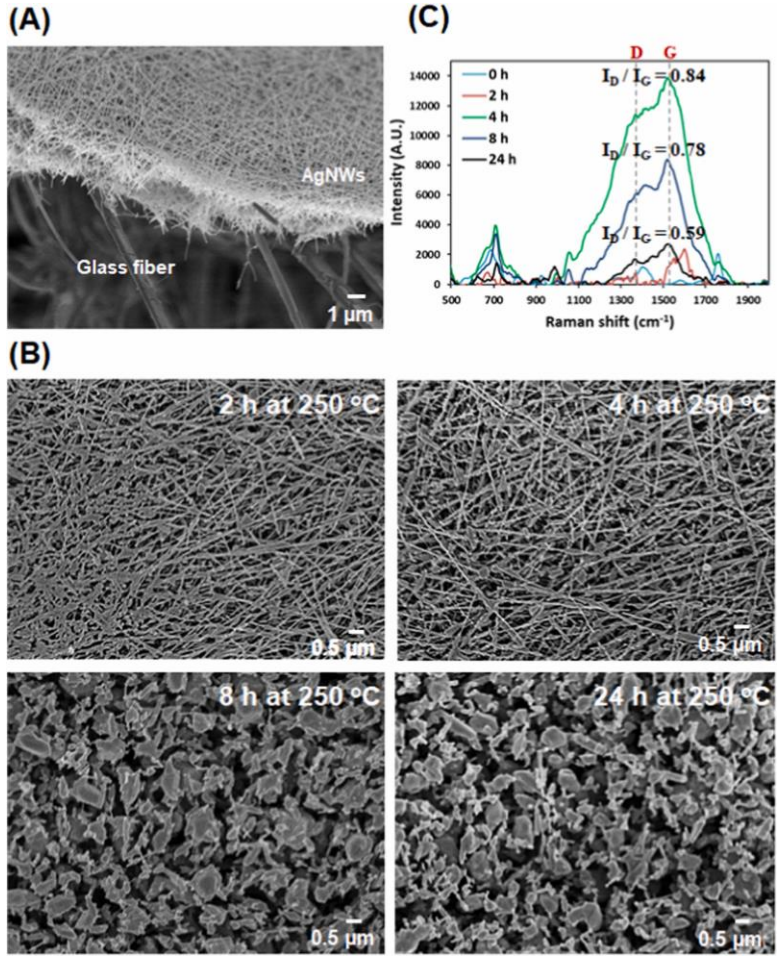

## SERS Detection




*Anal Chim Aca. 2024;* ***1292****:342233 [Ref 3]*

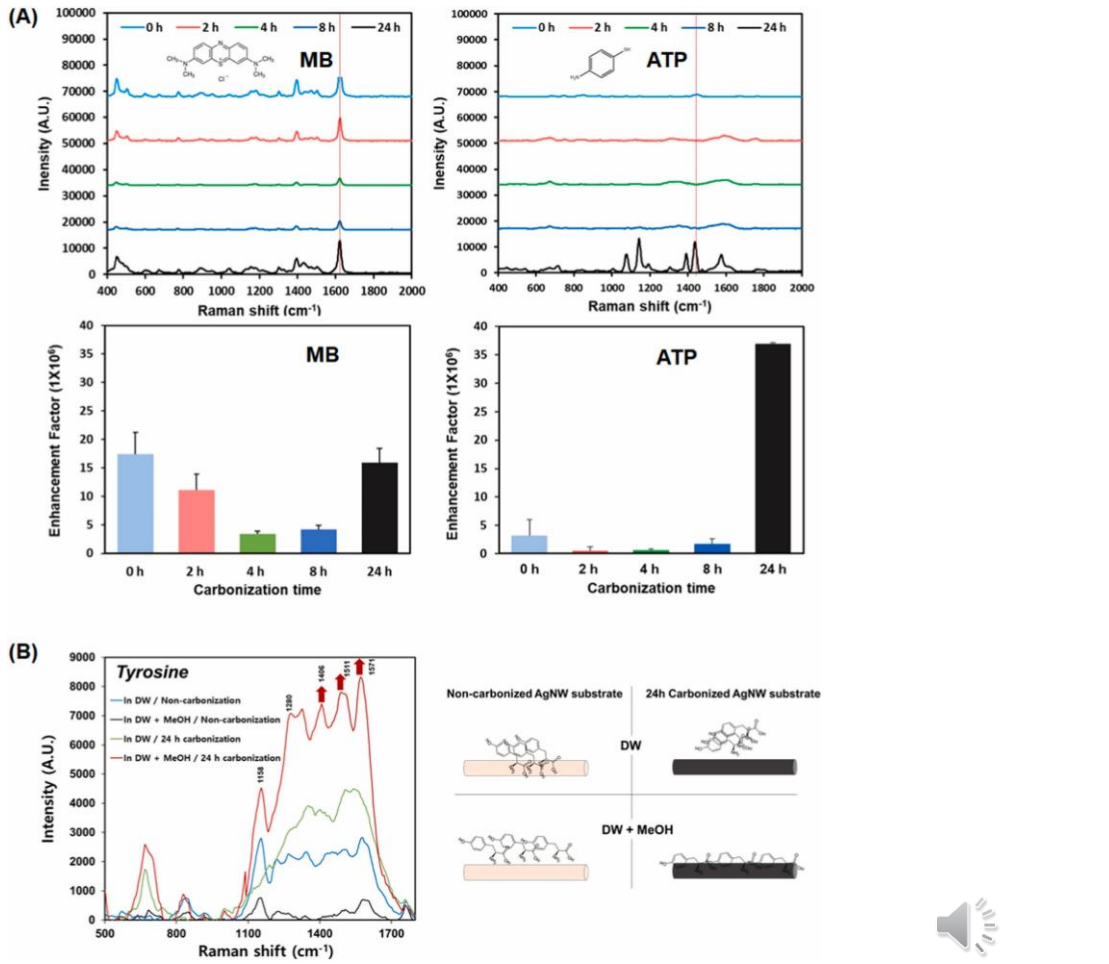
# Characteristics of Surface-Carbonized 3D SERS Sensor

## Characteristics of 3D SERS Sensor




Carbonization of surface stabilizers & deformation of silver nanowires

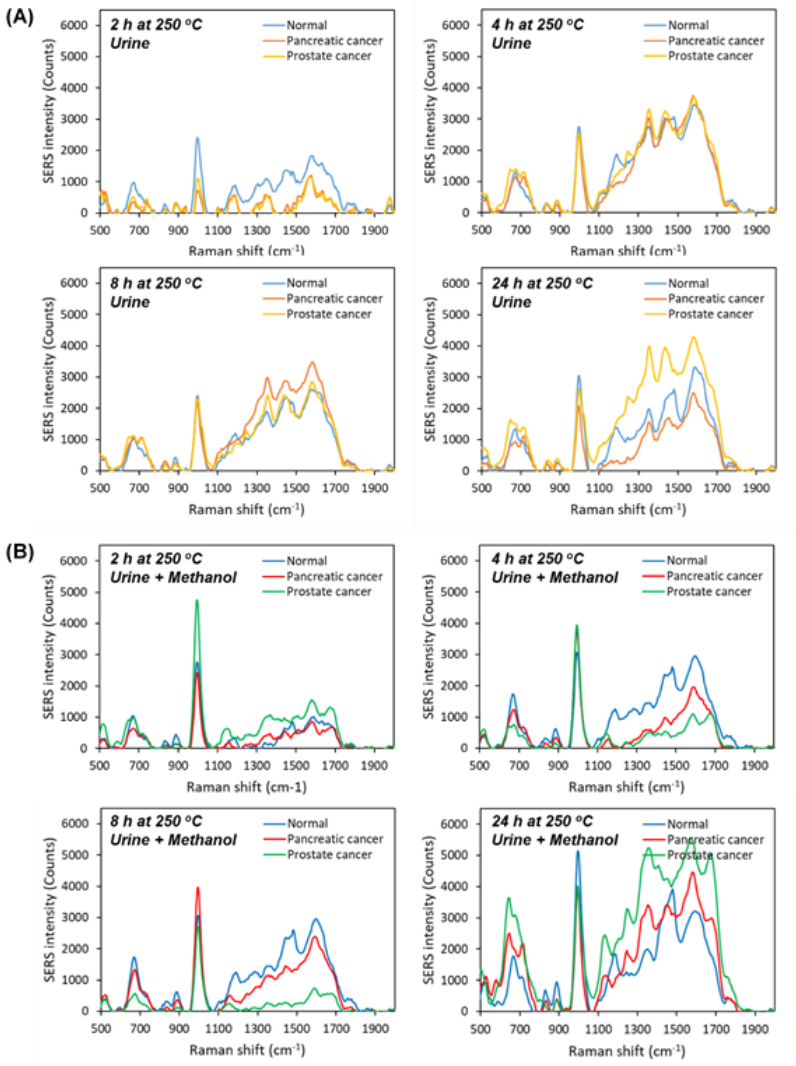
## Aromatic Molecules on Carbonized SERS Sensor

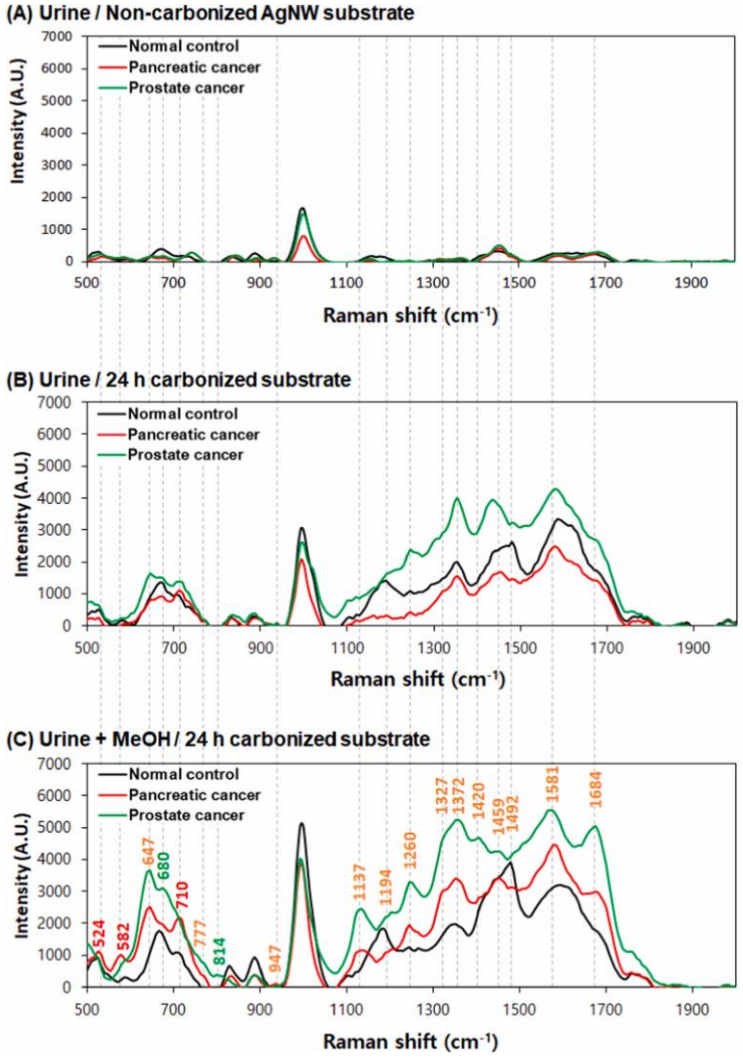


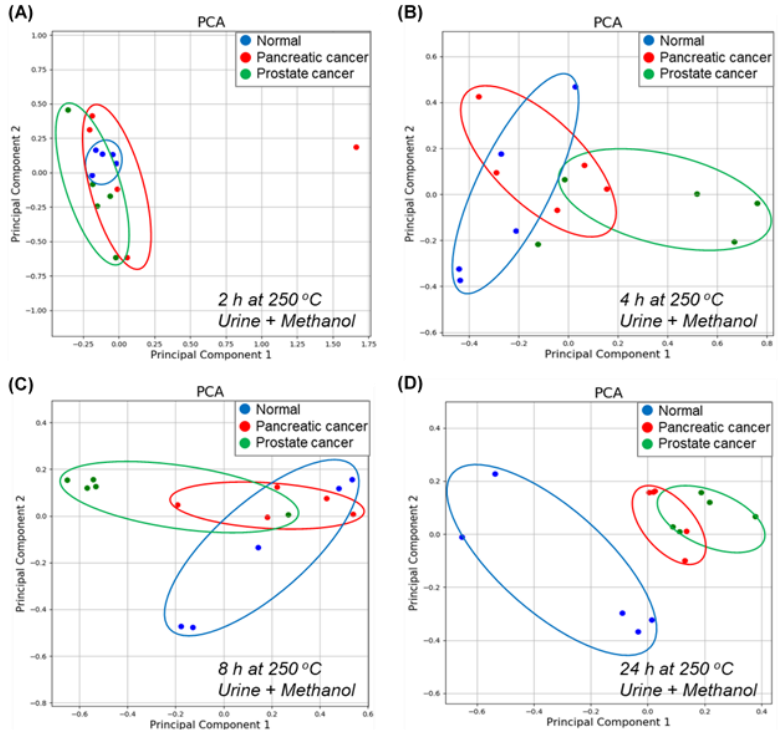

Enhancement of SERS signals of hydrophobic molecules depending on surface hydrophobicity

# SERS Spectra of Urine Samples

## SERS Spectra According to Sensor Surface Carbonization and Pretreatment method of Urine



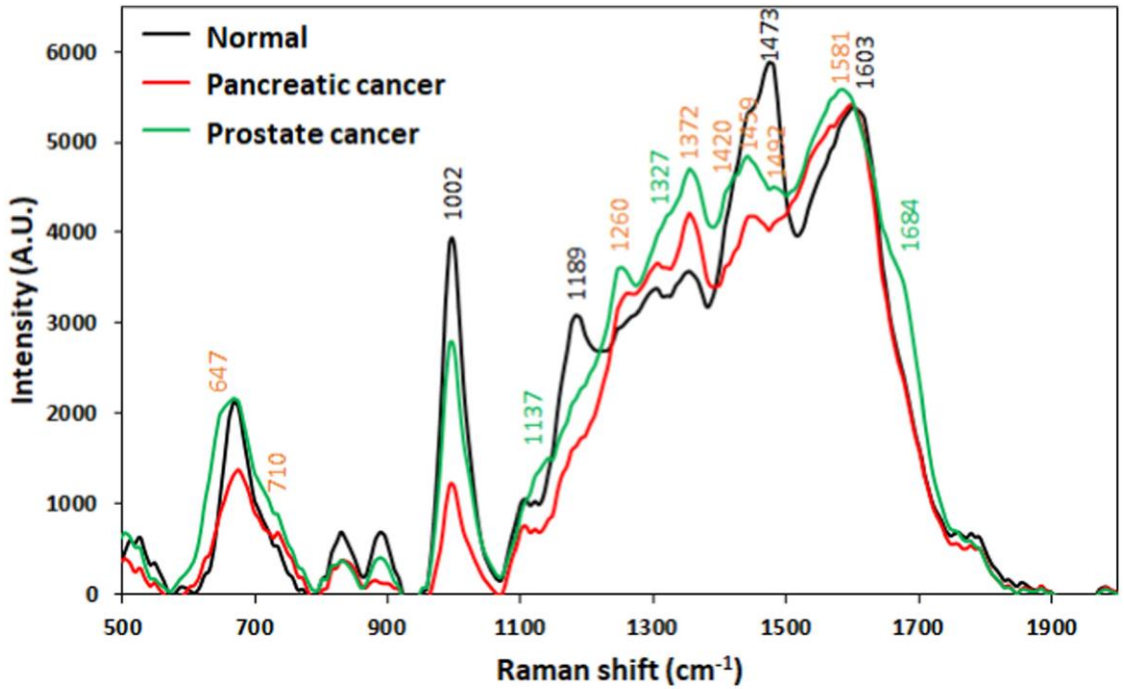




**Changes in SERS signals of urine from cancer patients depending on surface hydrophobicity**

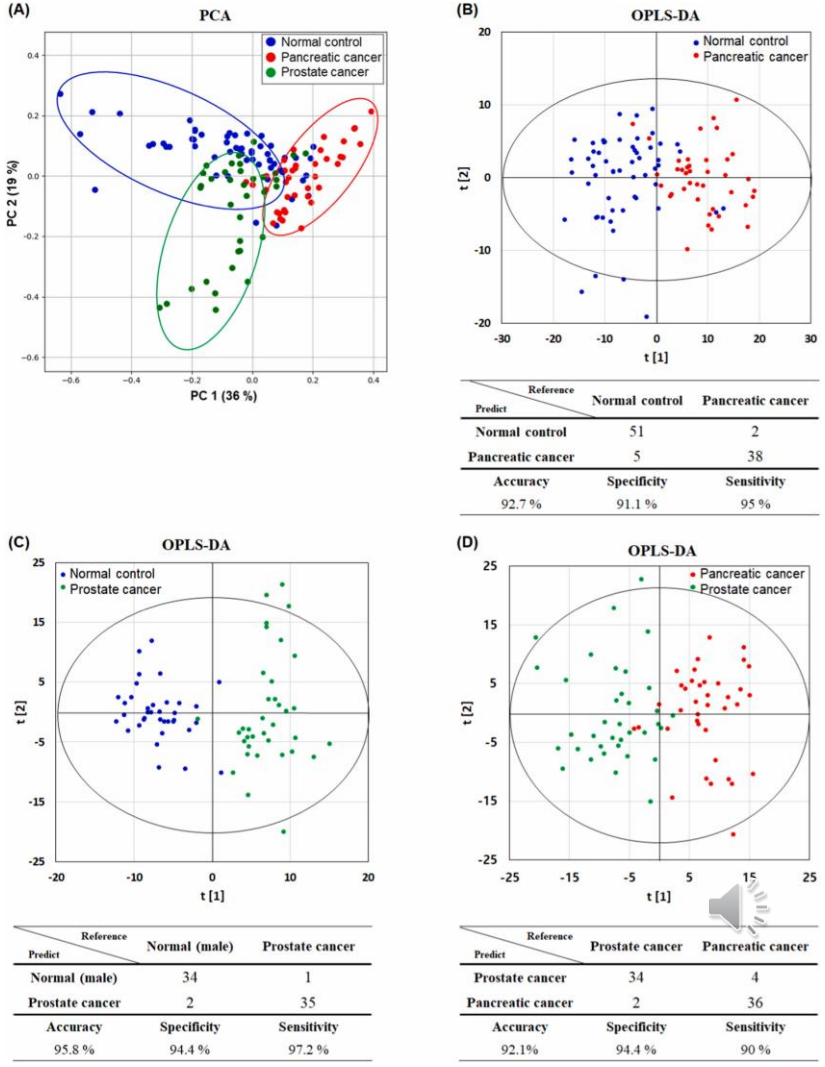
# Diagnostic Accuracy for Pancreatic and Prostate Cancer

## Pancreatic Cancer vs Prostate Cancer vs Normal Control




| | Pancreatic Cancer vs Normal | Prostate Cancer vs Normal | Pancreatic vs Prostate Cancer |
|---|---|---|---|
| Sensitivity | 95% | 97.2% | 94.4% |
| Specificity | 91.1% | 94.4% | 90% |

## Diagnostic Accuracy




| Predict \ Reference | Normal control | Pancreatic cancer |
|---|---|---|
| Normal control | 51 | 2 |
| Pancreatic cancer | 5 | 38 |

| Accuracy | Specificity | Sensitivity |
|---|---|---|
| 92.7 % | 91.1 % | 95 % |

| Predict \ Reference | Normal (male) | Prostate cancer |
|---|---|---|
| Normal (male) | 34 | 1 |
| Prostate cancer | 2 | 35 |

| Accuracy | Specificity | Sensitivity |
|---|---|---|
| 95.8 % | 94.4 % | 97.2 % |

| Predict \ Reference | Prostate cancer | Pancreatic cancer |
|---|---|---|
| Prostate cancer | 34 | 4 |
| Pancreatic cancer | 2 | 36 |

| Accuracy | Specificity | Sensitivity |
|---|---|---|
| 92.1% | 94.4 % | 90 % |

# SERS Spectra Comparison of Aromatic Metabolites

## Expected Metabolites for Pancreatic and Prostate Cancer

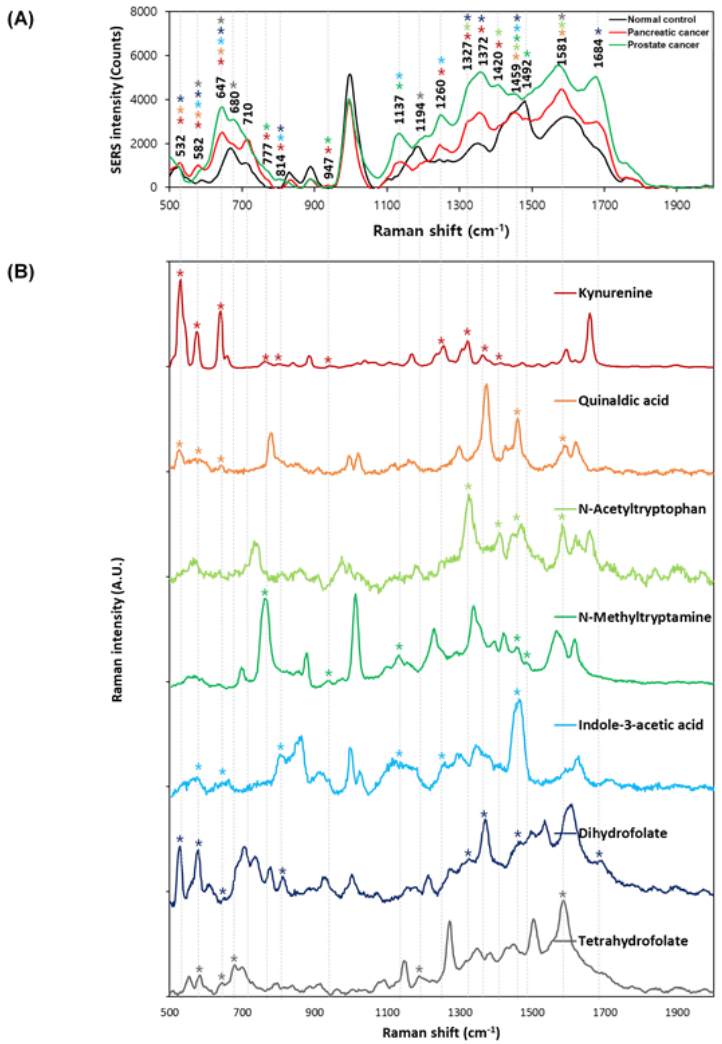
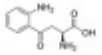
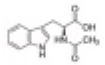
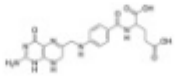
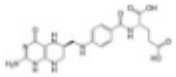



Table 1. Aromatic metabolites expected to exhibit elevated levels in the urine of patients with cancer.

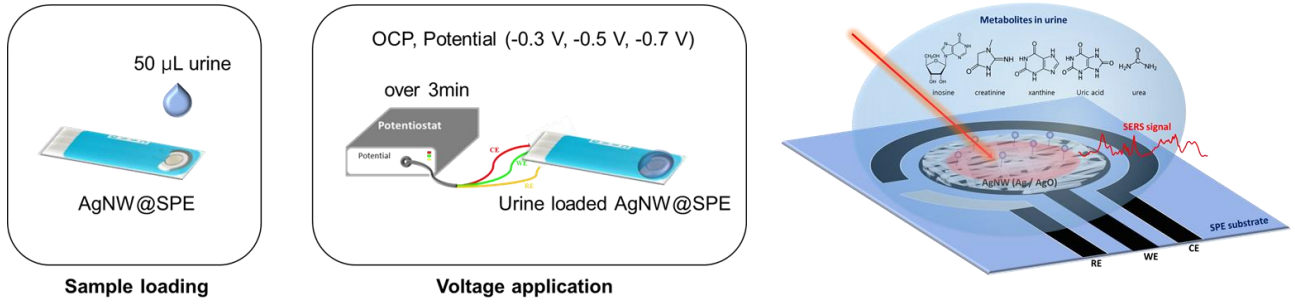
| Classification | Metabolite | Structure | Matched Raman peaks ($cm^{-1}$) of cancer patients |
|---|---|---|---|
| Tryptophan metabolites | Kynurenine |  | 532, 582, 647, 777, 814, 947, 1260, 1327, 1372 1420 |
| | Quinaldic acid |  | 532, 582, 647, 1459, 1581 |
| | N-Acetyltryptophan |  | 1327, 1420, 1459, 1581 |
| | N-Methyltryptamine |  | 777, 947, 1137, 1459, 1492 |
| | Indole-3-acetic acid |  | 582, 647, 814, 1137, 1260, 1459 |
| Folate metabolites | Dihydrofolate |  | 532, 582, 647, 814, 1327, 1372, 1459, 1684 |
| | Tetrahydrofolate |  | 582, 647, 680, 1194, 1581 |

# PART 2.

## Enhancing the identification of pancreatic cancer through surface-enhanced Raman scattering of electro-chemically separated urine components

# Electrochemical(EC)-SERS Sensor for Urine analysis

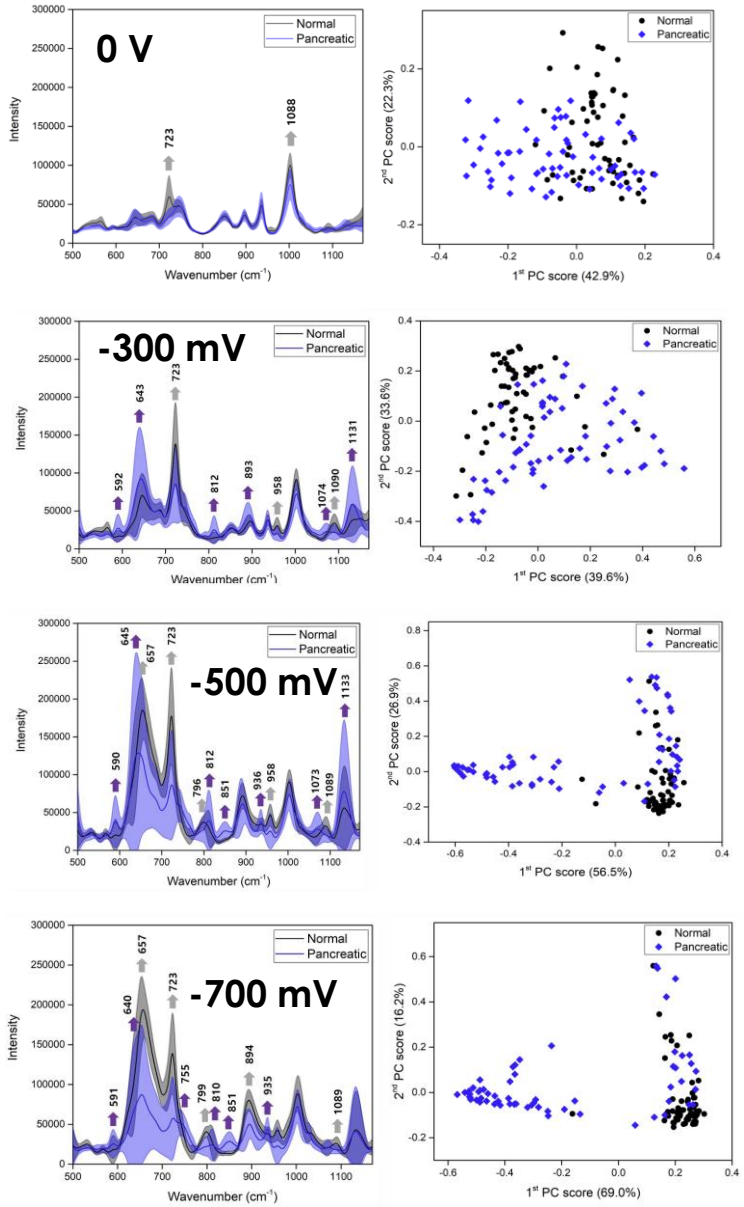
## EC-SERS Sensor for Separated Urine metabolites



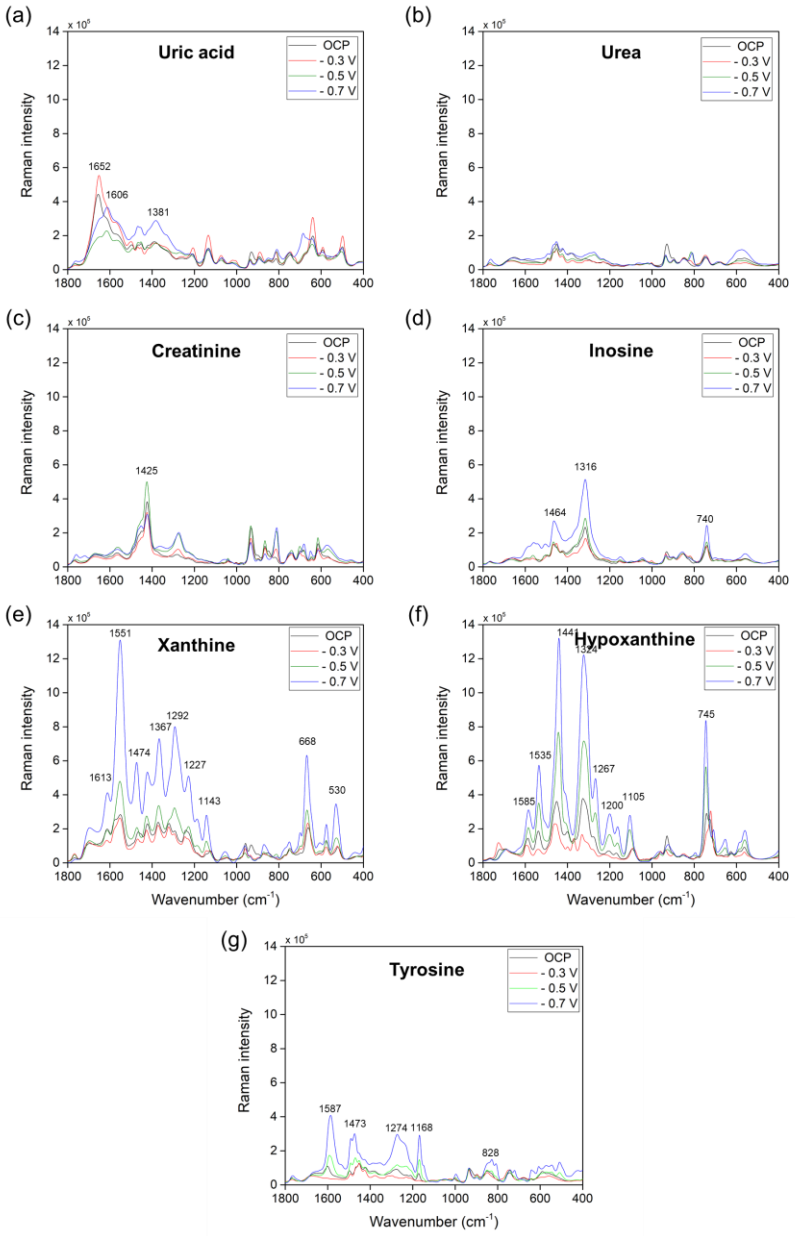

## Sensitivity and Specificity Based on Applied Potential

| | Accuracy | Sensitivity | Specificity |
|---|---|---|---|
| OCP | 85.7 % | 91.9 % | 78.9 % |
| 300 | 91.6 % | 93.5 % | 89.5 % |
| 500 | 91.6 % | 93.5 % | 89.5 % |
| 700 | 92.4 % | 90.3 % | 94.7 % |

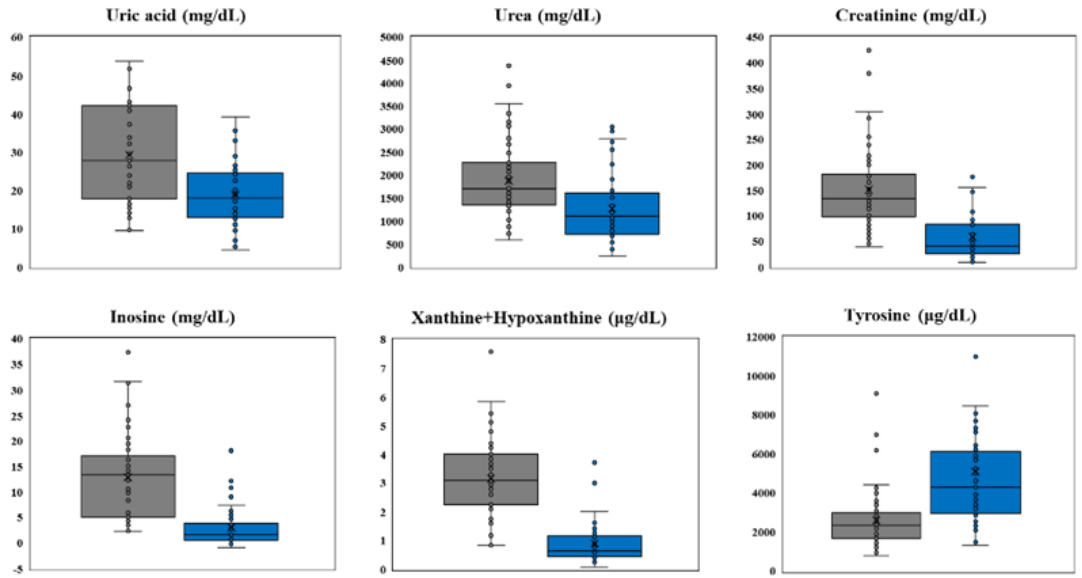
**Best pancreatic cancer diagnostic accuracy at -700 mV**



# SERS Spectra of Urinary Metabolites




## Metabolites Quantification by ELISA



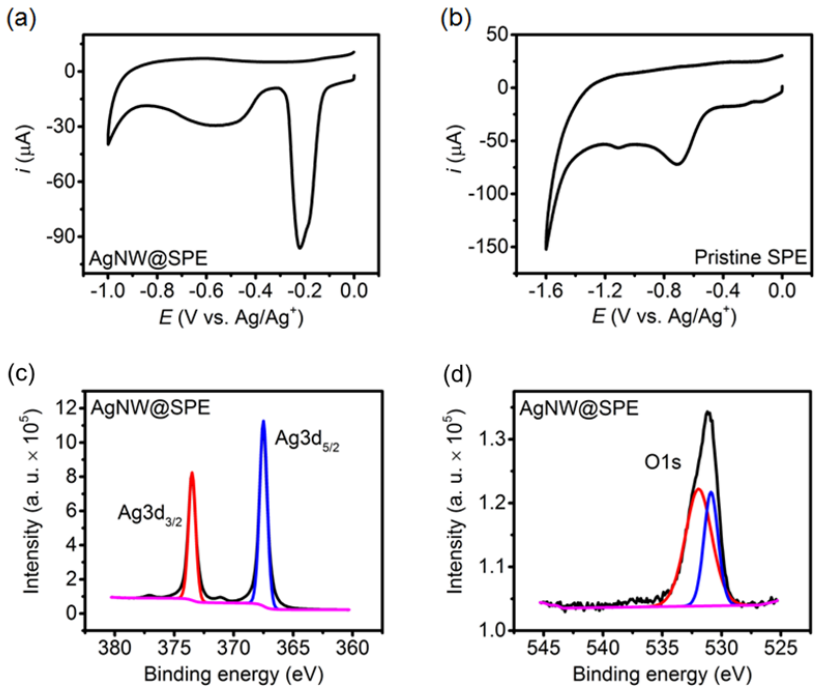

**Related Metabolite Levels in Urine of Pancreatic Cancer Patients**

**Decreased: Uric acid, Urea, Creatinine, Inosine, Xanthine, Hypoxanthine**

**Increased: Tyrosine**

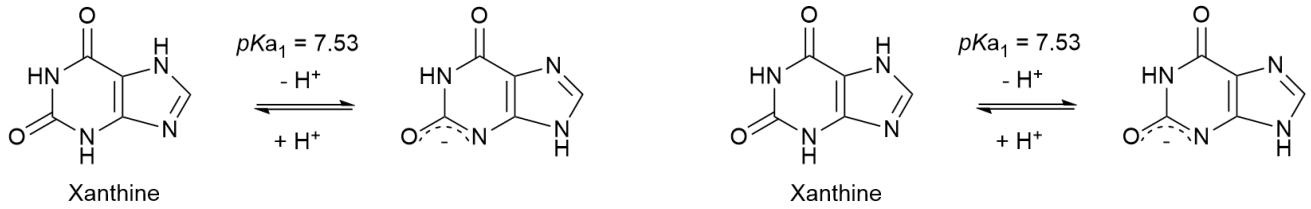
# Mechanistic Analysis of EC-SERS Diagnostics

## Cyclic Voltammetrys of only a 0.1 M NaNO3 solution



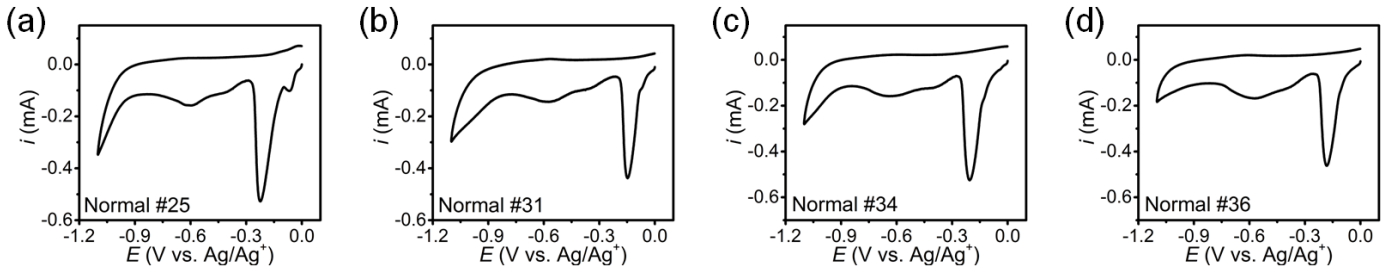

**In pancreatic cancer patients, silver nanowire oxidation is inhibited due to specific molecule adsorption, suggesting xanthine adsorption is blocked.**

## Ionization equilibria and the corresponding pKa values

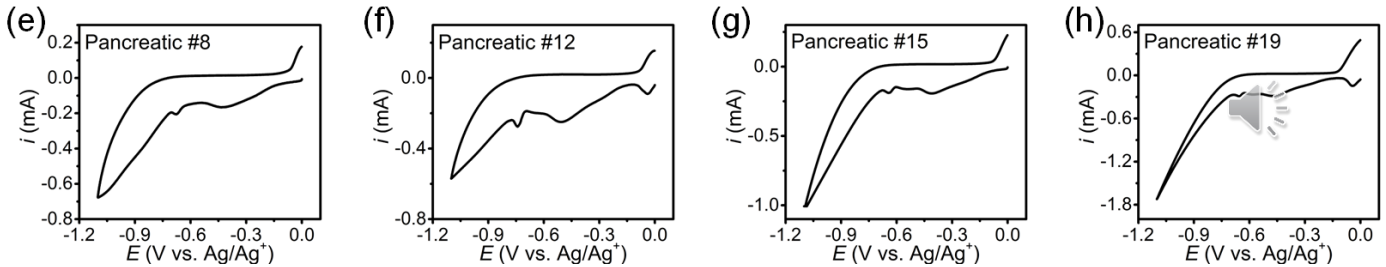



## Cyclic Voltammetry of normal and pancreatic cancer

*Normal Controls*




*pancreatic cancer*



# Conclusion

**The surface property changes of the SERS substrate or the applied voltage for SERS facilitated metabolite separation and adsorption in urine, maximizing the differences in label-free SERS spectra of urine metabolites for early pancreatic cancer diagnosis, which is challenging to detect. This approach enhanced diagnostic sensitivity and specificity to over 90% in both patient and control groups.**

**- Future Work**

1) **Expanded Clinical Validation :** Further validation of the SERS-based diagnostic platform using a larger and more diverse patient cohort to assess its robustness and accuracy across different stages of pancreatic cancer.
2) **Application to Other Cancers :** Exploration of the potential of this method for early detection and diagnosis of other difficult-to-diagnose cancers, such as liver or ovarian cancer, through urine metabolite profiling.
3) **Longitudinal Studies :** Conducting longitudinal studies to monitor changes in urine metabolite profiles over time, providing insights into disease progression and response to treatment.

# References

[1] Yu L, Li K, Zhang X. "Next-generation metabolomics in lung cancer diagnosis, treatment and precision medicine: mini review," Oncotarget., vol. 8(70), pp.115774-115786, Nov.2017, doi: 10.18632/oncotarget.22404.

[2] Vermeersch KA, Styczynski MP. "Applications of metabolomics in cancer research," J Carcinog. Vol. 12, pp. 9, Jun.2013, doi: 10.4103/1477-3163.113622.

[3] Yu HJ, Jang E, Woo A, Han IW, Jeon HG, Linh VTN, Park SG, Jung HS, Lee MY. "Cancer screening through surface-enhanced Raman spectroscopy fingerprinting analysis of urinary metabolites using surface-carbonized silver nanowires on a filter membrane," Anal Chim Acta. Vol. 1292, pp. 342233, Mar 2024, doi: 10.1016/j.aca.2024.342233.