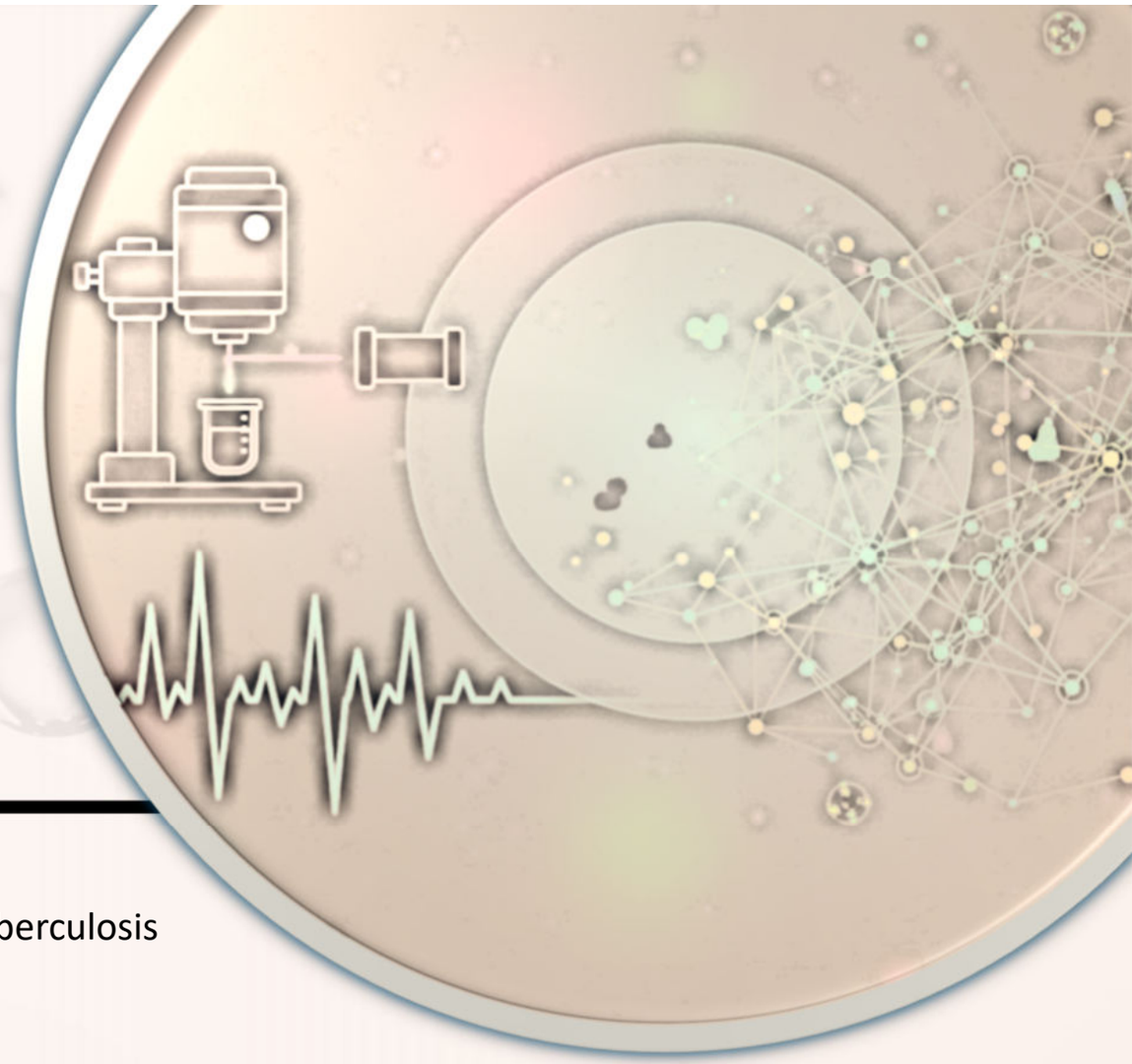
KHON KAEN UNIVERSITY

Thesis Progression Title

Optimization of serum and plasma concentration in sample preparation step using Raman spectroscopy and multivariate analysis



Thesis Title

Comparative feasibility of plasma, serum, and other clinical specimens for Tuberculosis diagnosis using Raman spectroscopy and machine learning

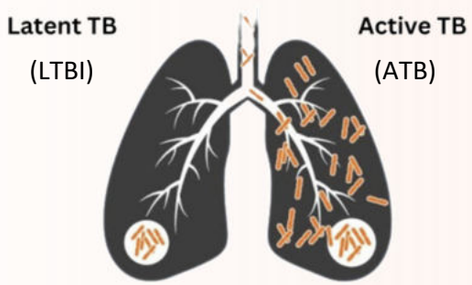
Mr. Patipan Wongboothong

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

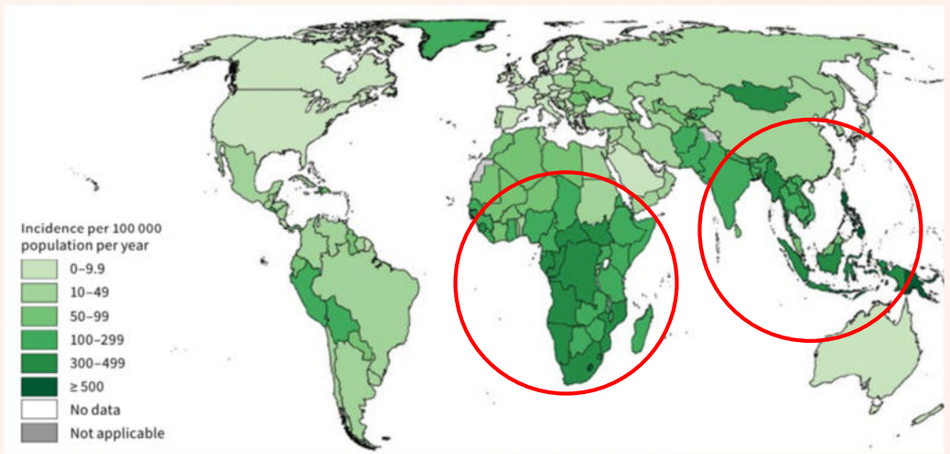
Advisor: Prof. Kiatichai Faksri, Ph.D

Tuberculosis

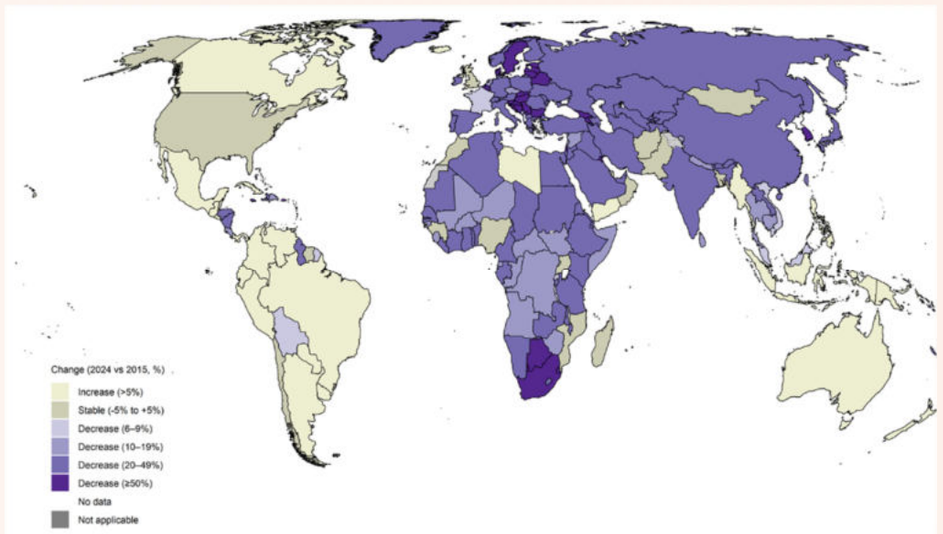
- TB is an illness caused by organisms of the *Mycobacterium tuberculosis* (MTB) complex.
- A total of 10.8 million people fell ill with TB, and 1.25 million people died from TB in 2023 worldwide.
- 22 billion US$ is needed annually for TB prevention, diagnosis, treatment, and care.
- Approximately one-quarter of the world's population has latent tuberculosis infection (LTBI).
- WHO ranks TB as one of 15 causes of death worldwide.
  (World Health Organization, 2025)



Type of tuberculosis



Estimated TB incidence rates, 2024



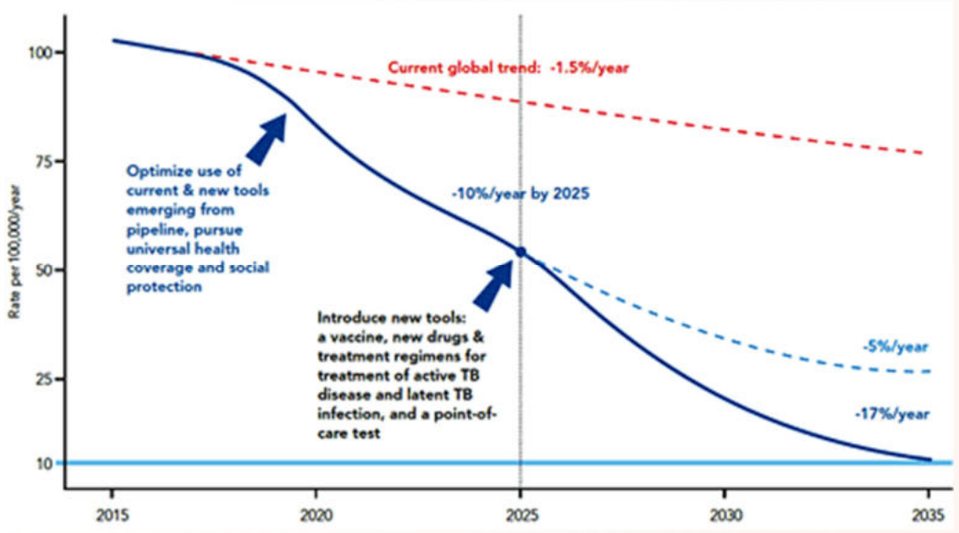
Change (%) in the estimated TB incidence rate, 2024 compared with 2015

World health statistics 2025
https://tradingeconomics.com/thailand/incidence-of-tuberculosis-per-100-000-people-wb-data.html

Introduction

Tuberculosis

Estimated TB incidence rates, 2024



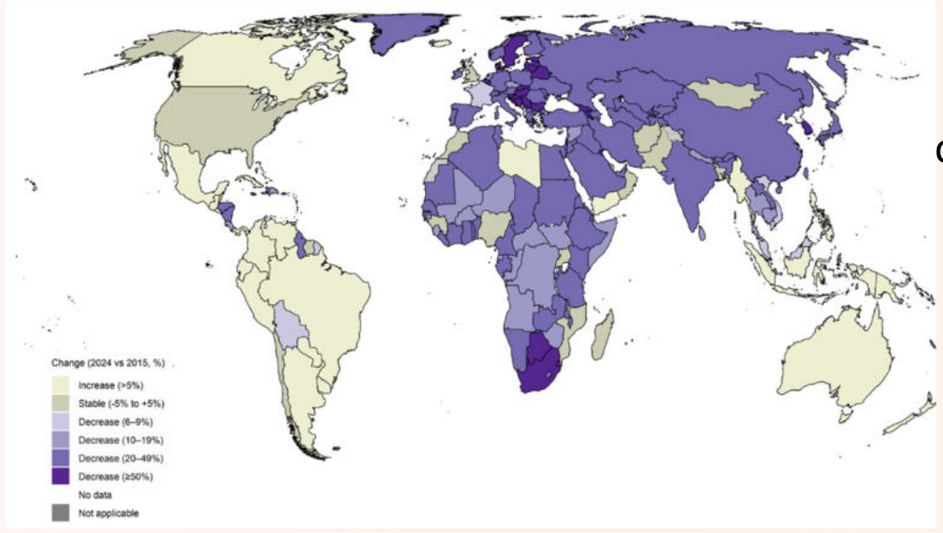
2015 (0%)

2020 (-20%)

2025 (-50%)

2035: End TB

Goal of End TB strategy



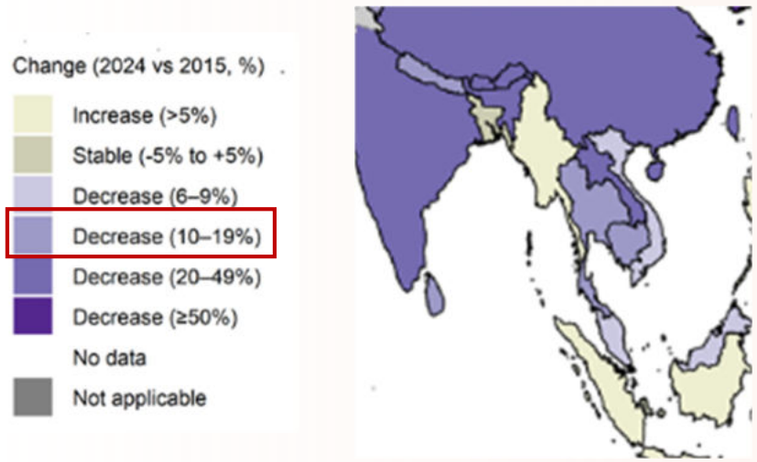
Change (%) in the estimated TB incidence rate, 2024 compared with 2015

Only few countries is close to or reached the 2025 milestone of the End TB Strategy.

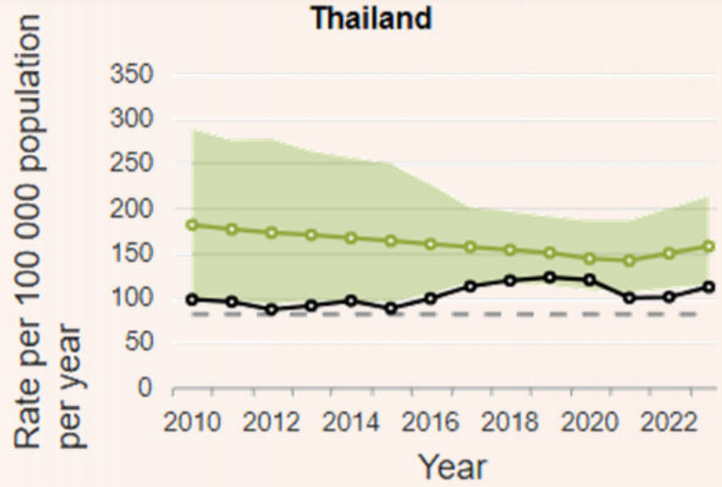
World health statistics 2025
https://tradingeconomics.com/thailand/incidence-of-tuberculosis-per-100-000-people-wb-data.html

Introduction

Tuberculosis



Change (%) in the estimated TB incidence rate, 2024 compared with 2015



Situation in Thailand

Thailand is a High TB burden country, with approximately 103,000 cases (143 in 100,000 population) (2021)

- TB mortality rate in Thailand is high, averaging over 30 deaths per day or around 11,330 deaths yearly (11% estimate mortality).
- Only about 72,000, or 65%, are diagnosed. The remaining 35%, represent unreported TB patients, posing a significant risk of spreading the infection.

Problem

- 1/4 of the population is a reservoir
- There is a gap that can't reach those 35% in the population.

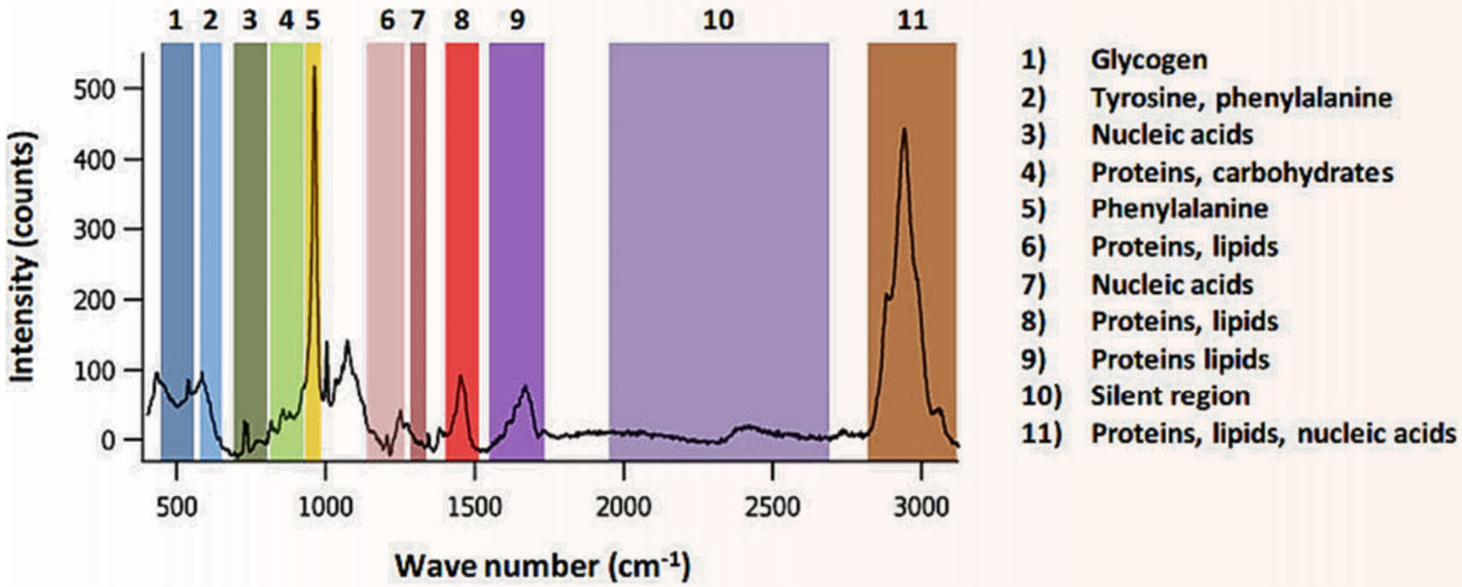
(Tuberculosis profile Thailand 2021, WHO).

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)

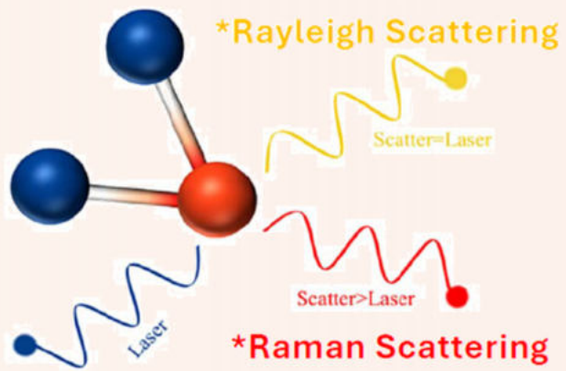


Figure represents a schematic diagram of the simple principles of the Raman scattering effect.

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)

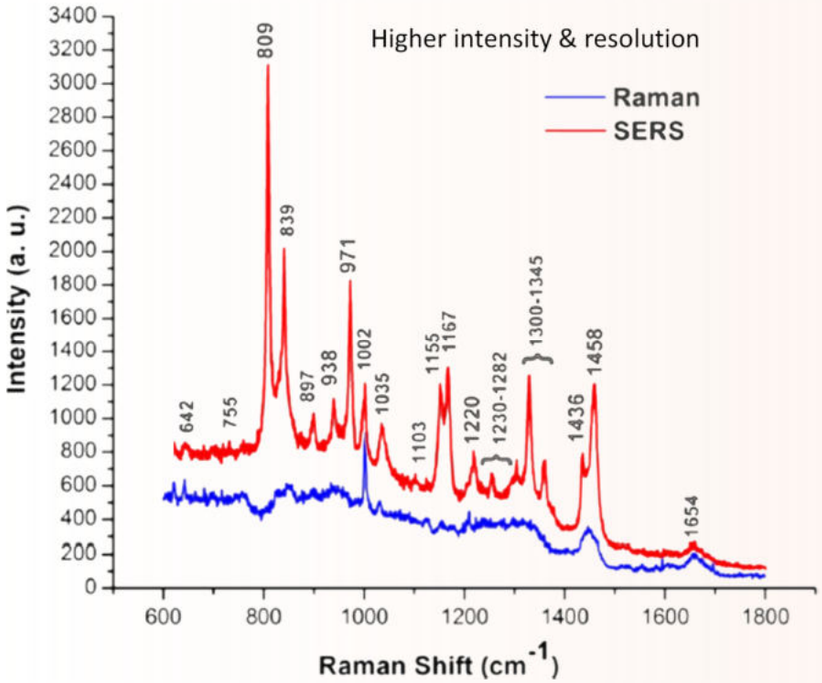


Figure represents different between SERS and Raman spectra

- 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.

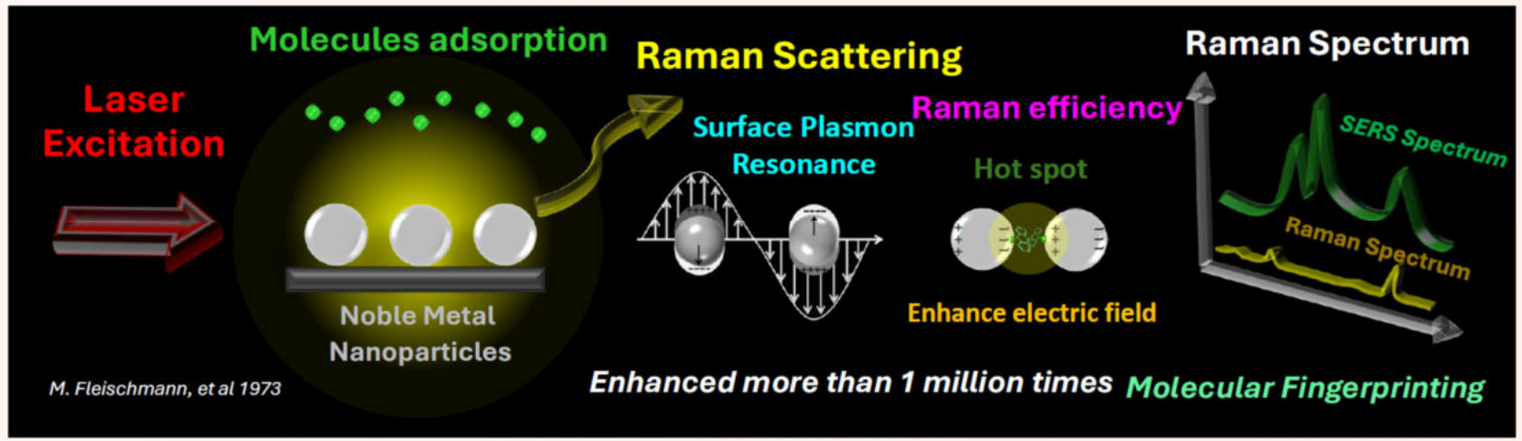


Figure represent mechanism for enhanced Raman signals from SERS (Nuntawong N., 2024)

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

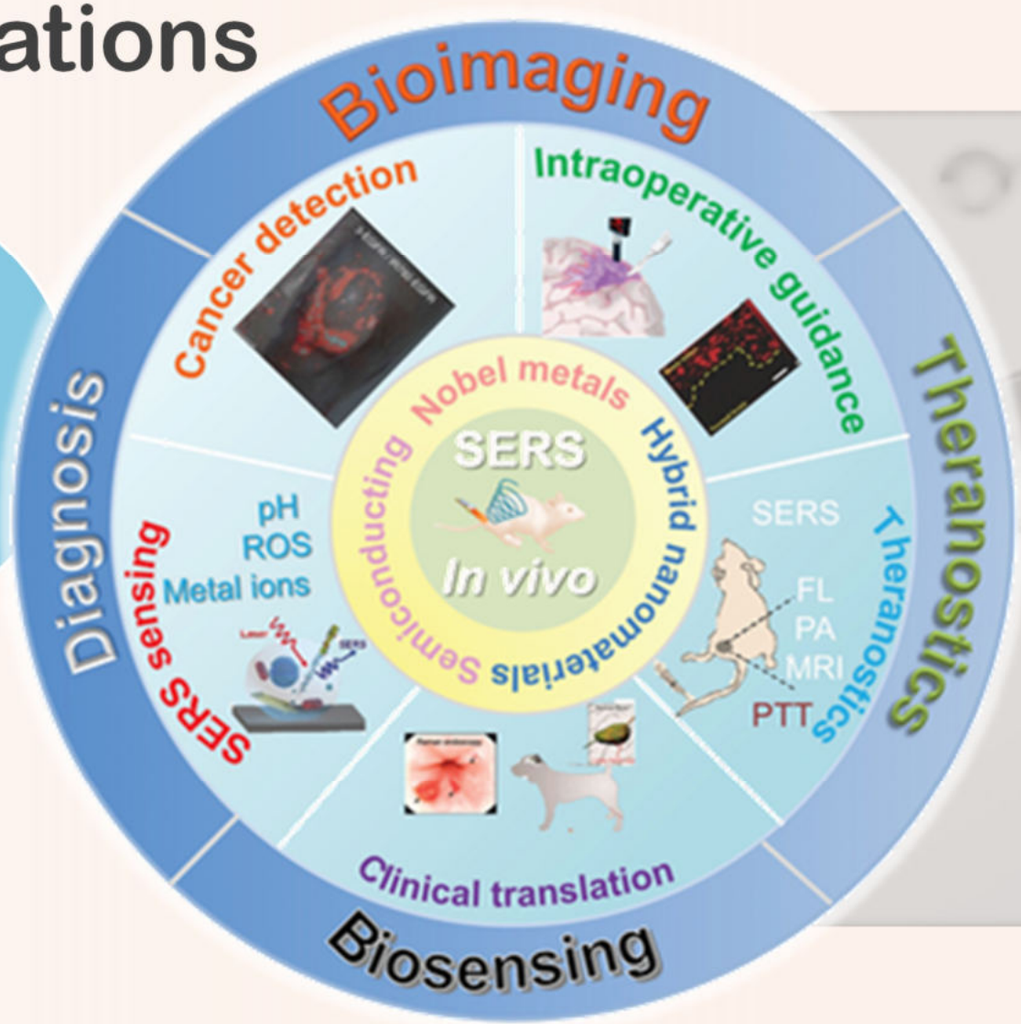
Cancer detection

Bacterial diagnosis

Viral detection

Disease diagnosis

Metabolite detection



Conceptual Framework

Problem

TB is a major global health crisis

Limitations of the current diagnostic

Latent tuberculosis diagnosis and reservoir for active disease

Process

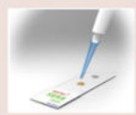
Invasive (Venous serum vs venous plasma)

Less-invasive (Fingertip serum vs venous serum)

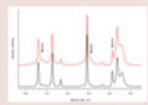
Non-invasive (Saliva vs Tongue swab)

ATB
LTBI
HC

Sample Preparation



Data Analysis



SERS spectra

Outputs

Sample preparation protocol

Raman spectra Database

Performance of TB screening in each sample types

Developing new screening tools

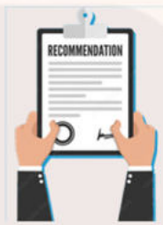
Knowledge and idea for further research

Outcomes

Model for Sample Preparation in Other Diseases

Easier to collect sample

Protocol recommendation in Clinical trial



Novel POCT for TB screening

Screening of large numbers of people in the community

Impact

Improving access to treatment

Enhance the overall health

Reduce costs and time

Promote economy

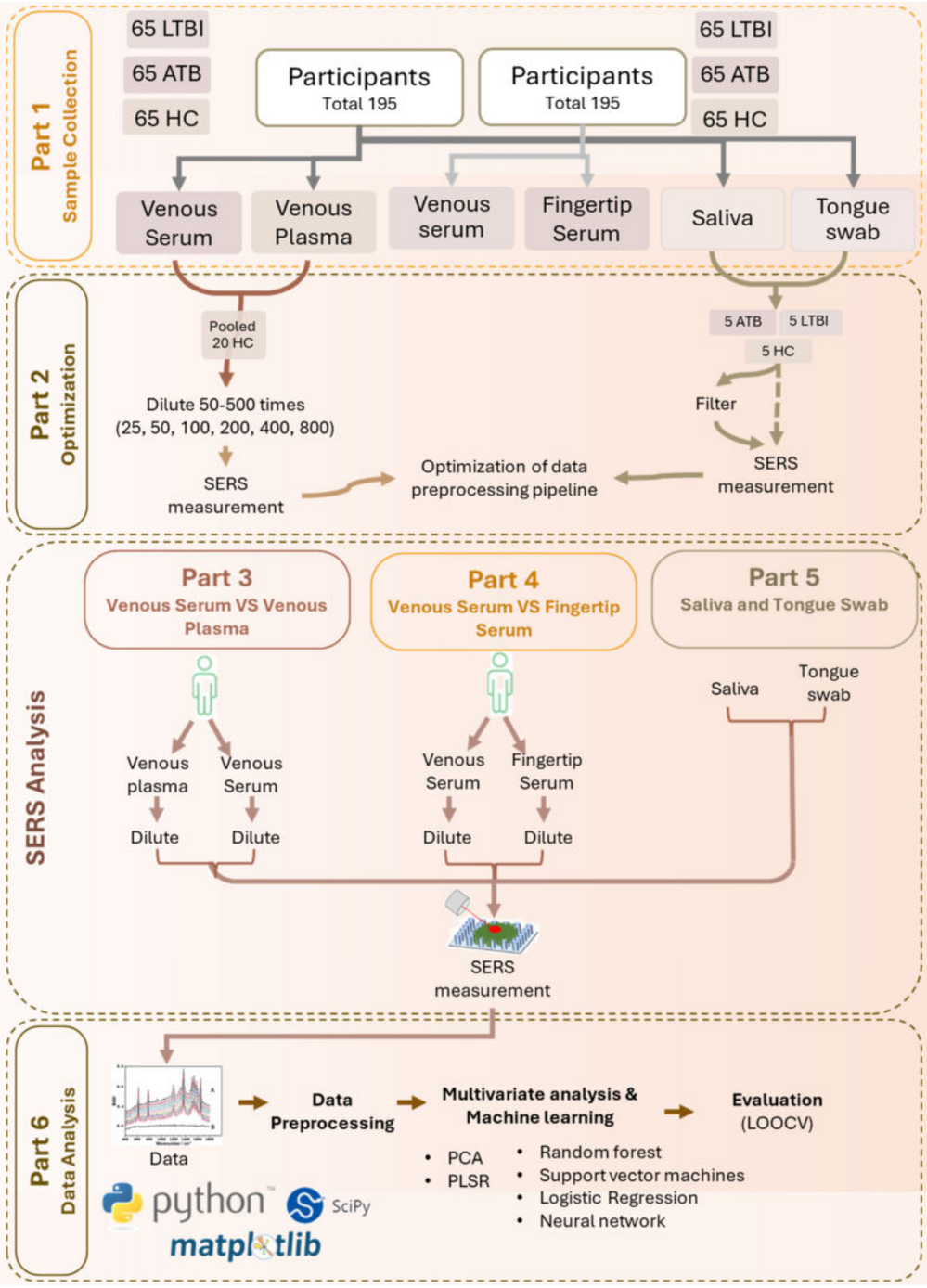
Objectives

To establish the optimal venous blood matrix by comparing the diagnostic performance of serum and plasma with SERS and machine learning to distinguish between TB groups.

To evaluate the feasibility of point-of-care translation by comparing fingertip serum to venous serum with SERS and machine learning to distinguish between TB groups.

To investigate the viability of screening by evaluating saliva and tongue swab samples with SERS and machine learning to distinguish between TB groups

Study Design



Method

Venous Serum

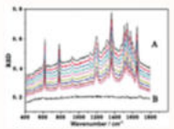
Venous Plasma

Pooled 20 HC

Dilute 50-500 times (25, 50, 100, 200, 400, 800)

SERS measurement

785 laser excitation

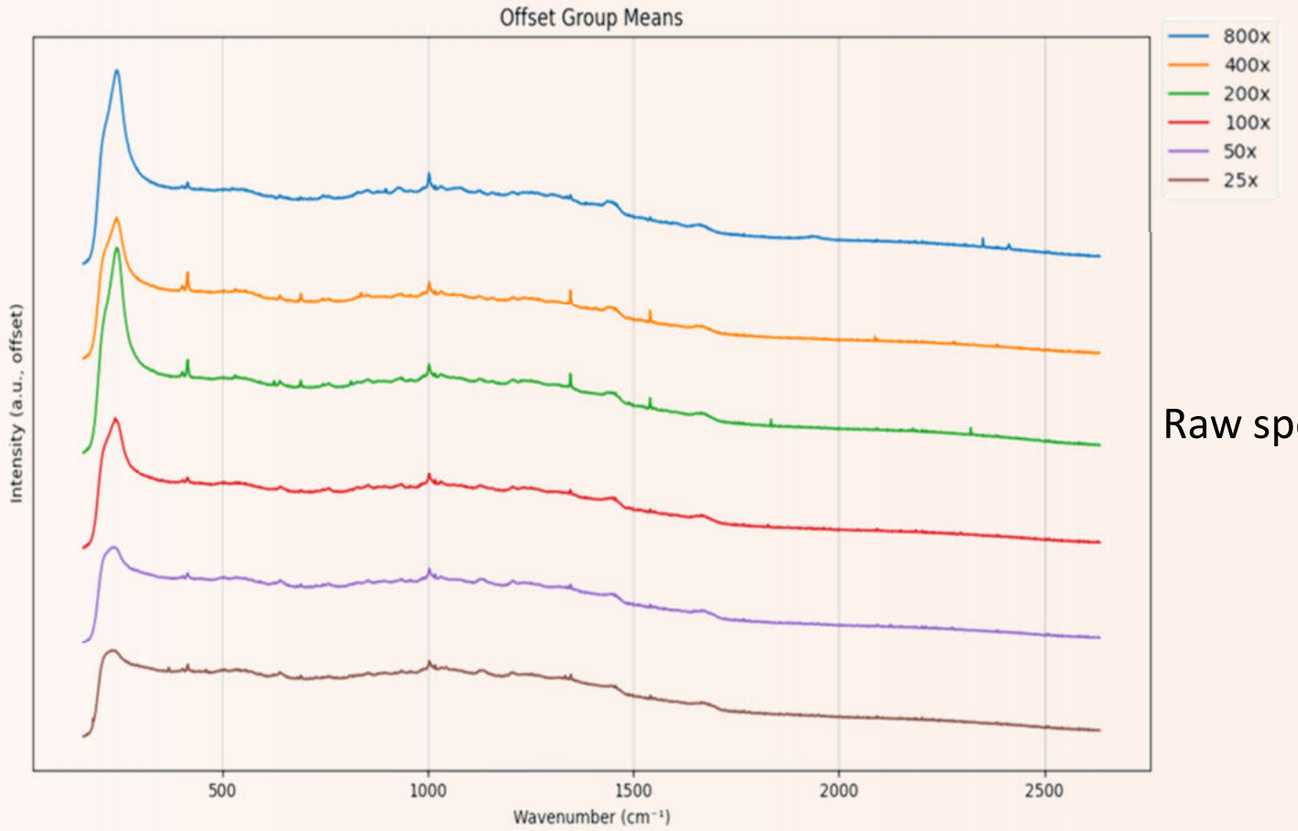


Data

Data Preprocessing → Multivariate analysis

- PCA
- PLSR

python™ SciPy matplotlib



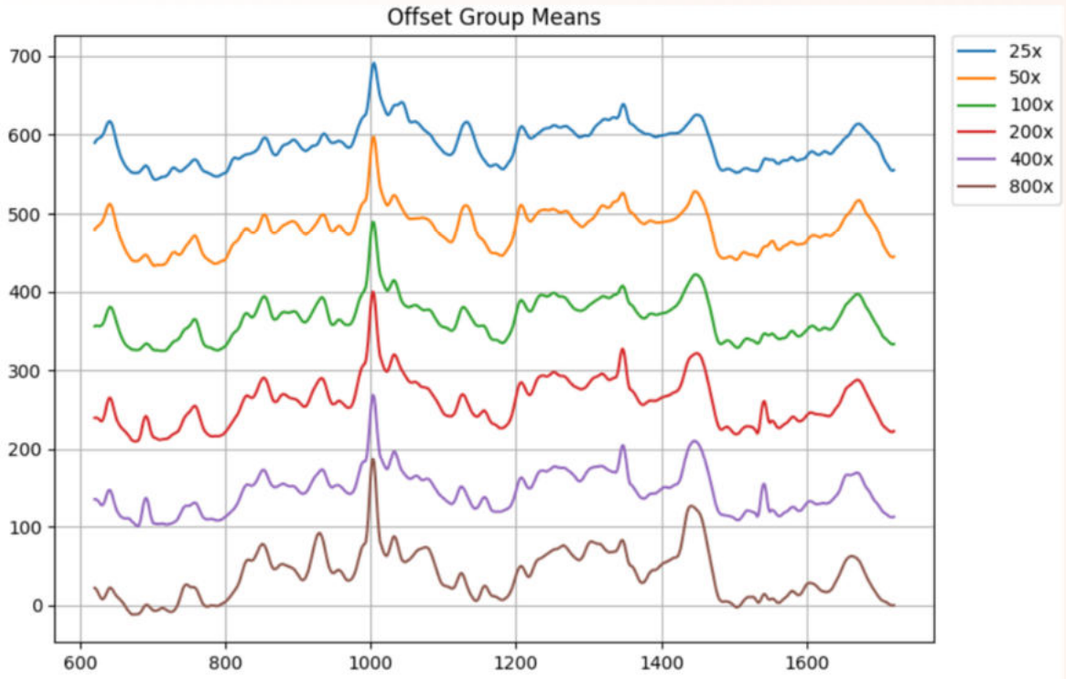
Raw spectra

Preprocessing Pipeline:

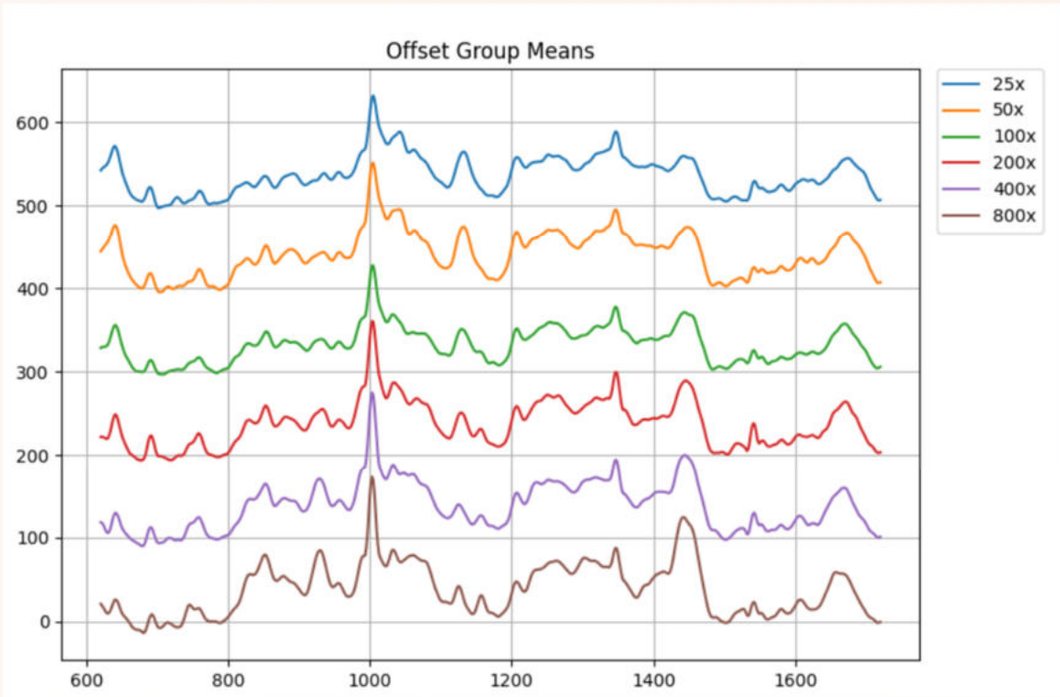
- Step 1: Spike Removal: Kernel: 5
- Step 2: Outlier Removal: SD Multiplier: 3.0
- Step 3: Smoothing: Window: 11, Polyorder: 2
- Step 4: Baseline Correction: Lambda: 100000, p: 0.01
- Step 5: Crop Spectrum: Start: 620, End: 1720

Result

SERS spectra



Serum SERS spectra (preprocessed)

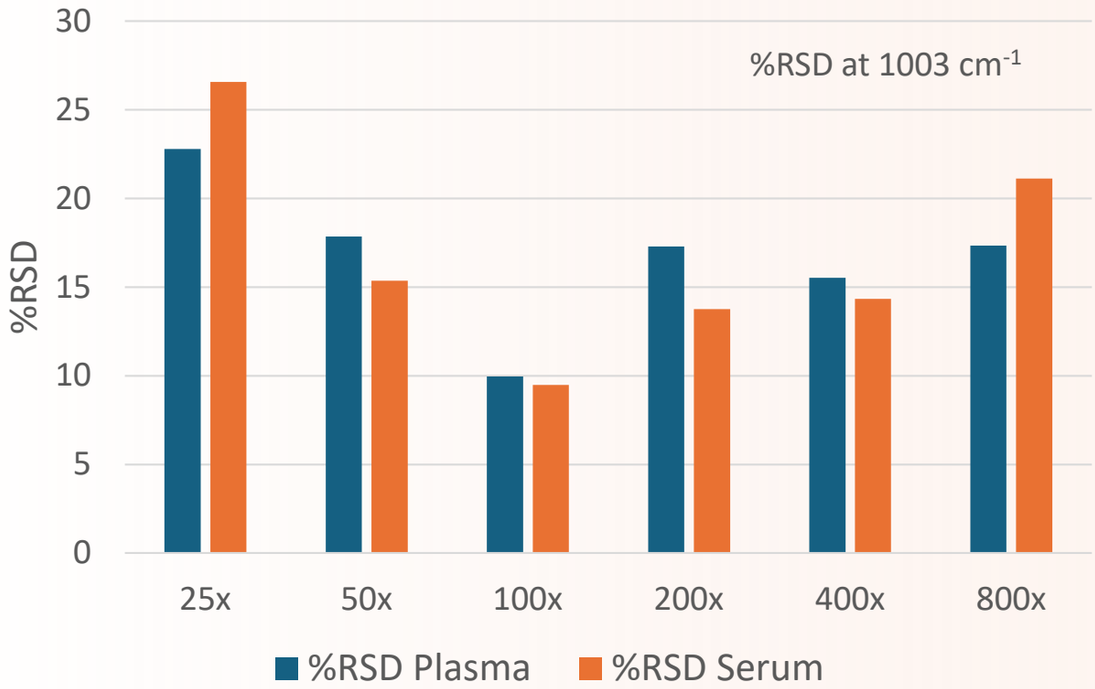


Plasma SERS spectra (preprocessed)

- SERS spectra of serum and plasma is similar in appearance
- At 1003 cm^{-1} is where peak is spiking the highest

Result

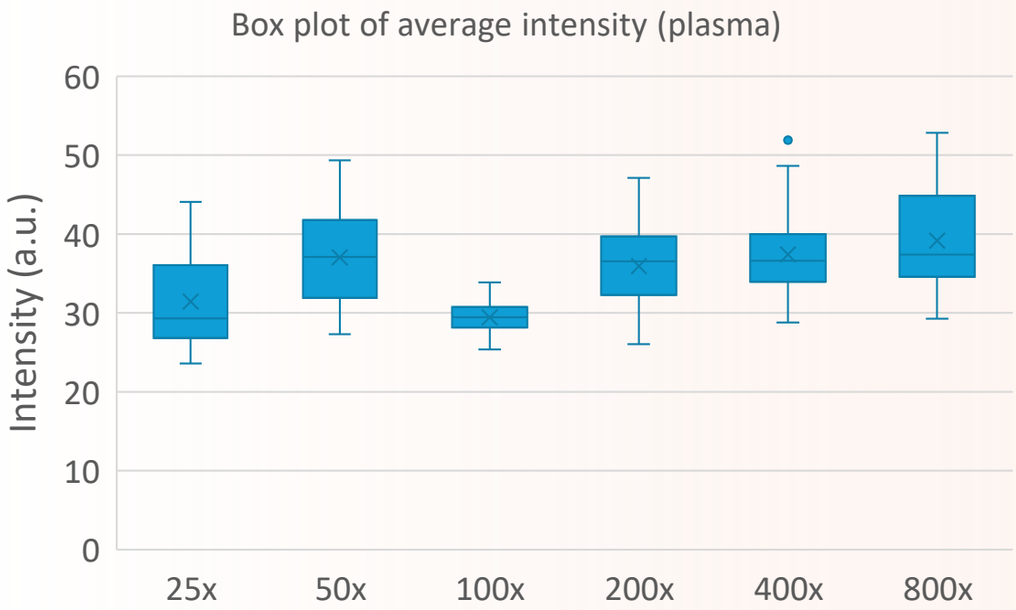
%RSD

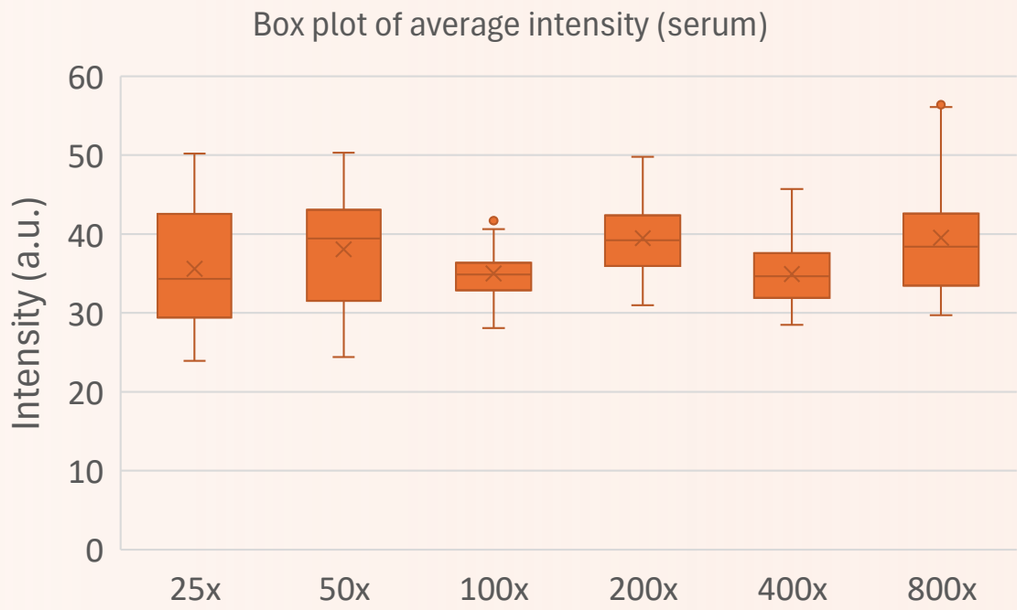


%RSD is calculated based on average intensity at 1003 cm^{-1}

The graph show that at concentration of 100, both plasma and serum achieve the lowest %RSD compared to other dilution.

Box plot of Average Intensity



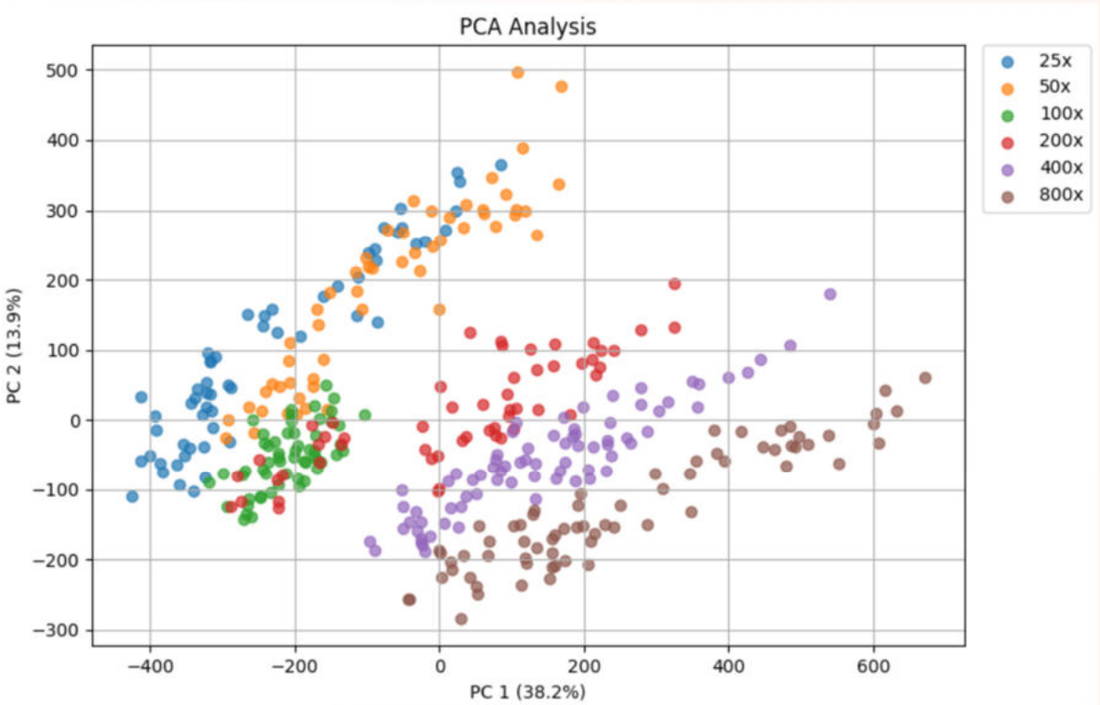


The intensity of all wavelength are average and plot into box plot.

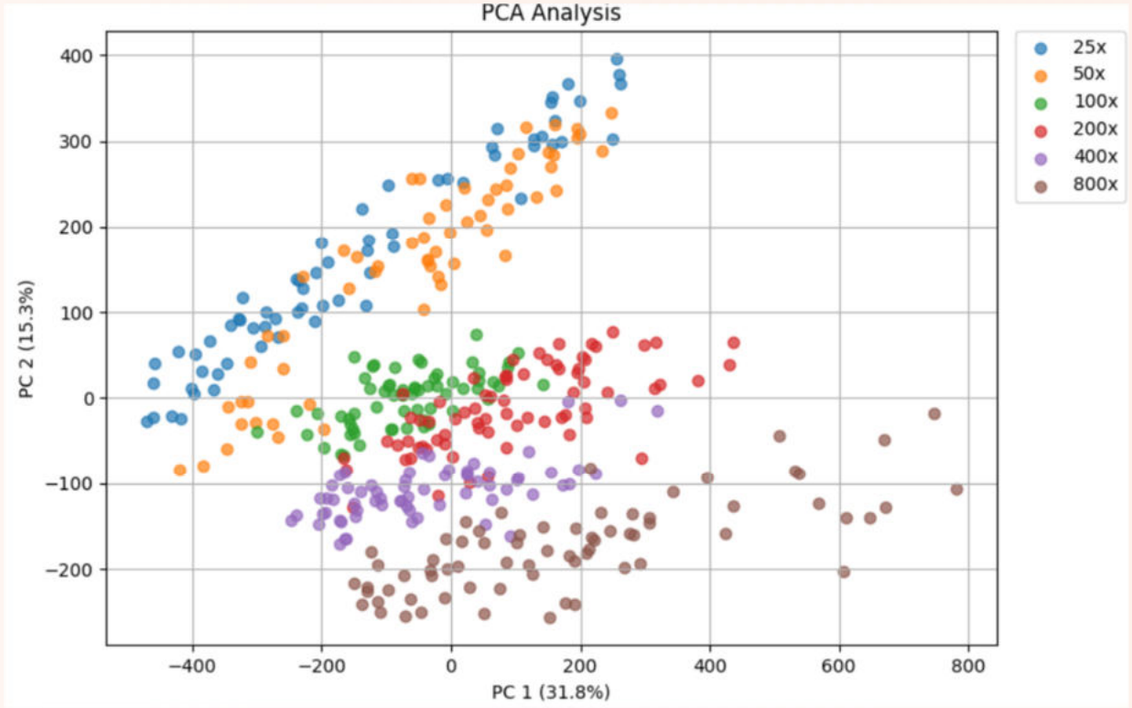
Both graph show at the concentration of 100 show the lowest Standard deviation, indicating the reproducibility of SERS spectra.

Result

PCA Analysis



Plasma PCA (preprocessed)



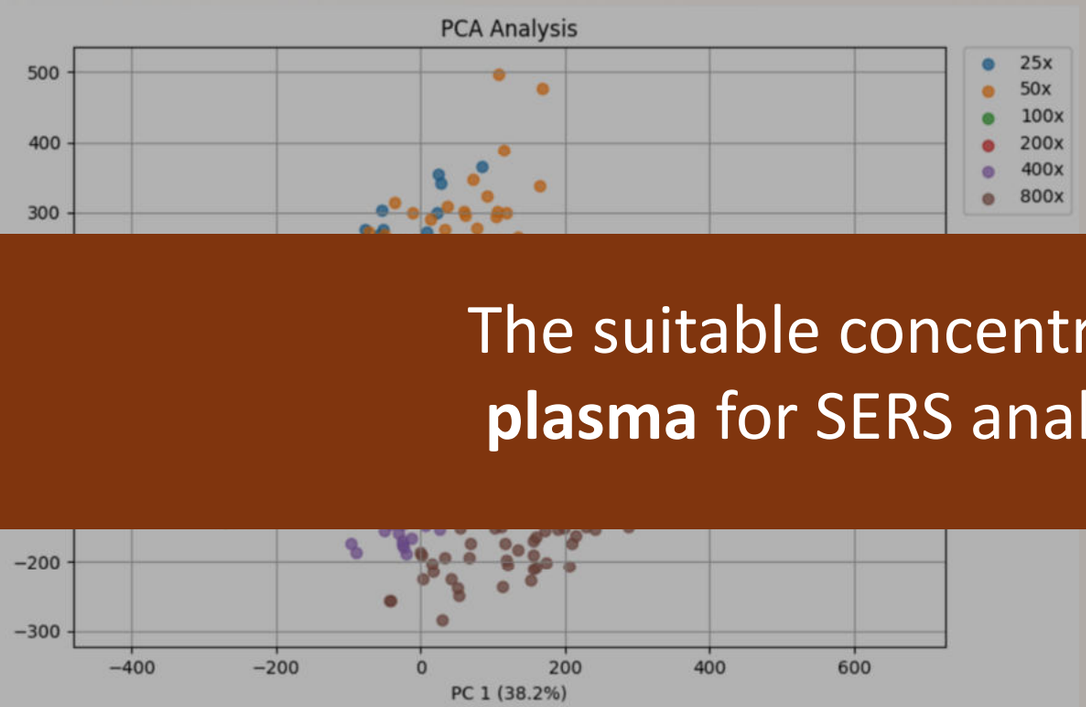
Serum PCA (preprocessed)

The result from PCA plots also support that at dilution factor 100 are the most tightly packed together.

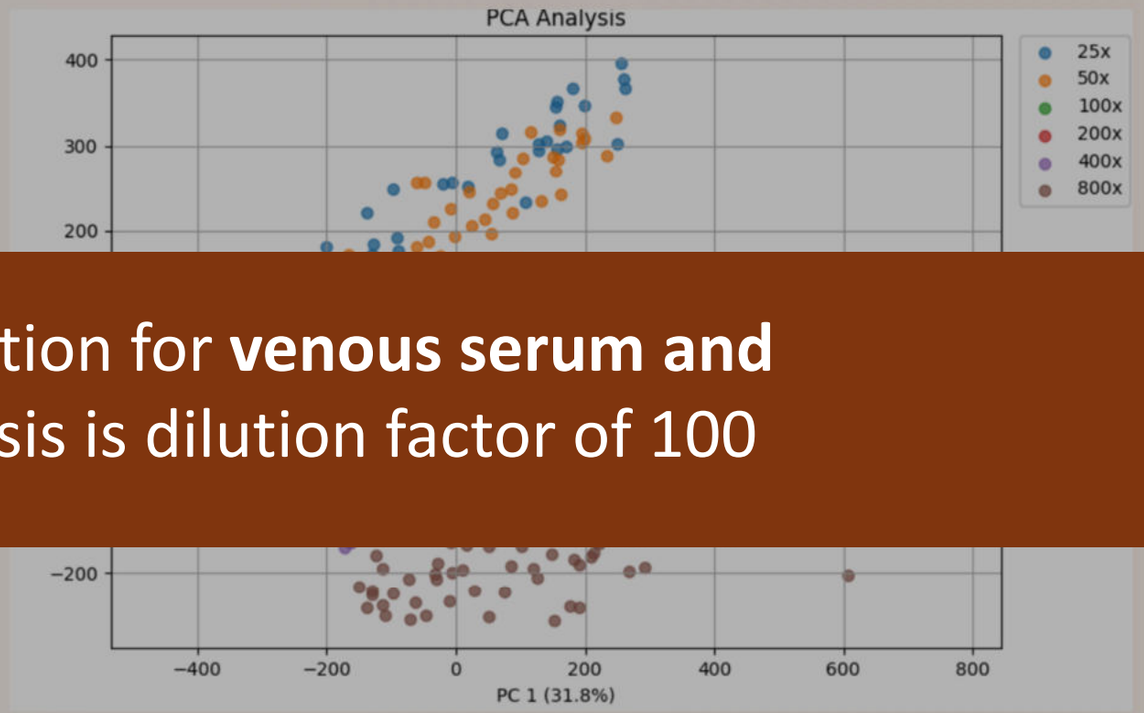
Clustering = Highly reproducible and consistency of spectrum

Result

PCA Analysis



Plasma PCA (preprocessed)



Serum PCA (preprocessed)

The suitable concentration for **venous serum and plasma** for SERS analysis is dilution factor of 100

The result from PCA plots also support that at dilution factor 100 are the most tightly packed together.

Clustering = Highly reproducible and consistency of spectrum

Thesis Plan

Activities	Time															
	2567		2568				2569				2570				2571	
	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2
Literature Review																
Proposal Writing																
Sample Collection																
Qualified Examination																
Comparison between Serum and Plasma																
Optimization of blood sample																
Optimization of preprocessing steps																
Dissertation Proposal Examination																
Sample preparation and SERS analysis																
Data analysis																
Manuscript 1 writing																
Comparison between Fingertip serum and Vein serum																
Sample preparation and SERS analysis																
Data analysis																
Manuscript 2 writing																
Comparison between saliva and tongue swabs																
Optimization of saliva and tongue swab preparation																
Optimization of preprocessing steps for Saliva and Tongue swabs																
Sample preparation and SERS analysis																
Data analysis																
Manuscript 3 writing																
Dissertation Defense Examination																

Acknowledgement

Advisor

Prof. Dr. Kiatichai Faksri

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MDKKU

Medical Microbiology

RCEID

Good Ventures

Thank you

for your attention