Raman Effect and its applications to Biosensing

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NECTEC-ACE 2019, 9 September 2019

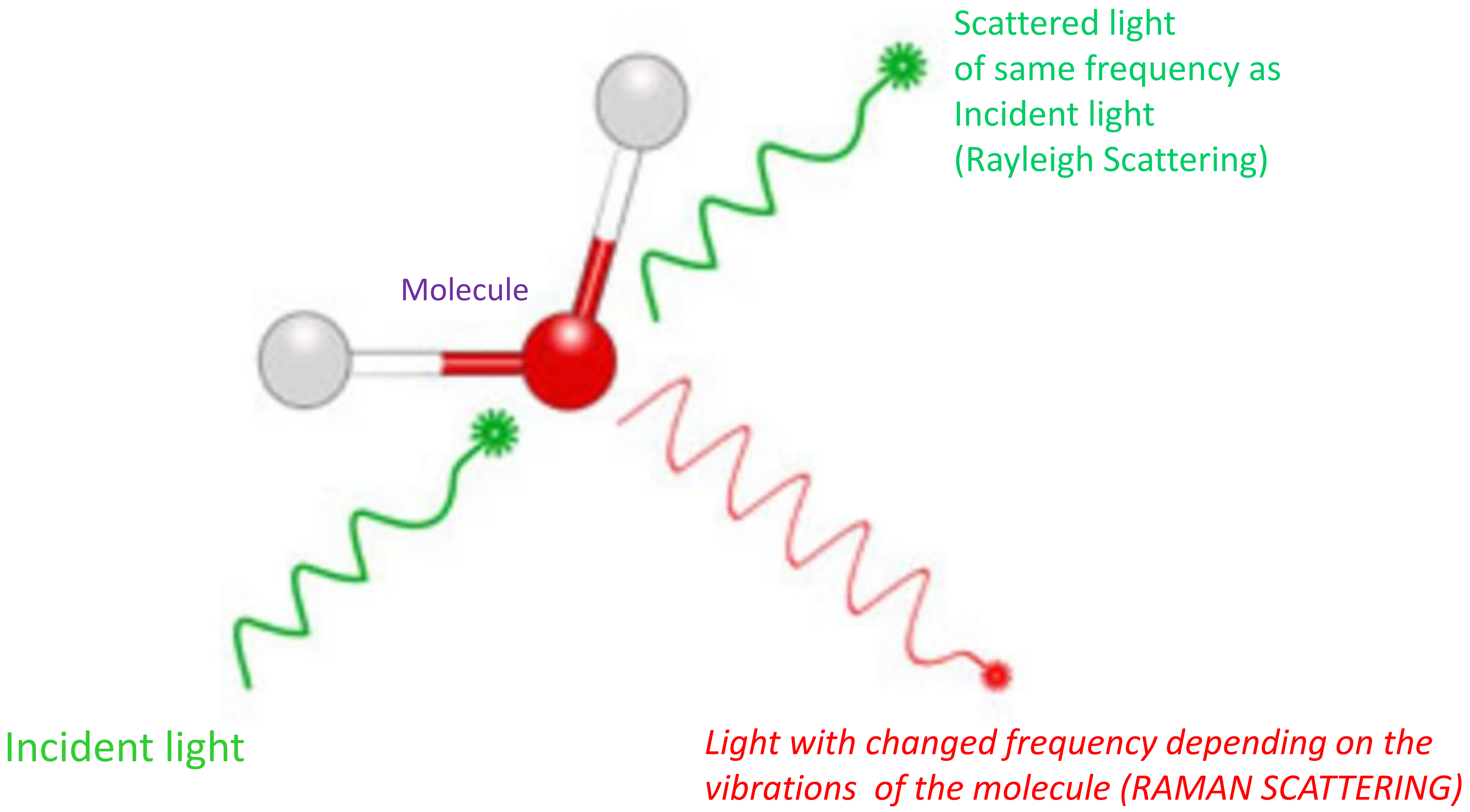
Discovered in INDIA by Sir C V Raman in 1928

The American Chemical Society and the Indian Association for the Cultivation of Science termed The Raman Effect an International Historic Chemical Landmark and said:
Sir C. V. Raman discovered in 1928 that when a beam of coloured light entered a liquid, a fraction of the light scattered by that liquid was of a different color. Raman showed that the nature of this scattered light was dependent on the type of sample present. Other scientists quickly understood the significance of this phenomenon as an analytical and research tool and called it the Raman Effect. This method became even more valuable with the advent of modern computers and lasers. Its current uses range from the non-destructive identification of minerals to the early detection of life-threatening diseases.

Sir C V Raman: 1888 – 1970

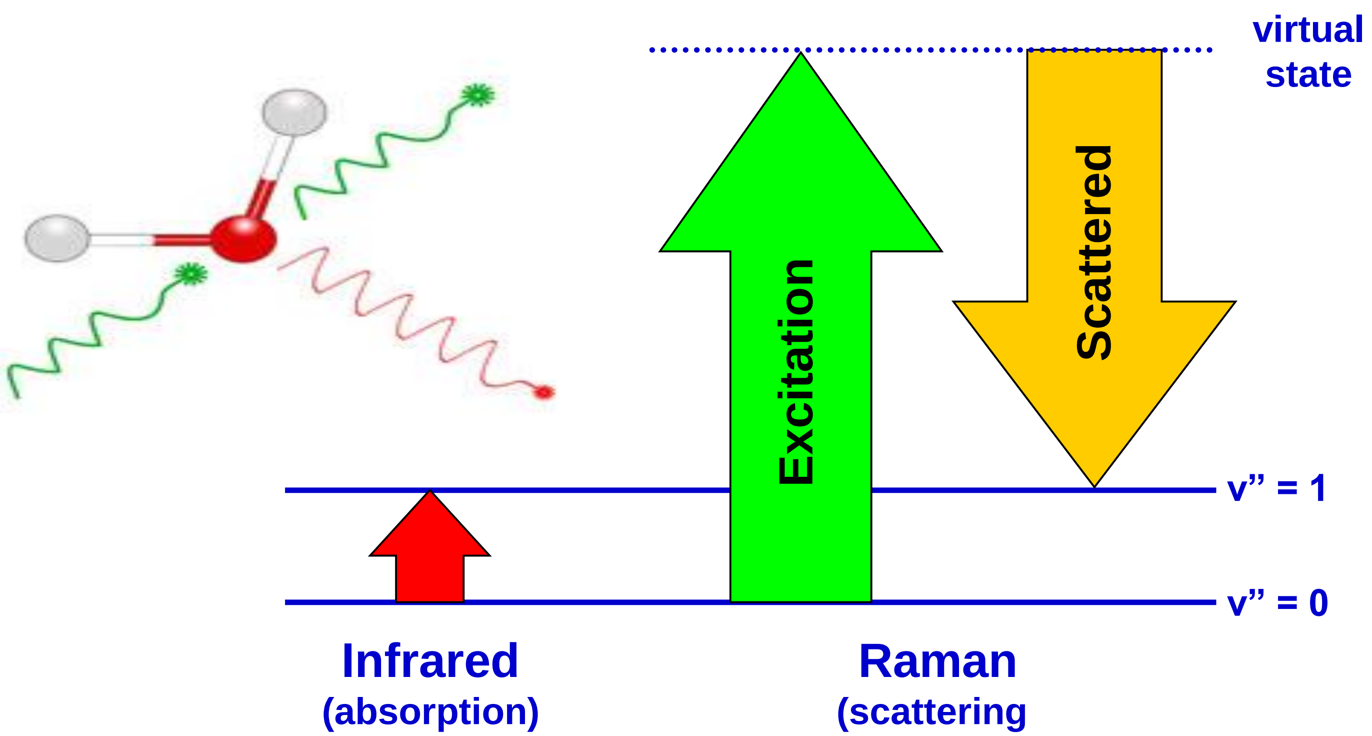
Raman was awarded the Nobel Prize for this discovery in 1930

http://www.acs.org/content/acs/en/education/whatischemistry/landmarks/ramaneffect.htm

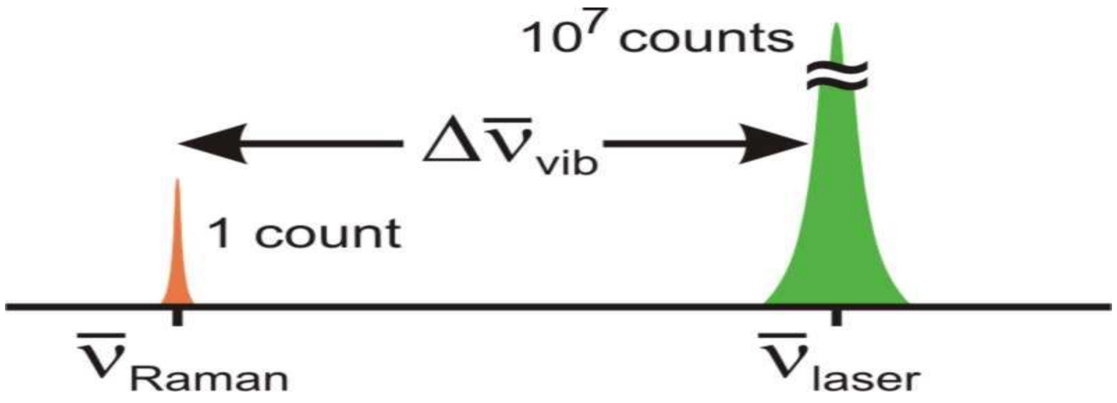

Scattered light
of same frequency as
Incident light
(Rayleigh Scattering)
Molecule
Incident light
Light with changed frequency depending on the vibrations of the molecule (RAMAN SCATTERING)

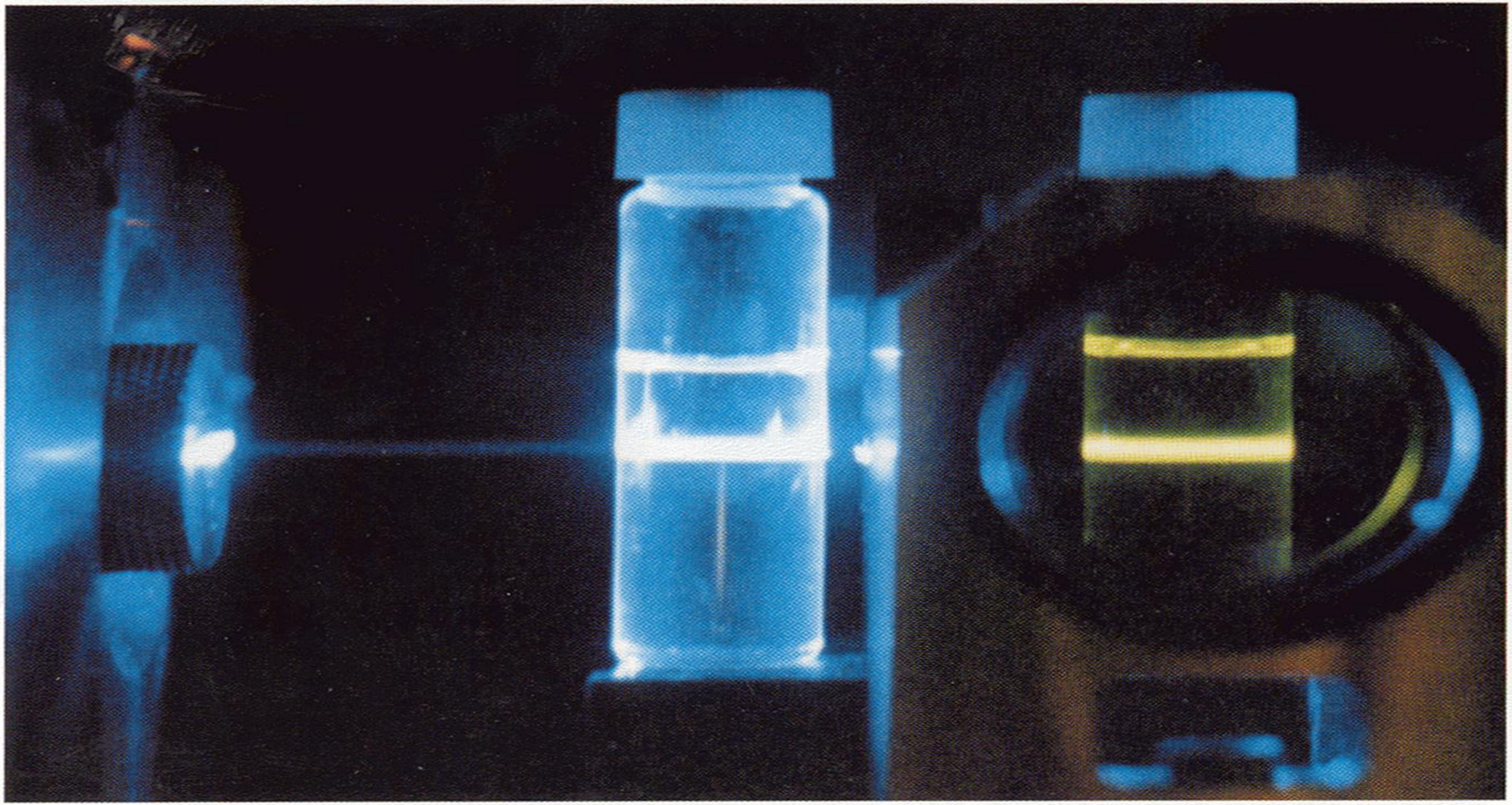
Raman Spectroscopy

1 in 10^7 photons is scattered inelastically



Rotational Raman
Vibrational Raman
Electronic Raman





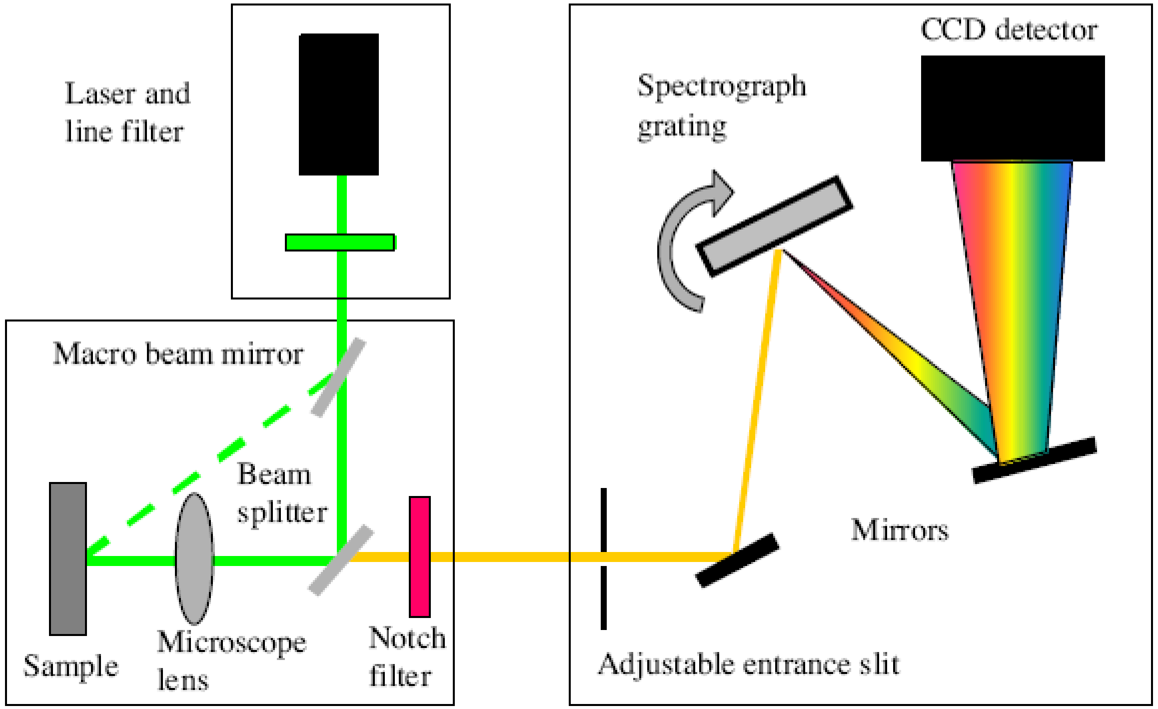
Raman scattering visible to the unaided eye, from a 488 nm laser beam passing through liquid cyclohexane. Left vial exhibits mostly Rayleigh scattering which obscures much weaker Raman scattering. Right vial is viewed through a 488 nm band rejection filter, which permits observation of longer wavelength Raman scattering.

The ease of using Raman spectroscopy has been made possible through numerous technological innovations since its discovery in 1928:

The first major breakthrough came with the development of the laser, which provided considerably more photons to generate Raman scattered photons and therefore improved sensitivity.
The 2-dimensional charge coupled device (CCD) detectors that soon followed, reduced the measurement time from hours to minutes.

The 1990s introduced two new optical elements that simplified Raman spectrometer optical designs. Notch filters eliminated the need for large or multi-stage spectrometers to physically separate the excitation laser Rayleigh scattering from the Raman scattering , and sharp optical cut-off filters allowed the design and use of 180° backscattering single ended probes.

Recent advances in miniaturization has made it possible to have hand held Raman Spectrometers.


Laser and
line filter
CCD detector
Spectrograph
grating
Macro beam mirror
Beam
splitter
Sample
Microscope
lens
Notch
filter
Mirrors
Adjustable entrance slit

Advantages:

Specificity: unique signature spectrum for each molecular species

Non destructive : sample is not damaged for Raman measurements

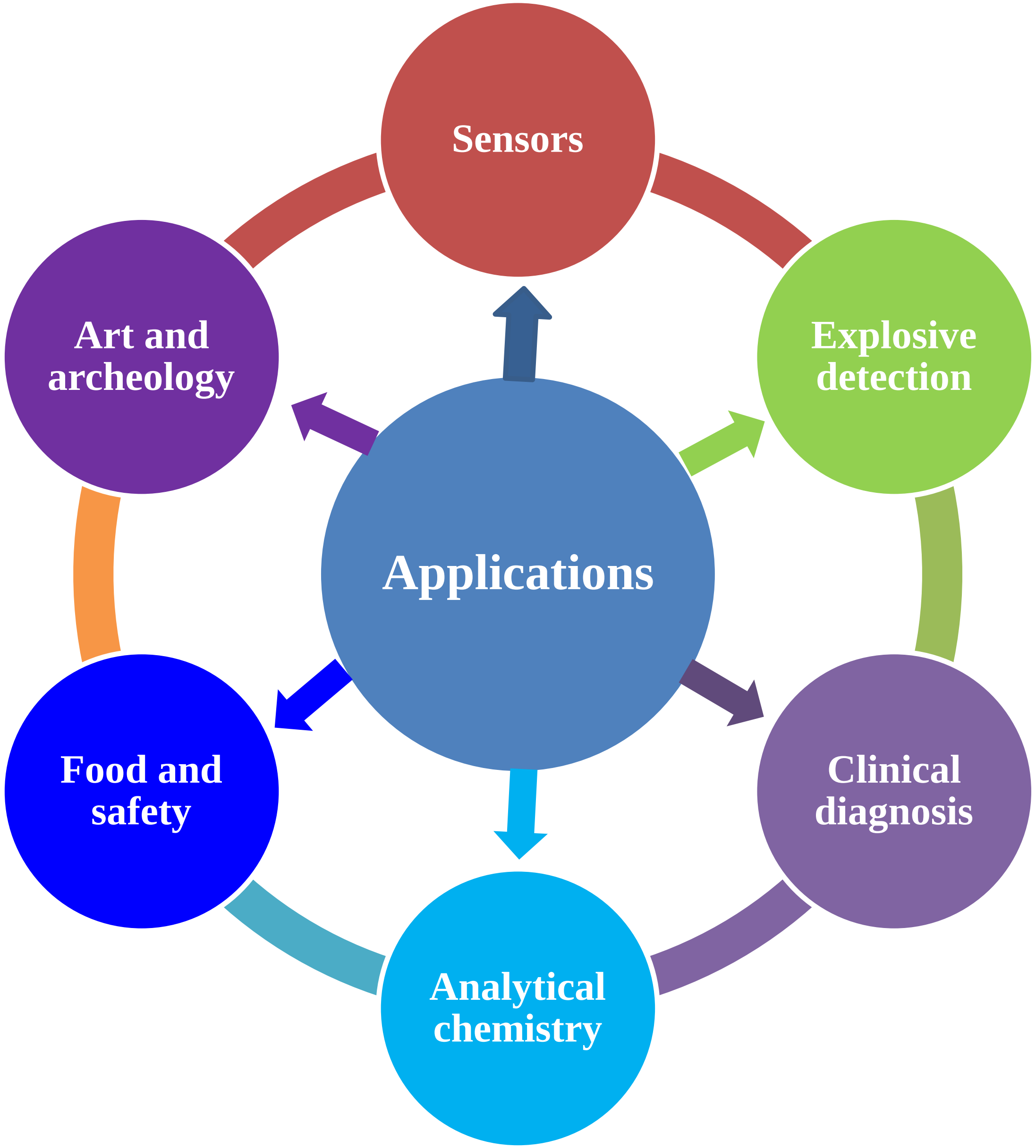
Sensitive : Even single molecule detection possible

Sample state: Aqueous and non aqueous samples can be analyzed

Quantitative detection of species possible

Sample preparation: Easy sample preparation

Applications of Raman Spectroscopy



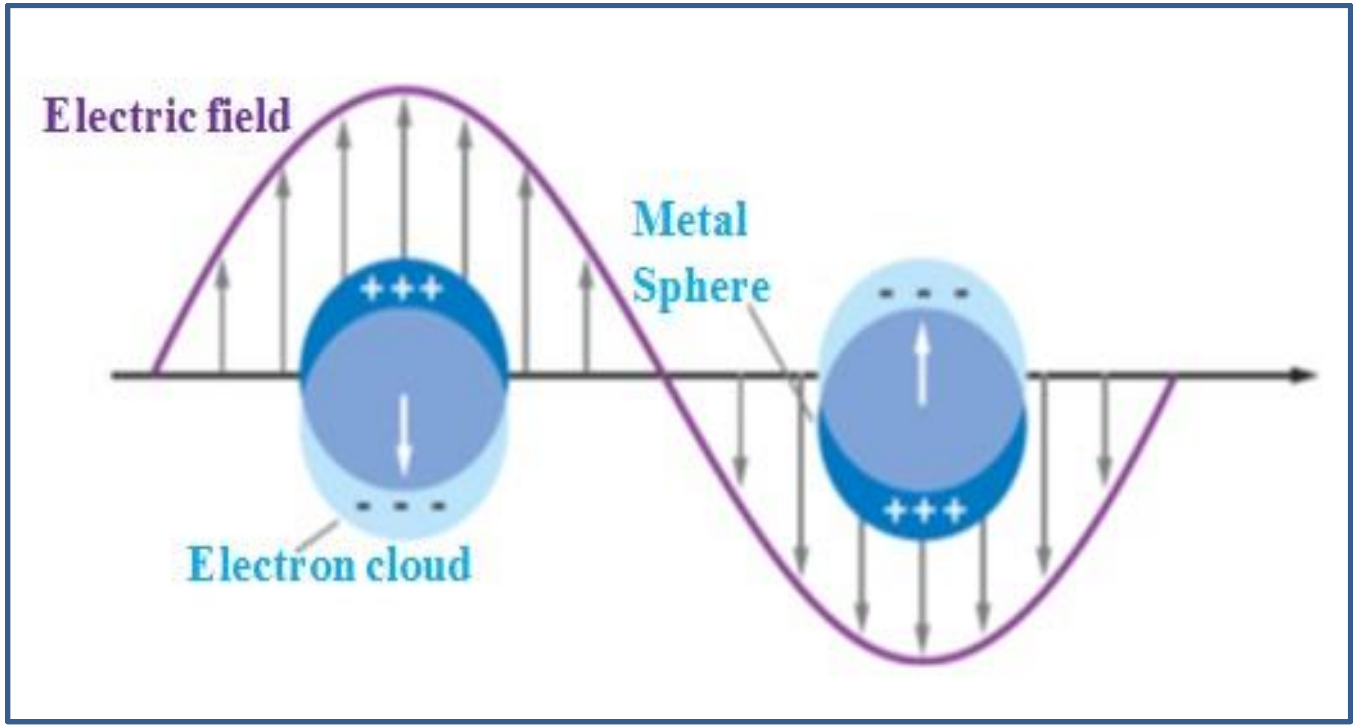
Raman lines from many bio molecules of interest are weak.

Several advancement of techniques has made it possible to study even weakly Raman scattering Molecules

One of them is the technique Surface Enhanced Raman Scattering where a surface plasmon on a metal nanoparticle enhances the Raman signal if the analyte molecule sits in the vicinity of the nanoparticle.

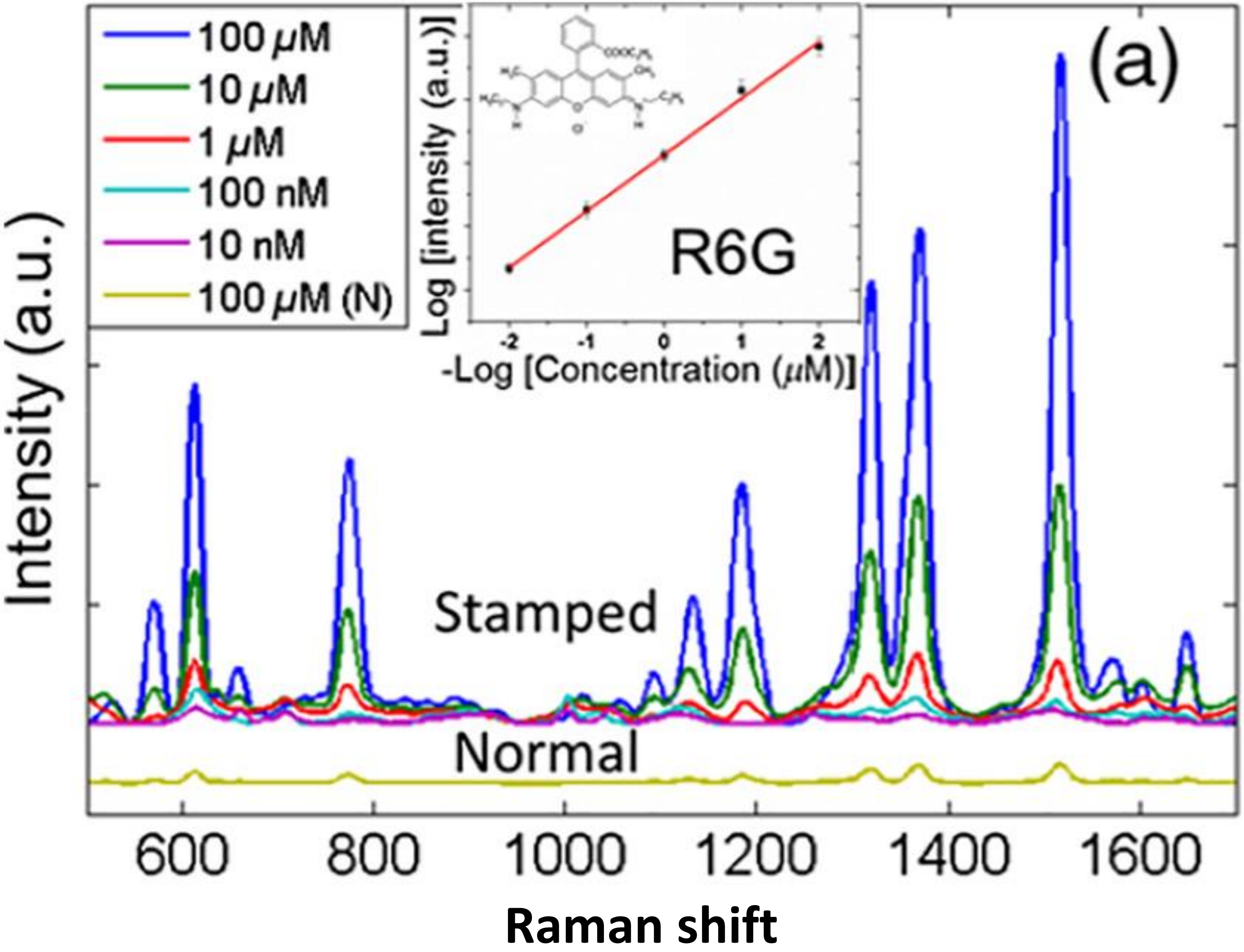
Raman intensity increases by 6 to 10 orders of magnitude.

Surface Enhanced Raman Scattering (SERS)



Very large increase in Raman Signal Intensity due to plasmonic enhancement

P.J. Hendra, M. Fleischmann and A.J. MecQuillan, "Raman spectra of pyridine adsorbed at a silver electrode" *Chem. Phys. Lett. 26 (1974) 163.*



100 μM
10 μM
1 μM
100 nM
10 nM
100 μM (N)
Log [intensity (a.u.)]
-2
-1
0
1
2
-Log [Concentration (μM)]
R6G
(a)
Intensity (a.u.)
Stamped
Normal
600
800
1000
1200
1400
1600
Raman shift

Bio-markers of several types of cancers detected by Raman spectroscopy:

Ovarian Cancer detection from blood serum

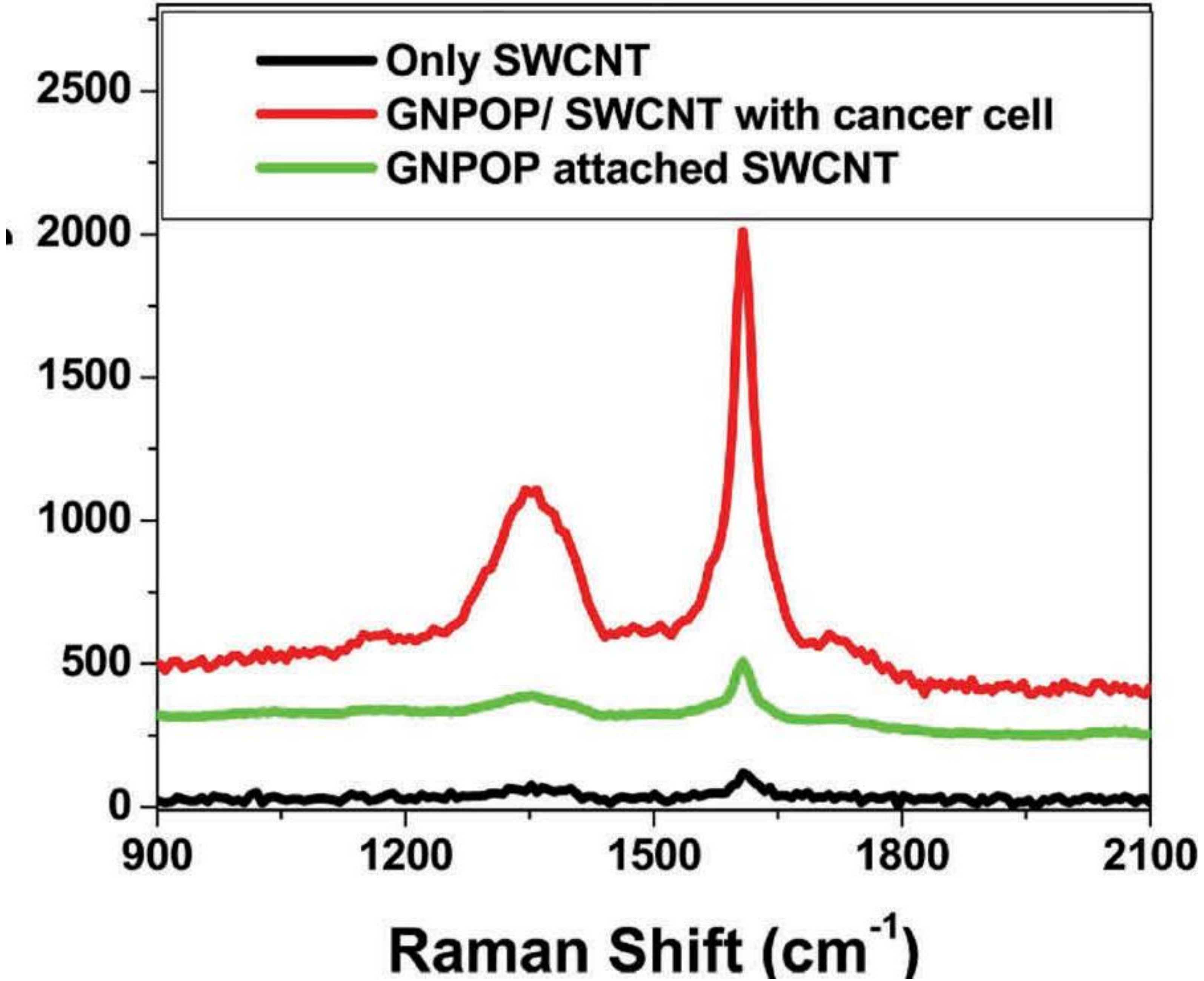
Prostrate Cancer

Breast Cancer

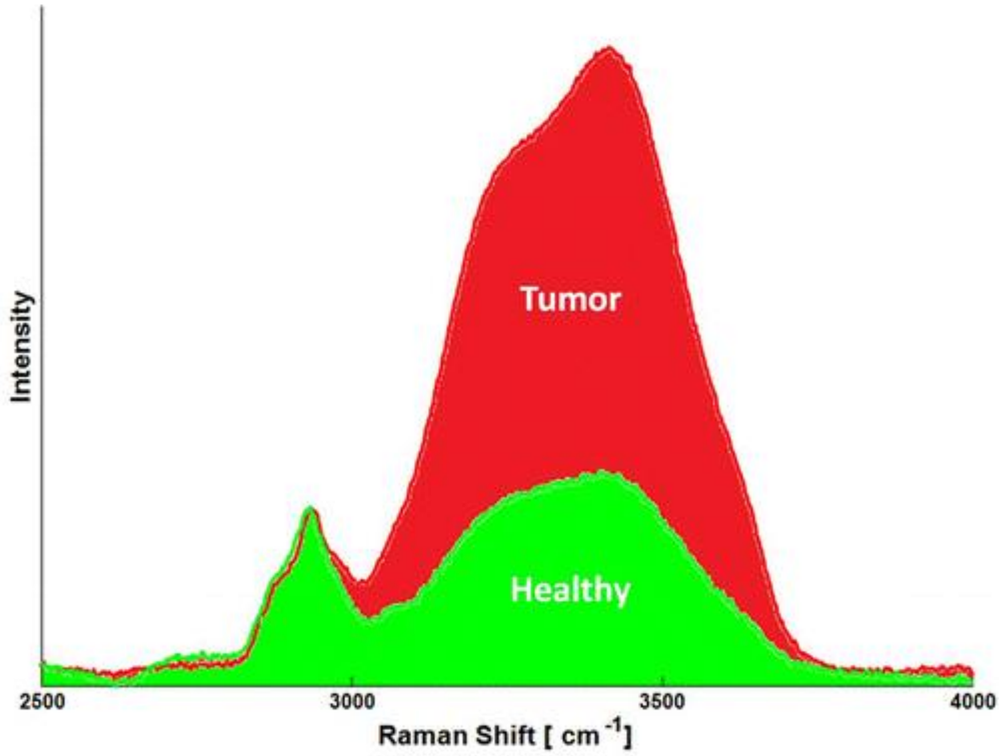
Pancreatic Cancer

Applications of Raman spectroscopy in cancer diagnosis
Gregory W. Auner et al.
Cancer and Metastasis Reviews (2018) 37:691–717

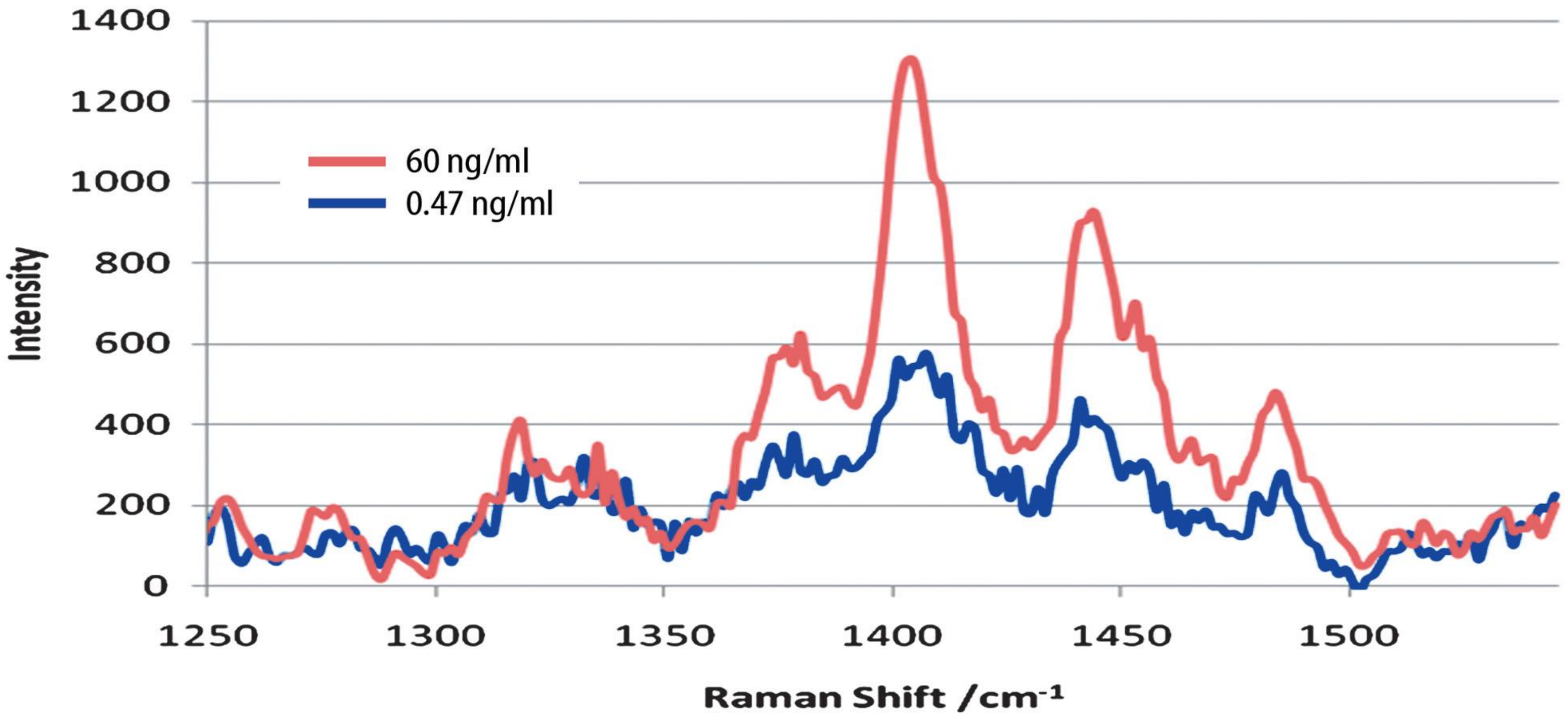
Early detection of cancer increases the survival chance



Detection of breast cancer using SERS: Beqa L, Fan Z, Singh AK, et al. ACS Appl Mater Interfaces. 2011, 3(9):3316–3324.



Discrimination between Oral Cancer and Healthy Tissue Based on Water Content Determined by Raman Spectroscopy
E. M. Barroso, R. W. H. Smits, T. C. Bakker Schut, I. ten Hove, A. Hardillo, E. B. Wolvius, R. J. Baatenburg de Jong, S. Koljenovi, G. J. Puppels, *Anal. Chem.*20158742419-2426



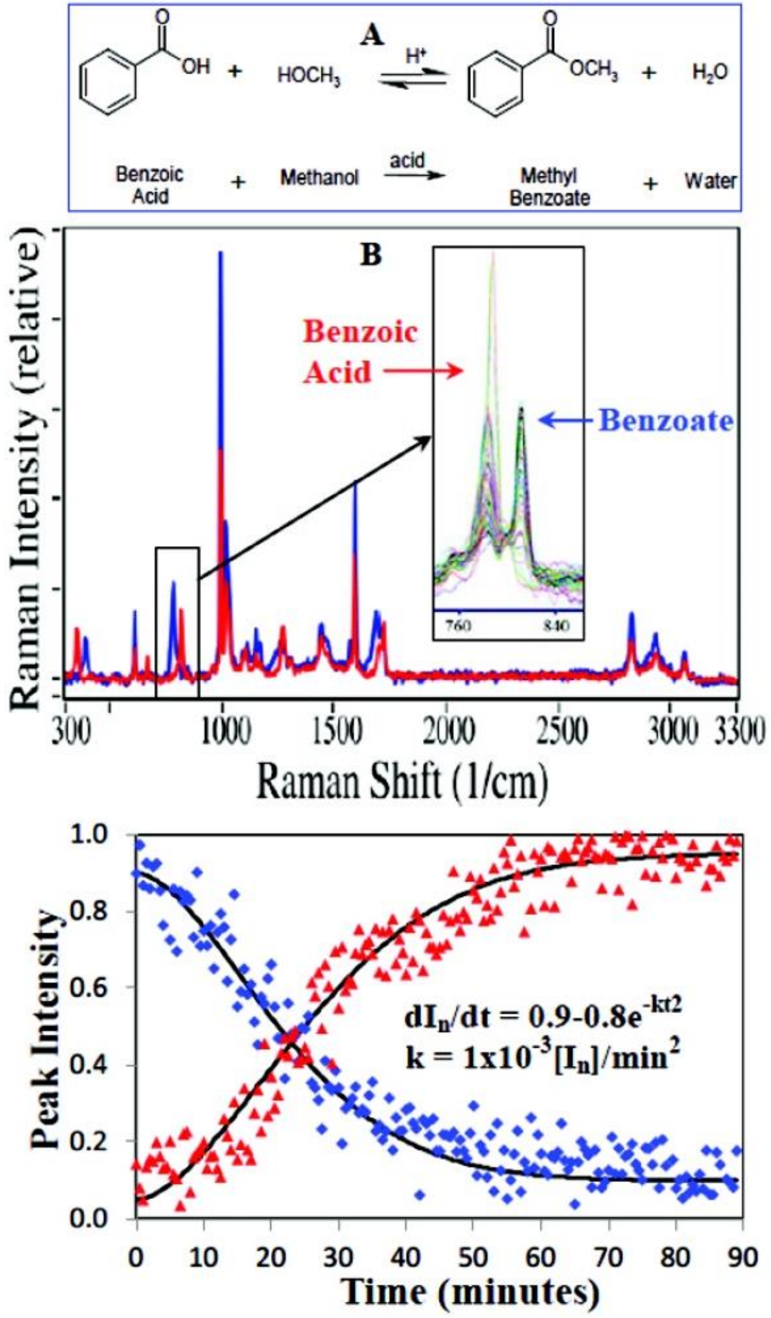
Detection of PSA for Prostate Cancer, Stevenson et al. Analyst, 2009, 134, 842–844 | 843

Pharmaceutical Applications

Once a potentially new drug is identified, the method to synthesize the drug is developed. Raman spectroscopy is ideal for monitoring reactant, intermediate and product concentrations, determining pathways, kinetics, mechanisms, end-points, and yields.

As an example, in Fischer esterification, the esterification of benzoic acid is performed to produce methyl benzoate. The reactant and product have unique spectra, with peaks at 780 and 817 cm^{-1}, respectively, which are ideal for real-time monitoring.

Also used for drug quality, product authentication, shelf life etc.



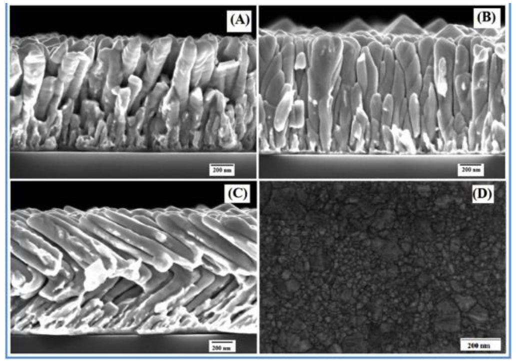
Stuart Farquharson, American Pharmaceutical Review, April, 2014

Tuberculosis determination using SERS and chemometric methods

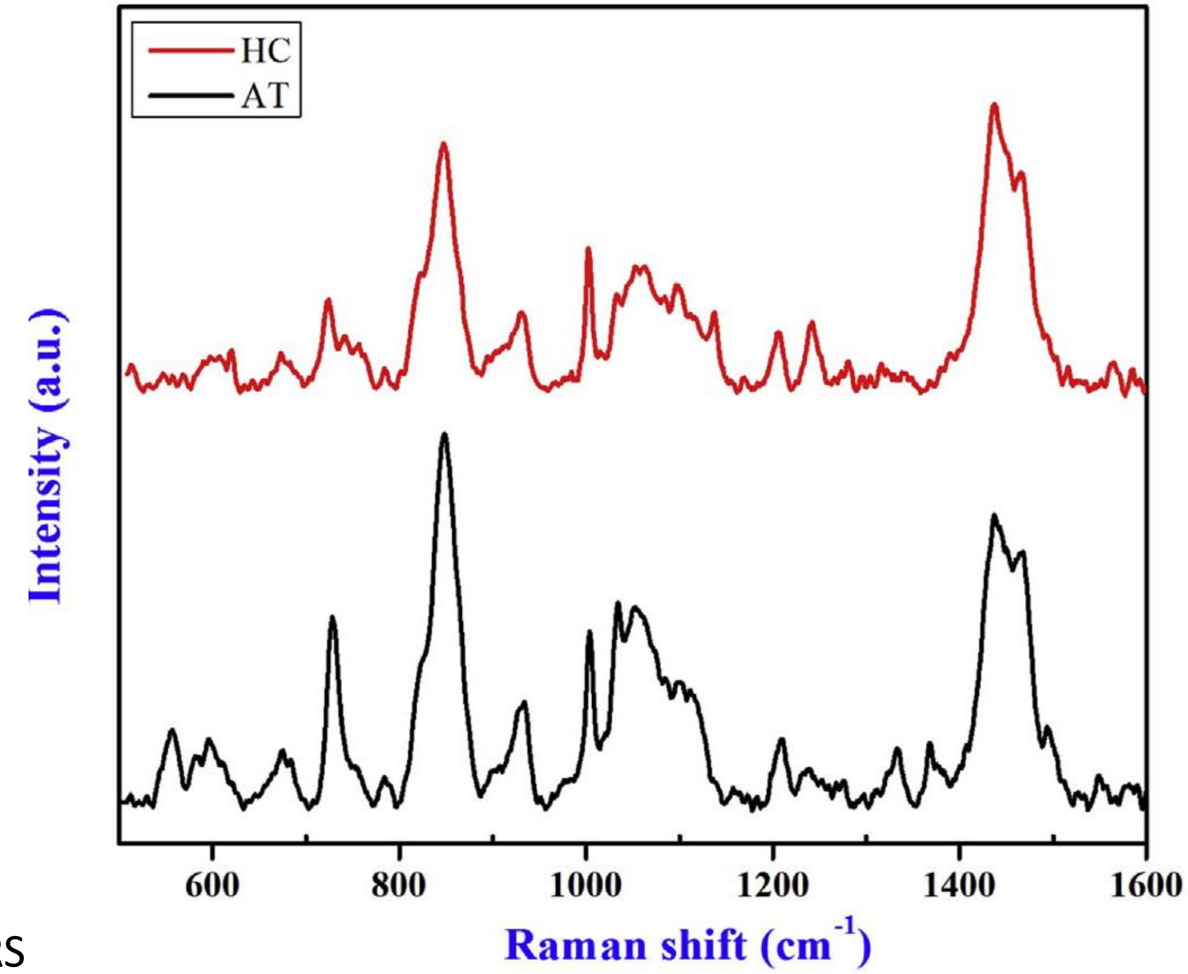
Tuberculosis, Volume 108, January 2018, Pages 195-200

RajuBotta, Pongpan Chindaudom, Pitak Eiamchai, Mati Horprathum, Saksorn Limwichean , Chanunthorn Chananonnawathorn , Viyapol Patthanasettakul, Benjawan Kaewseekhao, Kiaticha Faksri, Noppadon Nuntawong

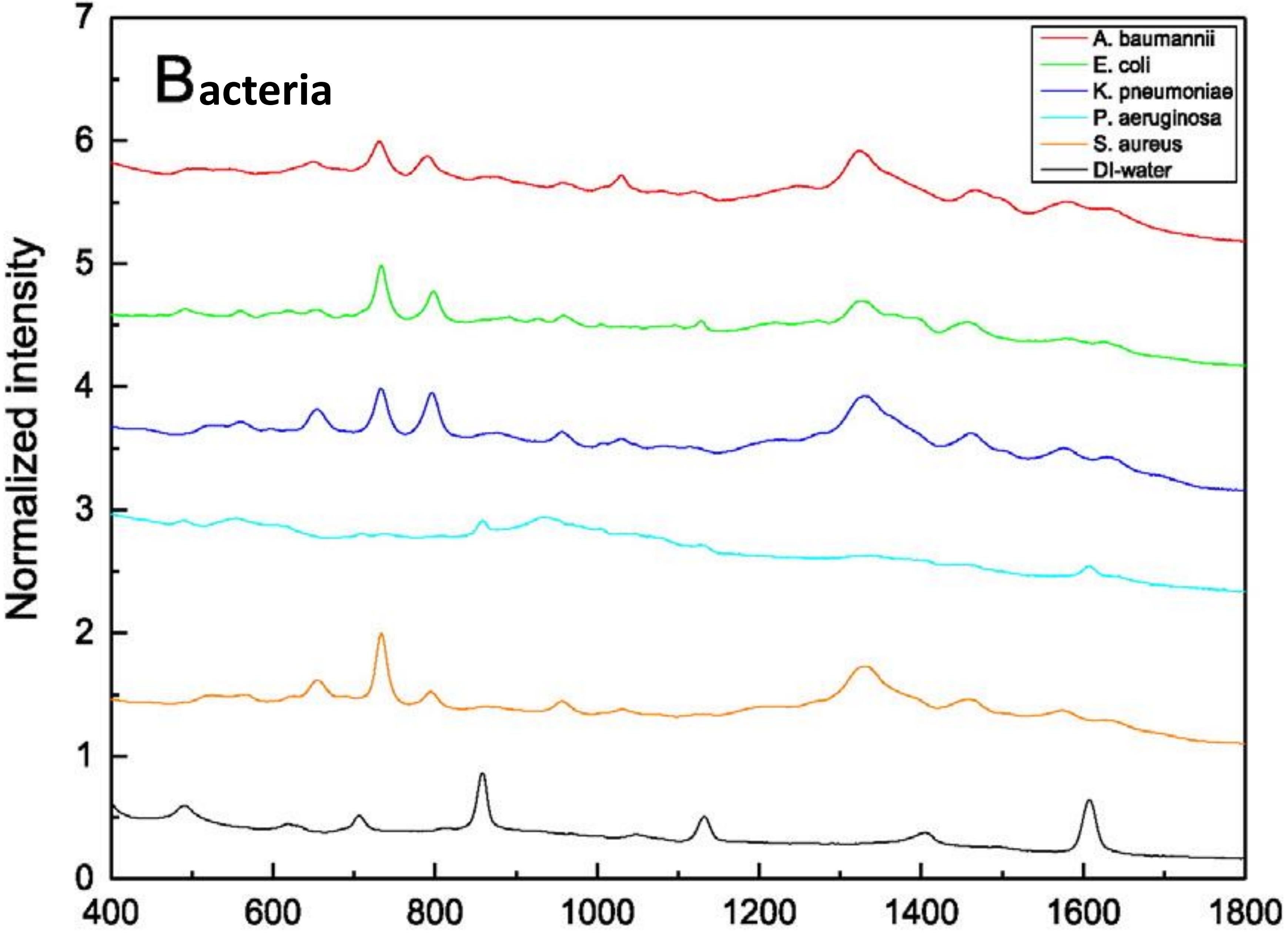
National Electronics and Computer Technology Center (NECTEC) ***and***
Research and Diagnostic Center for Emerging Infectious Diseases (RCEID), Khon Kaen University



Slant, vertical zigzag nanorods and nano particles of Ag as SERS substrates

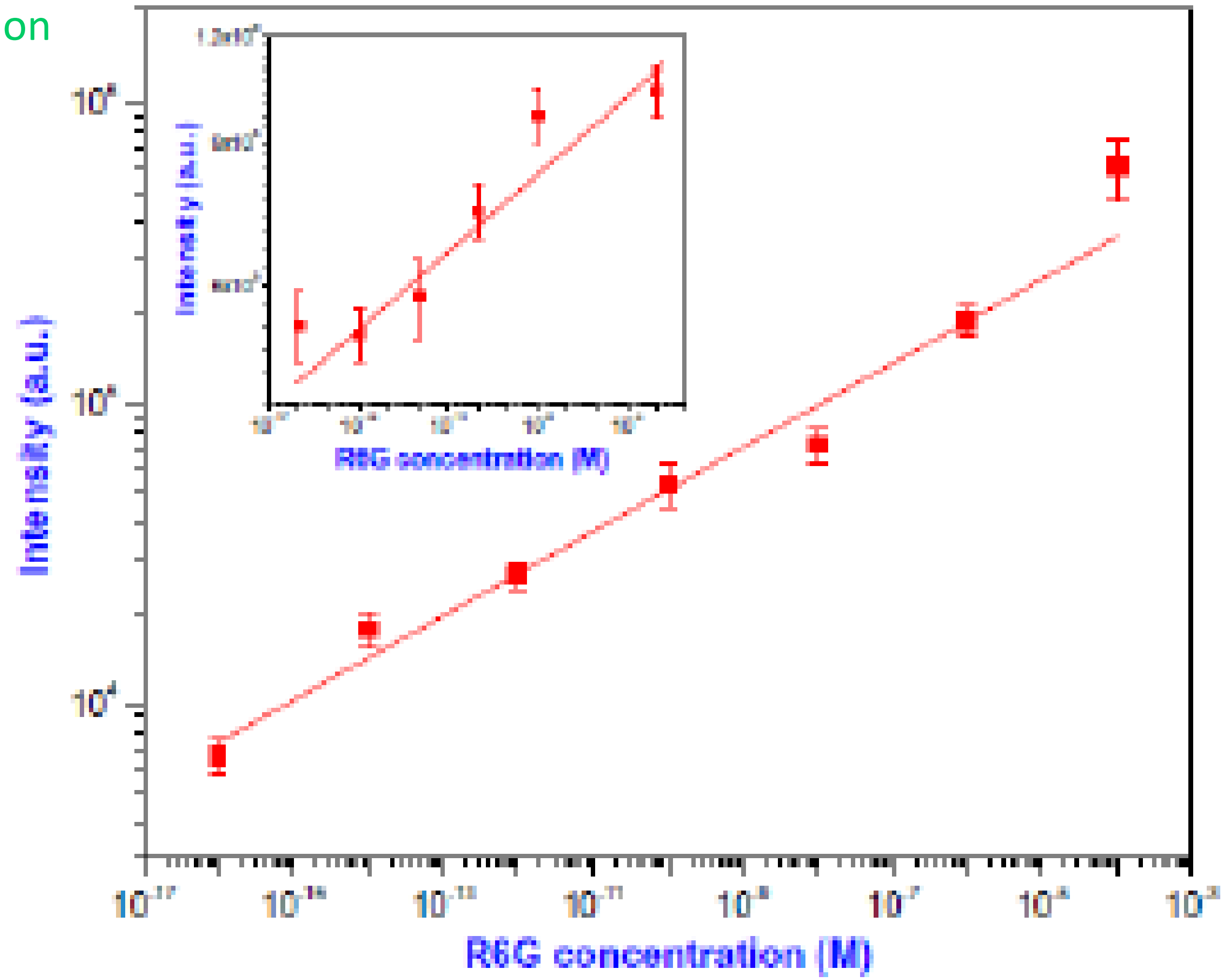


SERS spectra of active TB (AT) and healthy control (HC) samples with laser excitation wavelength of 785 n



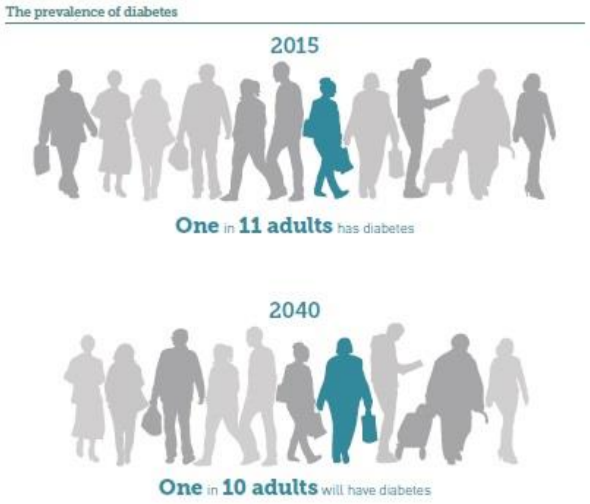
Bacteria
A. baumannii
E. coli
K. pneumoniae
P. aeruginosa
S. aureus
DI-water
Normalized intensity
0
1
2
3
4
5
6
7
400
600
800
1000
1200
1400
1600
1800

Single Molecule Detection



Raju Botta, G. Upender, R. Sathyavathi, D. Narayana Rao, C. Bansal, "Silver nanocluster films for single molecule detection using Surface Enhanced Raman Scattering (SERS)" Mat. Chem. Phys. 137 (2013) 699-703. (25 Citations)

Prevalence of diabetes in the world

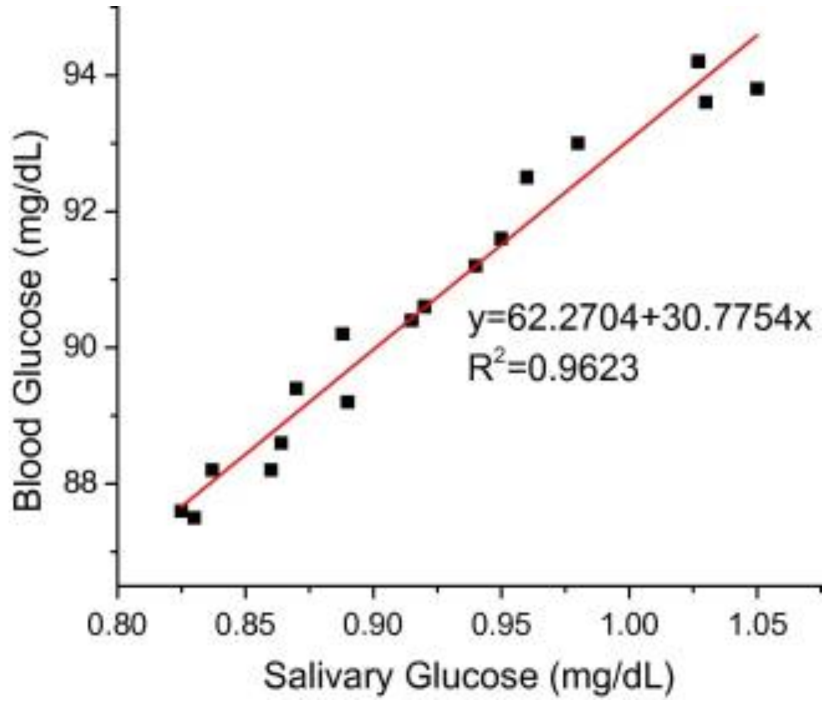


International Diabetic Federation (http://www.diabetesatlas.org)

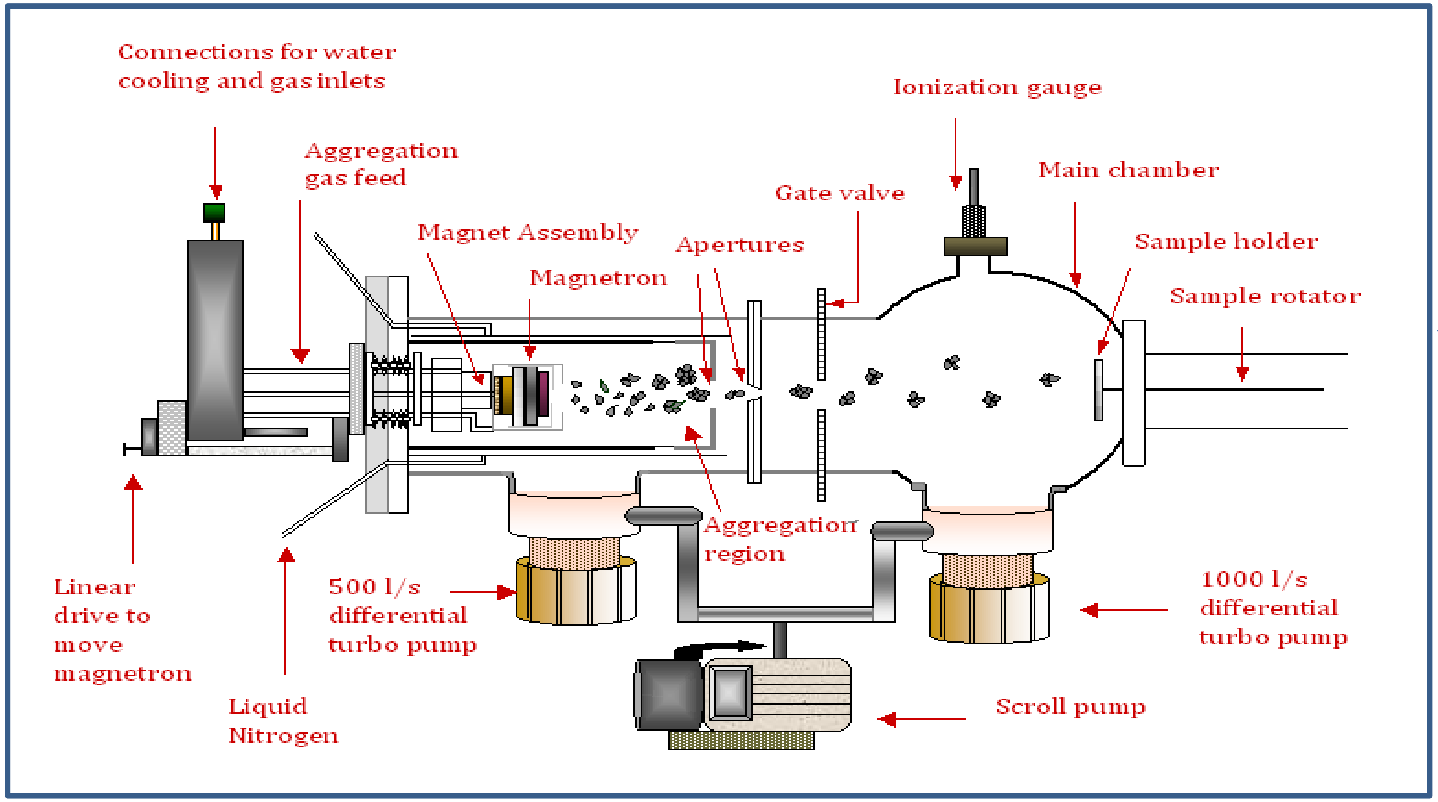
Dire need for a non-invasive glucose sensor for regular and painless monitoring of glucose levels in patients as well as for diagnosis of diabetes at an early stage to avoid future complications such as kidney failure, blindness, heart disease etc.

Present glucose monitors require piercing of body parts to draw blood which is a painful procedure

Our invention addresses this problem by making a sensor which requires body fluids such as saliva or tears that can be drawn from the body non invasively.

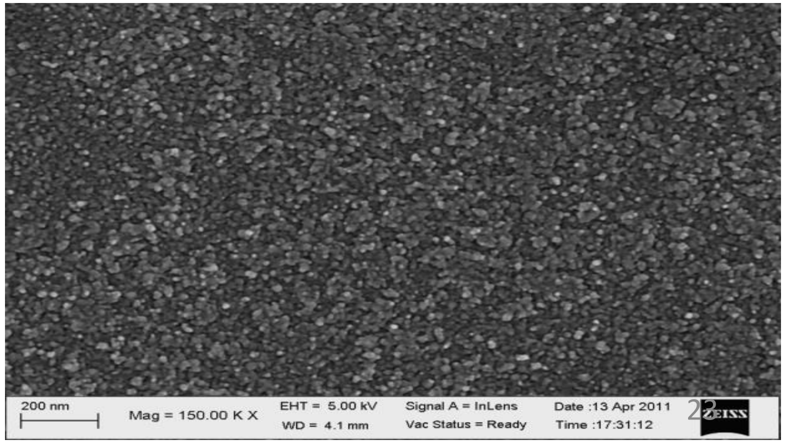


Correlation between blood glucose and salivary glucose, Zhang et al Sensing and Biosensing Research 4, 23-29 (2015)

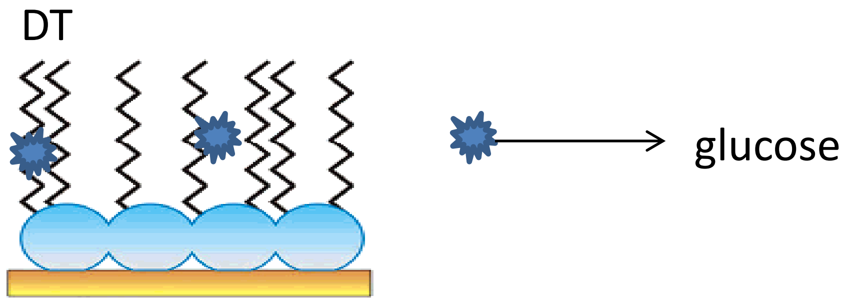


Block diagram of Nanocluster deposition system

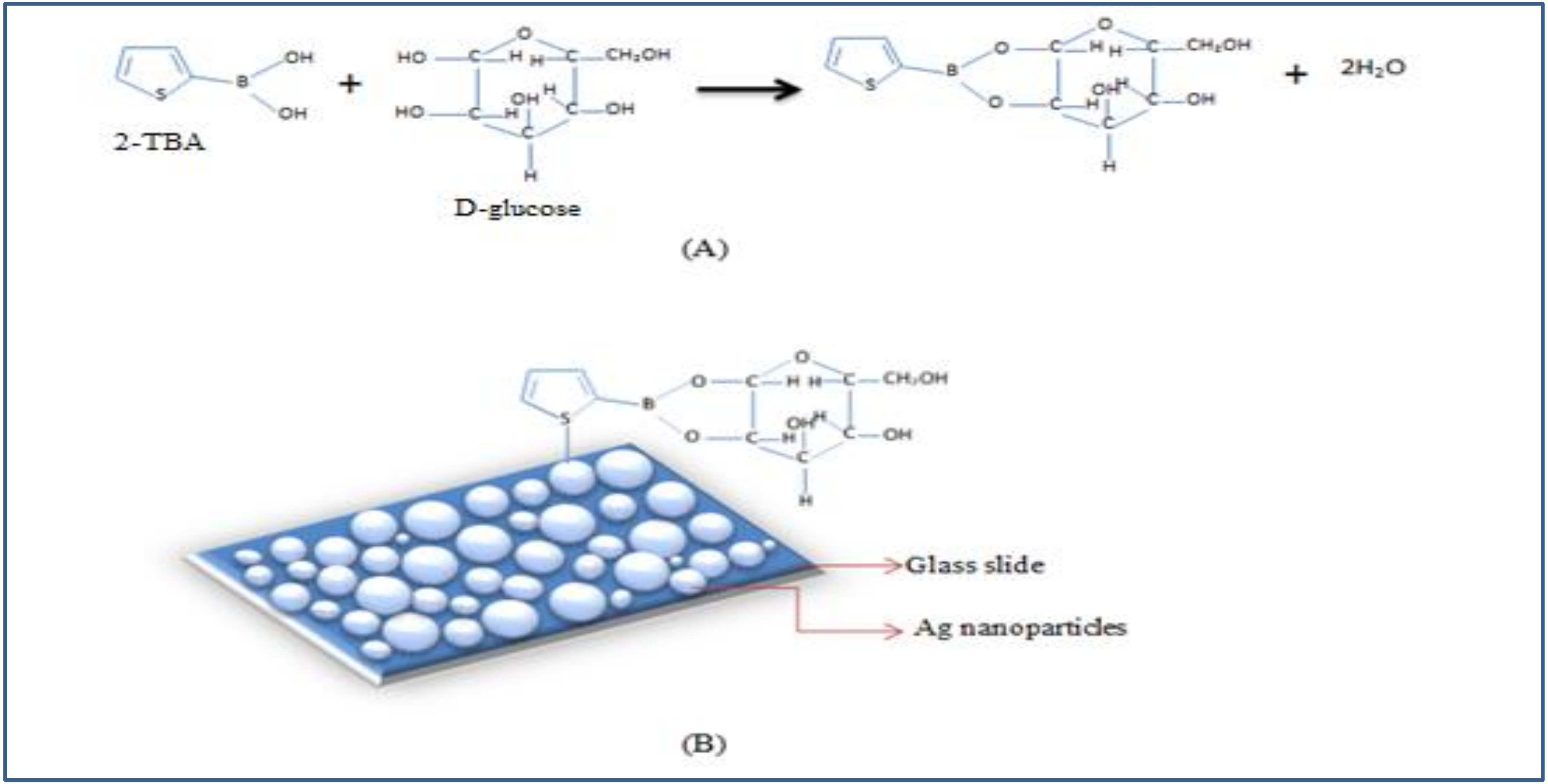
FESEM image of as-deposited Ag nanoclusters on glass substrates



- The detection of glucose by Surface Enhanced Raman Scattering (SERS) is a challenging problem because glucose molecules have a small Raman scattering cross-section and they have a low affinity for adsorption on metal nanoparticle surfaces.
- R.P. Van Duyne group proposed a method to bring glucose molecule near to metal nanoparticle surface by partitioning the nanoparticle surface with decanethiol (DT) so that glucose molecule can be trapped in the partition. In this method glucose signals are measured near the surface of metal nanoparticles.

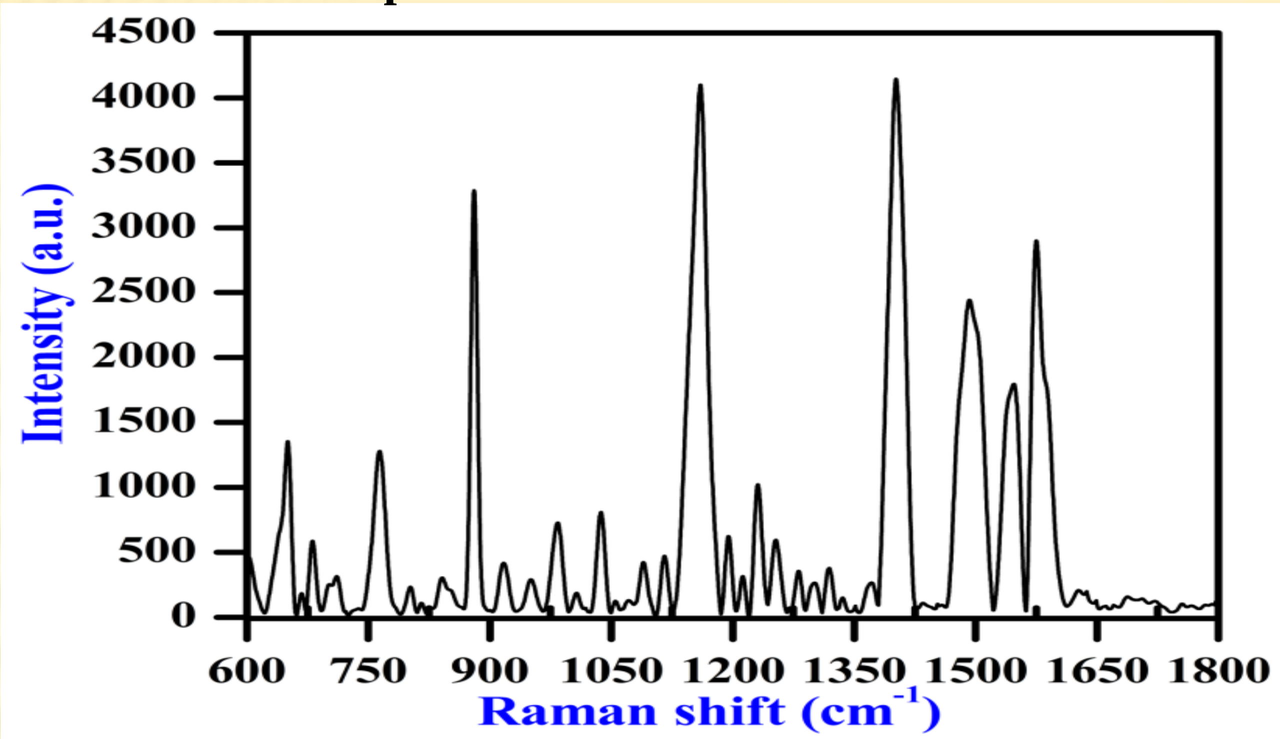


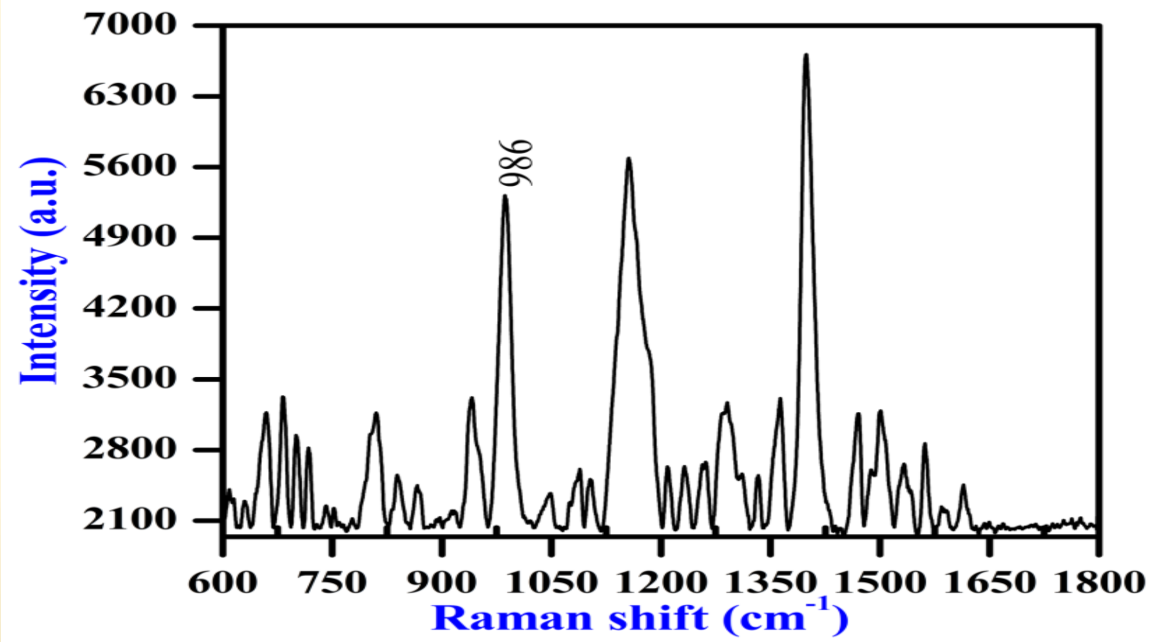
We have used a linker molecule 2 Thienyl Boric Acid that attaches to silver surface on one side as well as to D-Glucose on the other side



Schematic diagrams depicting (A) the bonding between the 2-TBA and D-Glucose and (B) the attachment of the 2-TBA+D-glucose molecule to the silver surface via Ag-S bond. Raju Botta, A. Rajanikanth, C. Bansal, *Sensing and Bio-Sensing Research 9 (2016) 13*

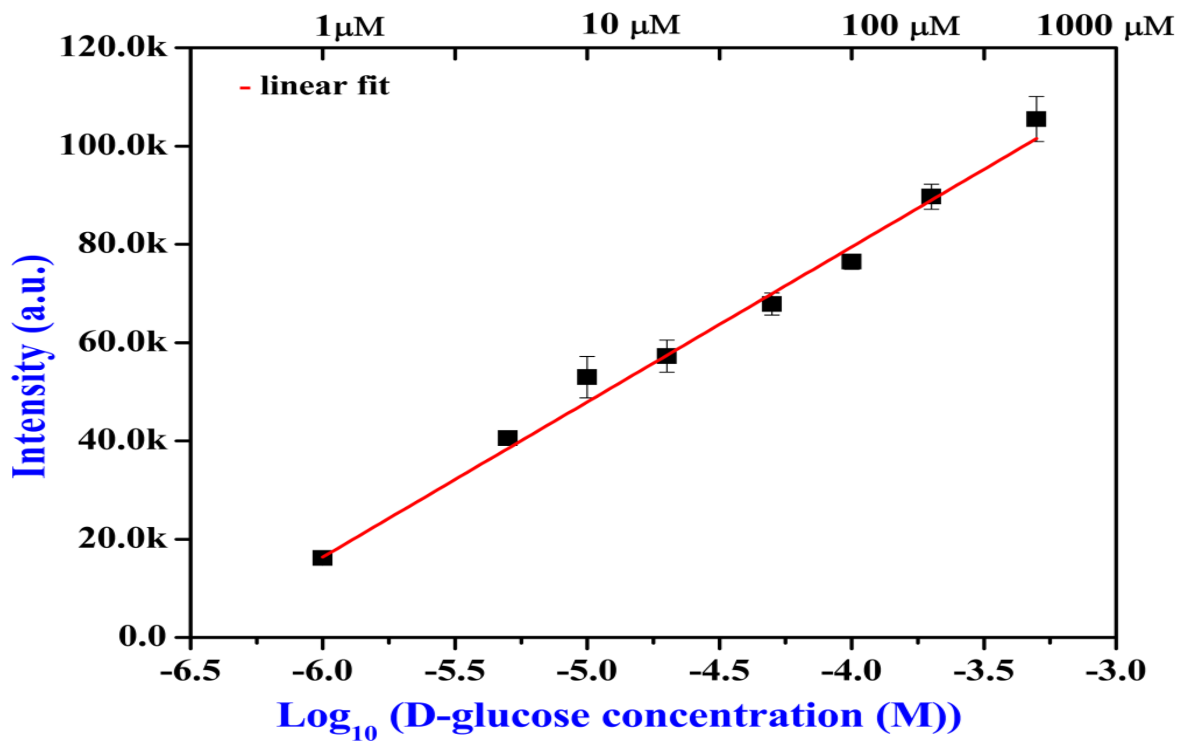
SERS spectrum of 0.1M 2TBA





SERS spectrum of 0.1M 2TBA+ 100µM D-glucose

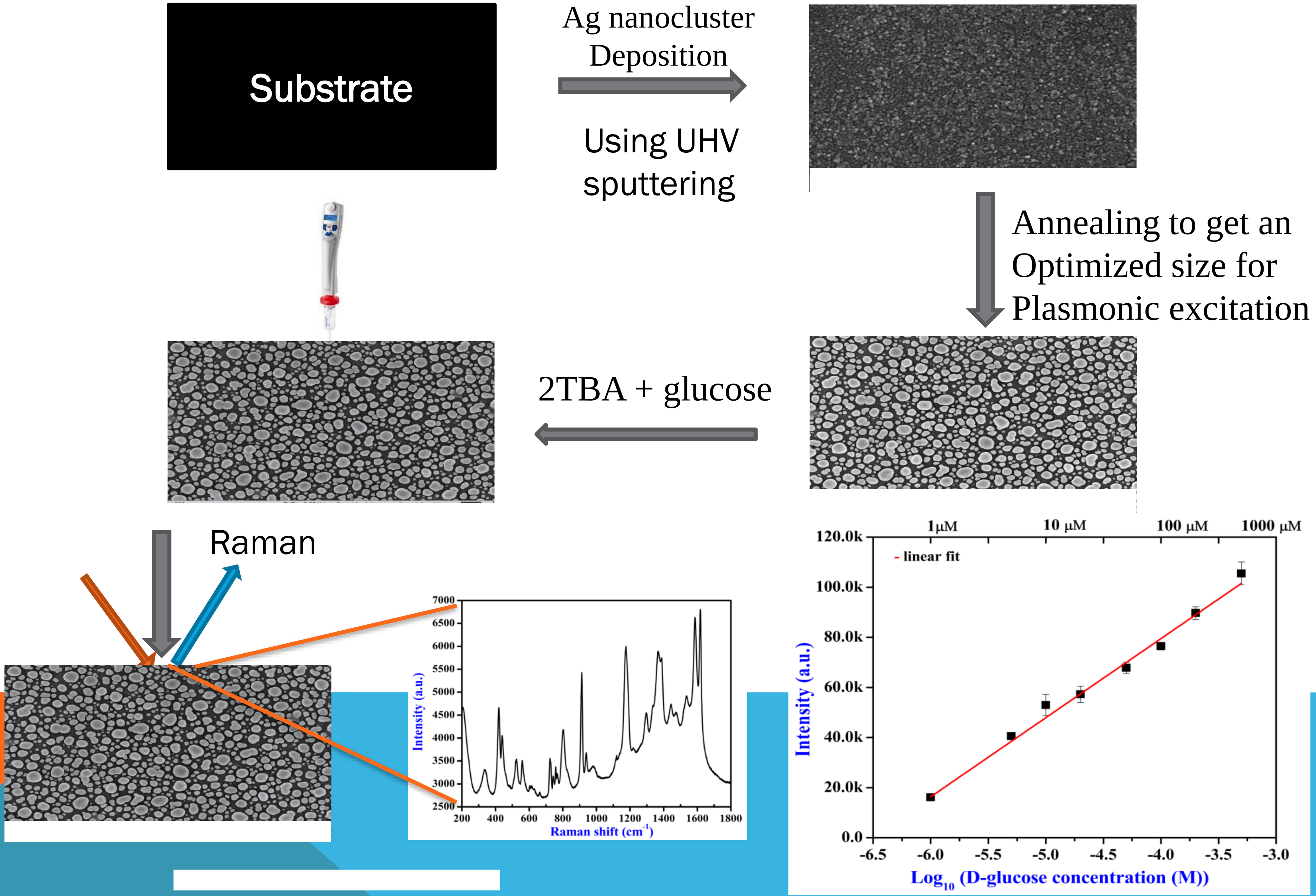
Quantitative analysis of D-glucose



concentration

- Typical glucose levels in blood are about 5mM/Liter
- Typical glucose levels in saliva are about 50μM/Liter.

Novelty



Indian patent application: 3146/CHE/2015 A

CONCLUSIONS

Raman spectroscopy with plasmonic intensity enhancement has emerged as a promising tool for bio-medical applications.

It has a bight future as diagnostic and monitoring method for cancers, bacterial Infections, non-invasive glucose sensing, drugs, pharmaceuticals, forensic applications, pesticides and in many other areas.

There is a need to develop a platform technology consisting of reliable and low priced SERS substrates and sensitive and simple Raman spectroscopic instrumentation.

KHOP-KHUN KHRAP