SURFACE-ENHANCED RAMAN SCATTERING (SERS) GENE PROBES FOR MEDICAL DIAGNOSTICS

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We describe the development of nanostructure technology for surface-enhanced Raman scattering (SERS) applications. We illustrate the development of a variety of sensors and a multi-label DNA mapping technique using SERS method and instrumentation. This research area involves the development of metallic nanoprobes that can produce SERS effect for ultrasensitive biochemical analysis. The intensity of the normally weak Raman scattering process is increased by factors as large as 10^6-10^{13} for compounds adsorbed onto a SERS substrate, allowing for trace-level detection. The SERS nanoprobe technology has been incorporated in several fiberoptic probe designs for remote analysis. As an example of the application of this technique to biomedical analysis, we show the use of DNA probes based on SERS labels for gene detection and DNA mapping. The detection method uses nanostructured metallic substrates as SERS-active microarray platforms. The SERS probes can be used to detect DNA targets via hybridization to DNA sequences complementary to these probes. The probes do not require the use of radioactive labels and provide sensitivity, selectivity and excellent label-multiplex capability. The SERS technique has great potential for use in simultaneous multi-analyte labeling for biomedical imaging.

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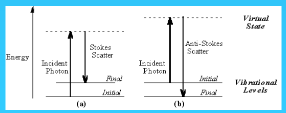
Motivation

- The development of practical and sensitive devices for screening multiple genes related to medical diseases and infectious pathogens is critical for early diagnosis and improved treatments of many illness.
- An important factor in medical diagnostics is rapid, selective and sensitive detection of biochemical substances, biological species or living systems at ultra-trace levels in biological samples, which often requires a detection method that is capable of identifying and differentiating a large number of biochemical constituents in complex samples simultaneously.
- Raman spectroscopy is rapid, nondestructive and highly compound-specific. It has multi-component analysis potential and requires little sample preparation, which allows on-line and in-field analysis.
- Raman scattering efficiency can be enhanced by factors $>10^8$ when a compound is adsorbed on or near special metal surfaces. The enhancement provided by surface-enhanced Raman scattering helps to bridge the sensitivity gap between the fluorescence and Raman techniques, therefore, the SERS gene probes could offer a unique combination of performance capabilities and analytical features of merit.

Principle of Raman spectroscopy



Raman scattering intensity:

$p = \alpha E$

p = 1st order transition electric dipole

α = Transition polarizability of the molecule

E = Incident electric field magnitude

- Raman effect forms a characteristic Raman spectrum – in effect a molecular fingerprint.
- A limitation of normal Raman spectroscopy is low sensitivity. Raman scattering efficiency can be enhanced by factors $>10^8$ when a compound is adsorbed on or near special metal surfaces, a phenomenon known as SERS.

Mechanisms of Plasmonics and Surface-Enhanced Raman Scattering (SERS)

Plasmonics refers to the research area of enhanced electromagnetic properties of metallic nanostructures. The term plasmonics is derived from "plasmons", which are the quanta associated with longitudinal waves propagating in matter through the collective motion of large numbers of electrons. Incident light irradiating these surfaces excites conduction electrons in the metal, and induces excitation of surface plasmons leading to enormous electromagnetic enhancement of spectral signatures [such as surface-enhanced Raman scattering (SERS) and surface-enhanced fluorescence (SEF)] for ultrasensitive biological detection and imaging.

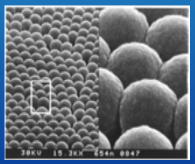
Electromagnetic Enhancement (E Factor)

- Incident radiation (primary field) induces oscillation of conductance electrons in the metal surface, generating a secondary field.
- When incident radiation at the plasma frequency, a resonant response of conductance electrons (surface plasmons) generates an enhanced secondary field.
  Plasma Frequency Factors:
  Type of metals
  Size of metal nanoparticle
  Shape of the metal nanoparticle
- Secondary fields can also be concentrated at submicron protrusions of a metallic surface-lightning rod effect.

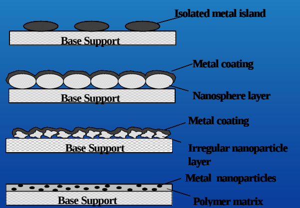
Molecular Enhancement (α factor)

- Charge transfer between the metal and adsorbate can enhance the transition polarizability.

Examples of SERS-active dyes and nanostructures



Scanning electron micrograph of silver-coated polystyrene nanospheres



A common feature of SERS substrates is atomically rough surfaces

Application of Raman (SERS) techniques to bioanalysis

Advantages

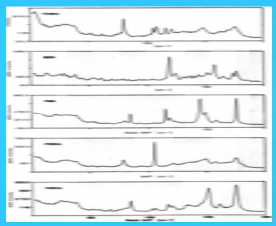
- Nonradioactive
- High spectral selectivity- Very narrow bands (<1 nm) may enable detection of multiple probes simultaneously

Disadvantage

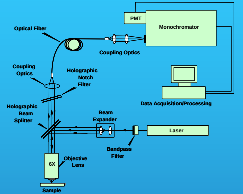
- Limited sensitivity

Surface-enhanced Raman scattering (SERS) is a potential solution

- SERS provides scattering enhancement factor of up to 10^8, making it competitive with fluorescence for certain trace analysis applications.
- SERS results from the adsorption of chemicals on a sub-micron textured surface
- Resonance can also enable up to 10^6 factor enhancement



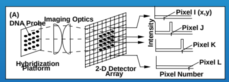
SERS spectra of various compounds, demonstrating minimal spectral overlap



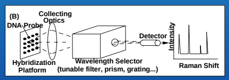
Schematic diagram of optical system for SERS detection

Modes of imaging and spectroscopy and their combination for multispectral imaging

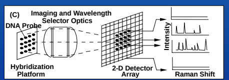
IMAGING: Intensity is recorded for every pixel at one single wavelength

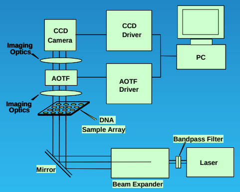


SPECTROSCOPY: Intensity is recorded for a single spot at multiple wavelengths

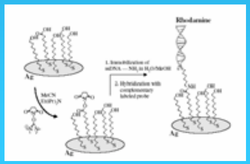


MULTISPECTRAL IMAGING: Intensity is recorded at multiple wavelengths for every pixel

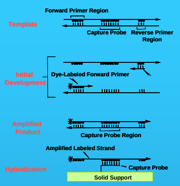




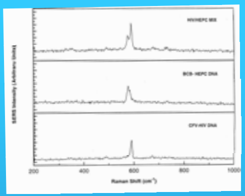
Instrumental system for 2-D multispectral imaging of a SERS gene array platform



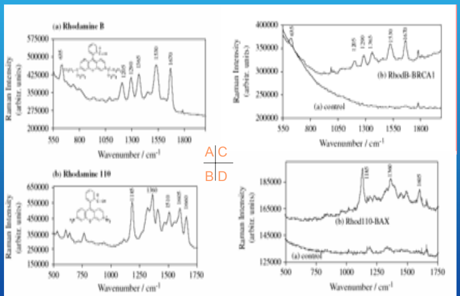
Covalent immobilization of single-stranded DNA on silver surfaces derivatized with alkanethiols. The succinimidyl derivative forms a labile ester that binds to the amino-labeled DNA. Subsequent hybridization with Rhodamine-labeled probes can be monitored by SERS



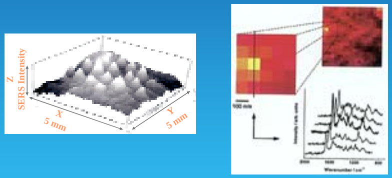
SERS gene probe development via PCR and hybridization for selective detection



Simultaneous detection of Human Immuno-deficiency Virus (HIV) and Hepatitis C Virus (HEPC) using SERS gene probe



SERS Spectra of A) Rhodamine B, B) Rhodamine 110, C) Breast cancer susceptibility BRCA2 gene fragment detected by Rhodamine B-labeled probe after hybridization, and D) Pro-apoptotic BAX gene fragment detected by Rhodamine 110-labeled probe after hybridization



(Left) Image of a 2D SERS signal pattern for p-aminobenzoic acid spots acquired with a multispectral imaging system. (Right) Near-field surface-enhanced Raman imaging of BCB-labeled DNA with 100-nm resolution

Plasmonic and SERS Probes for DNA/protein mapping inside cells

Targeted delivery of plasmonic and SERS-active nanoparticles to intracellular targets by liposomes. And targeting achieved by bioreceptors (e.g., antibody, DNA, enzyme) conjugated to nanoparticles

Conclusions

- The development of plasmonics and SERS methods and instrument for use in biomedical diagnosis and imaging is described.
- The SERS probes can be used to detect DNA targets via hybridization to DNA sequences complementary to these probes. The probes do not require the use of radioactive labels and have great potential to provide both sensitivity and selectivity. With the SERS gene technique, multiple samples can be separated and analyzed using multiple SERS labels simultaneously.
- Advanced instrumental systems designed for point-source spectral measurements and for multi-spectral imaging (MSI) are described. The MSI concept allows recoding the entire SERS spectrum for every pixel on the two-dimensional hybridization platform in the field of view with the use of a rapid-scanning solid-state device, such as AOTF.
- Investigation of DNA/protein mappings inside cells is currently under way.

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