Detection of Glucose Levels Using Excitation and Difference Raman Spectroscopy at the IUSL

Introduction

Raman spectroscopy such as spontaneous Raman spectroscopy, coherent anti-stokes Raman spectroscopy, and stimulated Raman gain spectroscopy are widely used in physics, chemistry and biology fields for investigations of vibrational modes. To our best knowledge, very few applications of Raman spectroscopy for the in-vitro and in-vivo patient medical diagnostics have been reported because of low signal levels and strong background levels. The conventional Raman techniques for detection of body level constituents are generally associated with long exposure time (several ten minutes), high power laser pump fluency which is much above the safety limitation of the laser illumination for human body applications, and strong noise background. Conventional Raman is too weak to determine analyte (glucose) level in humans due to the background.

Novel Raman Spectroscopy Investigation At IUSL

In order to apply Raman spectroscopy to achieve high sensitivity for glucose level detection for diabetes diagnosis, and other body level analyte detection in blood, we have investigated fingerprint Raman modes for glucose and other Raman-active blood analytes using a novel approach. In order to measure the body level of glucose and other blood constituents, we developed “low-power CW excitation Raman spectroscopy” and “difference Raman spectroscopy” methods described in our U.S. patents numbered 6,151,522 (Nov. 21, 2000) and 6,560,478 B1 (May 6, 2003), respectively.

Our purpose is to further the development of the novel approach to detect glucose and other biological analytes at low levels using the novel excitation and difference Raman process.

Fingerprint Raman Spectrum Of Glucose

The fingerprint Raman spectrum of glucose was measured in our lab under a pump of a 5 mW laser. The eight Raman modes of 436.4, 525.7, 854.9, 911.7, 1065.0, 1126.4,

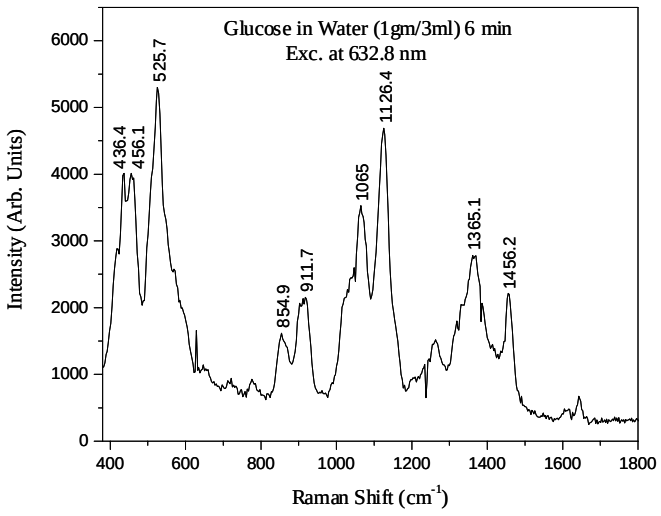

Fig.1 Spontaneous Raman spectrum of glucose. The eight Raman modes of 436.4, 525.7, 854.9, 911.7, 1065.0,1126.4, 1365.1 and 1456.2 cm^{-1} can be used as fingerprint of Glucose.

1365.1, and 1456.2 cm^{-1} were obtained and are considered as the fingerprint Raman lines of blood glucose as shown in Fig.1. The measurements were performed with glucose concentrations varied from 1 g/ml to 10^{-2} g/ml, and the results show that the intensities of the Raman lines decrease proportionally to the concentration of the glucose. This suggests that, in principal, the concentration lever of blood glucose can be determined by measuring its Raman spectrum. However, in practice, the concentration of human blood glucose is as low as ~0.7-1.1 $x10^{-3}$ g/ml for normal persons and >1.1 x 10^{-3} g/ml for diabetes. The spontaneous Raman intensity from such low concentration glucose is too weak to be measured within reasonable exposure time. In our measurements, the Raman signal could not be realized when the concentration of glucose decreases below 10^{-2} g/ml even the exposure time is as long as 20 minutes.

Increasing the pump power can increase the Raman intensity, but high-power pump is not suitable for the in-vitro and in-vivo diagnostic purpose. A group at MIT has measured spontaneous Raman spectra of blood glucose with a concentration above the physiological level of glucose in the human bodies using a 830 nm diode laser pump with the laser power of 150 mW at the sample position and a spectral acquisition time of 5 minute. Although their pump power is much higher above the safety limitation, the glucose concentration of the blood sample used in their measurements is still much above the physiological level. It is obvious that the conventional spontaneous Raman technique is hard or even impossible to be used to detect the physiological concentration of blood glucose at safe limit.

In contrast to the conventional Raman spectroscopy, we introduce a new novel Raman approach called “cw excitation Raman spectroscopy“. The method is described in our patent, which teaches the use of short detecting times (a few tens of seconds). In addition, the “cw excitation Raman spectroscopy” technique uses low-power cw laser pump and probe sources at levels over four order of magnitude lower than those which are generally associated with pulsed coherent Raman measurements. This can be achieved by using diode lasers.

Our second Raman technique namely “difference Raman spectroscopy” uses a novel method to reduce substantially the effect of strong background signal formed by tissue, and to improve the detection of fingerprint Raman lines formed by blood analytes.

Low-Power CW Excitation Raman Spectroscopy Device

The CW excitation Raman spectroscopy method can be used to detect in-vitro and in-vivo the concentration level The “CW excitation Raman spectroscopy” method employs two sources; a low-power CW pump and a low-power CW Stokes (or

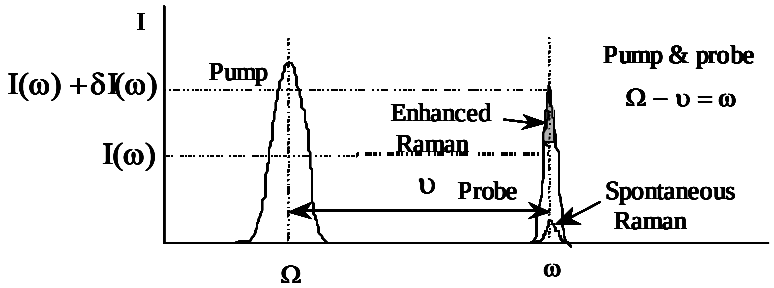

Fig.2 Schematic diagram showing the enhancement of Raman signal when The difference between the pump (Ω) and probe (ω) frequencies is coincident With a Raman vibrational mode frequency (v) of a molecule.

anti-Stokes) probe beams. The frequency of the pump beam is changed, while the frequency of the probe beam is fixed. The pump beam is used to induce the Raman emission, while the probe beam serves to reveal the Raman modes. Both the pump and the probe light traverse a Raman-active medium in collinearity. When the difference between the pump (Ω) and probe (ω) frequencies is coincident with a Raman vibrational mode frequency (ν) of the medium, the weak spontaneously Raman light will be amplified by several orders of magnitude (10 to 10^4) due to the pump photon flux. Gain is achieved as shown in Fig.2.

The large gain is observed only for the Raman lines, which have high Raman cross-section and narrow spectral bandwidth. The high sensitivity and high resolution of the "CW excitation Raman spectroscopy" enable the observation of Raman spectra using short detecting times.

The advantages of using two low-power laser sources, fast detecting times and better sensitivity enable the cw excitation Raman technique to be an effective means to obtain direct fingerprint Raman spectra in biomedical diagnostics applications. In fact, the low-power "CW excitation Raman spectroscopy" can be used as a potentially powerful tool for in-vitro and in-vivo applications for diagnosing diabetes, heart disease, hepatitis, and other diseases by measuring the excitation Raman modes of glucose, cholesterol, SGOT / SGPT, and other corresponding analytes in blood. This technique can also be used to diagnose the breast, cervix, uterus, ovarian cancers and other organ cancers by measuring the fingerprint excitation Raman spectra of breast, cervix, uterus, ovarian tissues and other organ tissues.

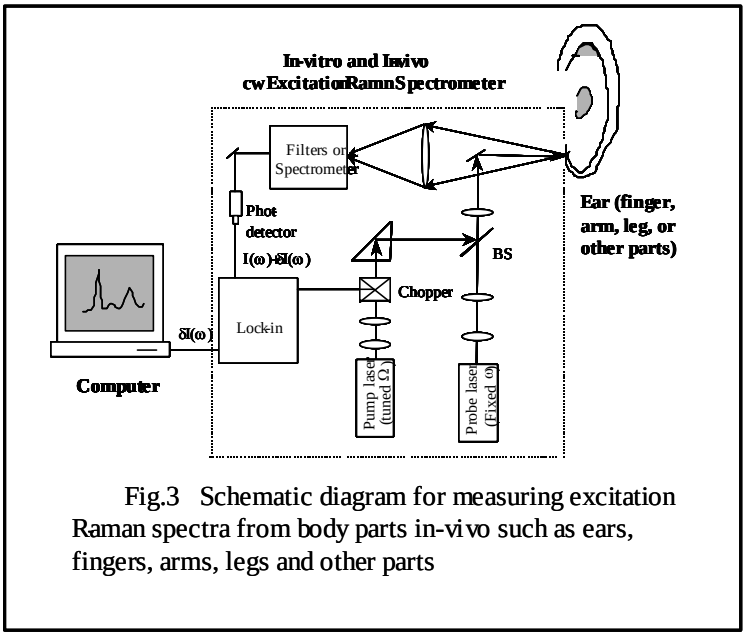

Fig.3 Schematic diagram for measuring excitation Raman spectra from body parts in-vivo such as ears, fingers, arms, legs and other parts

The cw excitation Raman spectroscopy method can be used to detect in-vitro and in-vivo the concentration level of blood glucose by using low-power diode lasers as a probe and a low-power tunable dye laser or a diode laser as a pump. By tuning the pump wavelengths from 570 nm to 630 nm, and measuring the Raman gain at the probe wavelength of 632.8 nm, the excitation Raman peaks from 436.4 cm^{-1} to 1456.2 cm^{-1} should be observed. The modes of 525.7 and 1126.4 cm^{-1} which have high Raman cross-section and narrow bandwidth are expected to be enhanced with lager gain factors. The exposure time can be decreased to be a few ten seconds. The measured intensities of the excitation Raman peaks can be used to detect concentrations levels of the blood glucose, and diagnose diabetes.

The measurements can be done not only for the blood samples but also for the proper body parts in-vivo such as fingers, arms, ears, and/or legs. The schematic diagram for a device used to measure the fingerprint Raman spectrum of blood glucose from ears, fingers, arms, legs or other body parts using the “CW excitation Raman spectroscopy” method is shown in Fig.3. The backscattering geometry is used in the device for patient convenience. This in-vivo glucose concentration measurement without taking blood is extremely important for diabetes persons because these patients have problems to recover the injection injure due to taking blood tests.

Difference Raman Spectroscopy Device

To measure low concentration of analyte molecules in a strong background such as measuring the body glucose level in body, we propose to use “difference Raman spectroscopy” method.

The Raman spectra of glucose and other blood analytes can be more efficiently measured by modulating the blood quantity in an examining region of a human body part where the blood flowing can be decreased or increased. The method for improving the detection of Raman spectra from blood analytes includes the following steps:

Measure Raman spectrum of an examining area of a finger, ear, arm, leg, or other body part in the normal blood flowing condition. The measured Raman spectrum includes the contributions from both tissue components and blood analytes. Since the Raman line intensities from tissue components and structures are strong, it is hard to accurately determine the Raman lines of the blood analytes inside the body tissues.

Modulate the blood quantity in the same examining area by decreasing or increasing the blood flowing in the area. For example, pulling the examining area of a finger, ear, arm, leg, or other body part can decrease the blood flowing and blood quantity in the examining area.

Measure Raman spectrum of the same examining area in the less blood condition. The measured Raman spectrum contains the same contribution from the tissue components but much less contribution from the blood constituents.

Subtract the Raman spectrum obtained in the less blood condition from the Raman spectrum obtained from the normal blood flowing condition. The resulting spectrum, namely difference Raman spectrum, has better signal-to-noise ratio because the contribution of the tissue components and structures to the Raman spectrum is reduced due to the subtraction. The Raman modes of the blood analytes can be clearly determined from the difference Raman spectrum.

Compare the intensities at one or more of the Raman modes for blood analytes from the difference Raman spectrum to the appropriate standards to determine the levels of glucose and other blood constituents.

The preliminary non-optimized experimental results showing the improvement of the detection of the Raman spectra from blood analytes by the “difference Raman spectroscopy” are described in our patent (#6,560,478 B1).

The salient feature is that the blood glucose Raman modes between 770-914 cm^{-1} and 1018-1126 cm^{-1} do not appear in the Raman spectrum obtained in the less blood condition, but can be seen in the Raman spectrum obtained in the normal blood flowing condition. These Raman modes become more apparent and clear in the difference Raman spectrum as shown in Fig.4, from which the glucose level can be well determined. The experimental results also show that the intensities of the glucose Raman lines change after sugar ingested to the test person. It is clear that this technique is a potential candidate for the diabetes screening and diagnosis.

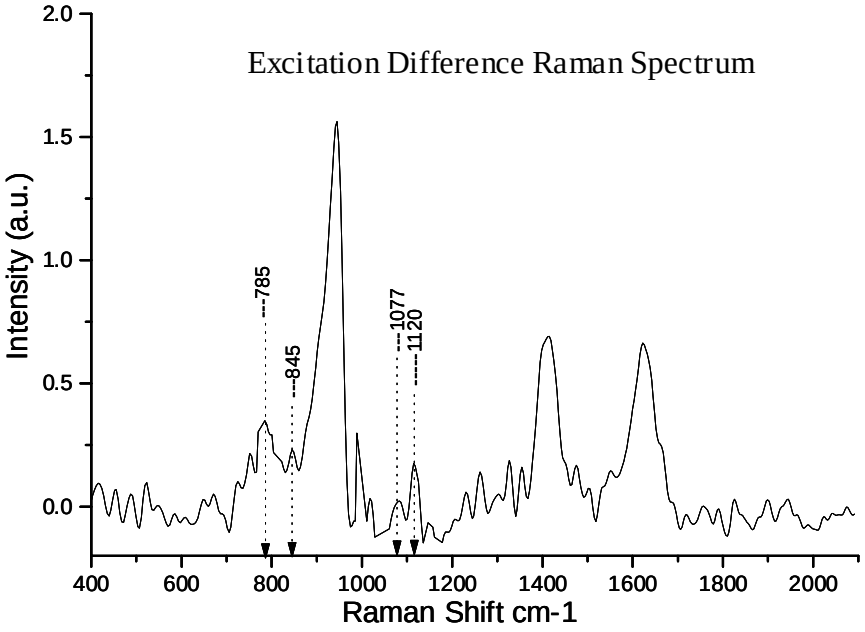

Fig. 4 The excitation difference Raman spectrum of a human finger obtained by subtracting the Raman spectrum with less blood flowing condition from the Raman spectrum with normal blood flowing condition. The blood glucose Raman modes between 770-914 cm^{-1} and 1018-1126 cm^{-1} become more apparent and clear in this difference Raman spectrum. It has better signal-to-noise ratio because the contribution of the tissue components (background) to the Raman spectrum is reduced due to the subtraction. Combining the difference Raman technique with the excitation Raman technique can improve the detection ability of the Raman modes associated with glucose and the glucose level in blood.

Research Plan

Our research plan over the next three years is to test the concept and develop the “cw excitation Raman spectroscopy” and “difference Raman spectroscopy” device for detection low levels of glucose and other molecules in the body blood stream as outlined above and in the patents. We are looking for industrial collaborators to support this R&D project, and commercialize the excitation and difference Raman instruments in the future.

The project will include the following three major phases:

Phase 1, Build a prototype excitation and difference Raman spectroscopy system using fixed and tunable diode lasers, and test the concept for glucose solution. (Year 1)

Phase 2, Test the prototype system on animals and humans, determine sensitivity and specificity, and design a compact system. (Year 2)

Phase 3, Develop and test a compact excitation and difference Raman unit based on human experimental results for clinical testing by companies. (Year 3).

Contact Information

Principal Investigator

R. R. Alfano, Distinguished Professor of Science and Engineering

212-650-5531, alfano@sci.ccny.cuny.edu

Project Leader:

Dr. W. B. Wang,

Tel. 212-650-5531, Fax: 212-650-5530, E-mail: wwang@sci.ccny.cuny.edu

Business Development

Alan Doctor

212-650-8265, doctor@ee.ccny.cuny.edu