❉ RESEARCH REPORT

Measurement of Blood Glucose Concentration by Near-Infrared Raman Spectroscopy

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G. R. Harrison Spectroscopy Laboratory, MIT

Introduction

In the field of clinical blood analysis, optical spectroscopy has begun to provide valuable alternatives to standard chemical and electrochemical tests. One biological analyte that has received considerable attention, especially with regard to transcutaneous measurements, is glucose. Spectroscopic techniques for glucose measurement currently being explored include near-infrared (NIR) diffuse reflectance of skin [Robinson et al., 1992; Marbach et al., 1993] and visible-excitation Raman scattering of aqueous humor through the cornea [Erckens et al., 1994]. In our laboratory, we are exploring the technique of NIR-excitation

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❉ SPECTROSCOPY LABORATORY RESEARCHERS
RECEIVE SCIENCE FOR ART PRIZE

Michael Feld (center) and Kyungwon An (right) receiving the "Vinci d' Excellence" for invention of the single atom laser from Francis D. Massie, Scientific Aattaché of LVMH, sponsor of the award. The two scientists were cited for overcoming conceptual and technological barriers in developing a laser device in which coherent radiation is generated through the interaction of a single excited atom with a single mode of the radiation field. The award ceremony took place in New York City on October 17.

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Measurement of Blood Glucose ...

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Raman spectroscopy for both in vitro and in vivo blood glucose analysis. Recently, the ability of the NIR Raman spectroscopic technique to perform quantitative optical histochemistry on artery tissue [Brennan et al., 1995] and to measure the concentrations of multiple biological analytes in aqueous solution simultaneously [Berger et al., 1995; Wicksted et al., 1995] has been demonstrated. Here, we demonstrate that NIR Raman spectroscopy can measure the amount of spiked glucose in human whole blood samples.

Materials and Methods

A reservoir of heparinized whole blood was drawn from one of the authors (AJB) and left at room temperature for two hours prior to measurements. Immediately before each Raman scan, two milliliters of blood were taken from the sample reservoir and spiked with a precise concentration of glucose ranging from 0 to 100 mM. In order to eliminate any spurious systematic correlation with glucose concentration, the sequence of concentrations was randomized. Glucose loss due to glycolysis was negligible.

NIR Raman spectra of blood samples were acquired at 830 nm excitation using a system equipped with holographic filters, optical fibers, f/1.8 spectrograph optics, and a liquid nitrogen-cooled CCD detector [Berger et al., 1995]. Blood samples were placed in quartz cuvettes for scanning on the NIR Raman system. Laser power at the sample was 150 mW. During each Raman scan, the blood sample was stirred by a miniature magnetic stir bar in order to minimize thermal effects from the deposited laser light. Spectral acquisition time was five minutes per sample. No sample degradation was observed in the course of the measurements.

The multivariate technique of partial least squares (PLS) [Geladi and Kowalski, 1986] was used to correct for spectral backround interferences and to quantitate the amount of glucose in each sample. In these experi-

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The Spectroscopy Laboratory houses two laser research resource facilities. The MIT Laser Research Facility, supported by the National Science Foundation, provides shared facilities for core researchers to carry out basic laser research in the physical sciences. The MIT Laser Biomedical Research Center, a National Institutes of Health Biomedical Research Technology Center, is a resource center for laser biomedical studies. The LBRC supports core and collaborative research in technological research and development. In addition, it provides advanced laser instrumentation, along with technical and scientific support, free of charge to university, industrial and medical researchers for publishable research projects. Write or call for further information or to receive our mailings, (617) 253-9774.

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❋ INSTRUMENTATION UPDATE

Streak Camera

A state-of-the-art streak camera (Hamamatsu model C5680) capable of time resolution of 2 ps in either synchroscan mode or high speed single shot mode is now in operation. It is being used in conjunction with our mode-locked Ti:sapphire laser in photon migration studies, such as time dependent fluorescence-based imaging to locate tumors in the human body. It will also be used in several physical science projects, including studies of luminescence of nanocrystallites and acoustic properties of thin films.

Multi-Wavelength EEM Spectrofluorimeter

A compact, state-of-the-art spectrofluorimeter for rapid acquisition of excitation-emission matrices (EEM's) of biological tissue has been developed. This unique system, fabricated for the first time, can provide a set of rapidly varying excitation wavelengths and collect multi-excitation fluorescence spectra. The system generates pulses of light at 10 different excitation wavelengths, using a pulsed nitrogen laser to pump different dyes placed in cuvettes on a rotating wheel. A white light reflectance spectrum is also collected at each cycle for normalization of the fluorescence spectra. This device has been specially configured for clinical use. As in past LBRC instruments for fluorescence data acquisition, an optical fiber probe is used to excite and collect spectra from tissue. Spectra are detected by a gated photodiode array detector. The system can collect fluorescence EEM's from biological tissue with a signal-to-noise ratio in excess of 50:1 in less than 500 ms.

Upgrade of CW Raman Facility

The CW Raman Facility, which uses argon and krypton ion lasers, is now optimized for excitation wavelengths at 647, 568, 514 and 406 nm. Addition of a back-thinned CCD detector has enhanced the sensitivity of the system. A variable temperature cell holder permits spectra to be collected at different sample temperatures in the range 10-80° C. Input and collection optics have been modified so as to make the system convenient for use. New users must participate in an initial training session followed by study of the software manuals.

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❋ RESEARCH REPORT

The Single Atom Laser - A Quantized Rabi Oscillator

Kyungwon An, Ramachandra R. Dasari and Michael S. Feld

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Every laser consists of two essential components: an optical resonator, usually composed of two highly reflective mirrors; and a laser gain medium, capable of amplifying light of a given wavelength. Conventional lasers require trillions of excited atoms or molecules to achieve laser oscillation. Why are so many radiators needed? What is the smallest number required to effectively amplify the light? Can a single atomic radiator be used? Development of a single atom laser has been an illusive scientific goal for many years. It was recognized that such a laser would exhibit fundamental properties not displayed by conventional lasers, which would provide valuable new insights into the interaction of light with matter. Single atom laser devices might also have an important impact on the field of opto-electronics, where there is a premium on developing sub-miniature light sources for information processing and display[*]. However, it was also known that simply scaling down the number of atoms in an ordinary laser to one atom would reduce the laser gain to such a minuscule level that laser threshold condition could not be satisfied.

A quite different and far more efficient approach to attaining single atom laser oscillation is based on the dynamical energy exchange between an atom and a resonator mode to which it is strongly coupled. Such a device, which we call the microlaser, has been demonstrated recently in the Spectroscopy Laboratory [An el al., 1994]. In this device the number of interacting atoms can be reduced below one while achieving a radiator-to-photon ratio of larger than one.

The operating characteristics of the microlaser differ from those of conventional lasers in important ways. First, the experiments show that under single atom conditions as many as ten photons can be stored in the resonator, and the device then emits several million laser photons per second. Second, neither spontaneous emission nor stimulated emission play a role in photon buildup. In addition, the device does not manifest the distinct threshold behavior exhibited by a conventional laser.

The microlaser is a macroscopic system which exhibits quantum mechanical features. It can be thought of as a giant molecule composed of a single atom and a pair of mirrors, and it exhibits resonant features in the output power as a function of the velocity of the injected atoms. This new device is important because of its conceptual simplicity, the insights it provides about the laser-atom interaction process, and a number of new possibilities which have now become possible.

The microlaser is composed of a resonator formed by two supercavity mirrors with extremely high reflectivity (99.9997%) separated by 1 mm, with a beam of atomic barium flowing through the gap between the mirrors (Figure 1). The barium atoms are raised to an excited state by sending them through an optical pump field before they enter the ☞

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[*] For a review of microcavity semiconductor lasers see Y. Yamamoto and R. E. Slusher, "Optical Processes in Microcavities", Physics Today, pages 66-73, June 1993.

Figure 1. Schematic of the microlaser.

The Single Atom Laser …

Figure 2. Rabi oscillation can be depicted in analogy to a rotating pendulum. An excited atom corresponds to an inverted pendulum. In our experiment, the atom undergoes a one-sixth rotation while traversing the empty cavity. If n photons are present its rotation is enhanced by a factor of $\sqrt{n+1}$.

Figure 3. Average number of photons in the cavity mode, <n>, as a function of the average number of atoms in the mode, <N>, compared to a quantum trajectory simulation.

resonator. The barium beam density is kept small enough so that one atom or less is inside the resonator at any moment. Laser operation is initiated when a barium atom traversing the cavity undergoes vacuum Rabi oscillation and emits a photon at 791 nm wavelength into the empty resonator. In our experiment the atom undergoes about one-sixth of a complete Rabi oscillation when it exits the cavity. Note that half an oscillation corresponds to the atom exiting the cavity precisely in its ground state. A one-sixth turn means that the exiting atom has a 25% probability of being transferred to its ground state (Figure 2). This is simply the probability for an excited atom to emit a photon into the cavity mode. In other words, for every four such atoms traversing the cavity, one is likely to exit in its ground state, leaving a photon behind. This photon is stored in the cavity until the next atom arrives. This new atom undergoes non-vacuum Rabi oscillation which is enhanced by the presence of the previously emitted photon. The emission probability is now increased to 45%, indicating that now a second photon is likely to be emitted after two atoms pass through the cavity. In this fashion the number of photons in the resonator builds up. For a lossless cavity, with an infinite storage time, this build-up would continue to infinity. An actual cavity has a finite storage time, and so in practice the build-up slows down as the number of photons grows, reaching an equilibrium when the number of photons per second coupled out of the cavity via mirror transmission is precisely balanced by the energy supplied to the system per unit time in the form of excited atoms.

In our experiment we observe an emission rate of millions of laser photons per second when the average number of atoms in the cavity is unity, corresponding to about 10 photons stored in the resonator (Figure 3). The system exhibits mild threshold behavior when the mean photon number becomes comparable to unity. As mentioned above, the emission probability is about 25% below threshold, and it is observed to jump to about 50% above threshold. This result is confirmed by a quantum trajectory simulation (solid line in Figure 3), based on a recently developed stochastic wave function approach

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The Single Atom Laser …

[Tian et al., 1993] which accounts for the random distribution of experimental parameters such as atomic velocity and position, the time of atomic arrival in the cavity, the spatial structure of the standing wave cavity mode, atomic spontaneous emission, and cavity decay.

It should be noted that although the threshold feature in our data resembles that of an ordinary laser (but with a much smaller slope change), its origin is quite different. In the microlaser, threshold is strongly correlated with the emission probability of the excited atoms, which starts from 25% and increases to 50% as the mean photon number grows. As mentioned above, under proper conditions the emission probability can be further enhanced. In fact, it oscillates as a function of the mean photon number. As shown in Figure 2, the atoms undergo Rabi oscillation, corresponding to a sinusoidally varying emission probability. Therefore, one can expect the single atom laser to exhibit multiple thresholds. In the vicinity of an emission probability minimum, the mean photon number does not change appreciably until the next threshold sets in. In other words, the system is self-stabilizing (Figure 4). As a result, the intracavity field exhibits photon number squeezing . Another interesting prediction of the simulations is that the observed threshold behavior is due to the standing-wave structure of the resonator mode. If this structure is eliminated the threshold behavior should disappear, resulting in thresholdless laser oscillation. Experiments to study these interesting points are under way.

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Figure 4.   Results of the quantum trajectory simulations. As the number of injected atoms increases, the intracavity photon number (a) saturates to a value that induces the injected atoms to undergo almost a complete Rabi oscillation (very small emission probability) and leave the cavity in the excited state (b). The photon number distribution function in the vicinity of an emission probability minimum is shown in (c).   A finesse of 10^7 is assumed.

<u>Please Post</u>

Lester Wolfe Workshops in Laser Biomedicine

Transcutaneous Diagnosis

Tuesday, November 28, 1995, 4:00-7:00 PM

Real-Time Confocal Microscopy in Skin
Robert H. Webb, MGH-Wellman Laboratories

Ouchless Blood Analysis Via Raman Spectroscopy
Andrew Berger, MIT Spectroscopy Laboratory

Intravital Microscopy of Solid Tumors
Rakesh Jain, Massachusetts General Hospital

Sub-Surface Imaging in Biological Tissue using Optical Coherence Tomography and Microscopy
Joseph A. Izatt, CaseWestern Reserve University, Cleveland

Room 6-120, Massachusetts Institute of Technology
77 Massachusetts Avenue, Cambridge

Refreshments at 5:20 P.M.

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Seminar on

MODERN OPTICS AND SPECTROSCOPY

FALL SEMESTER, 1995

September 19   Randall Hulet, University of Southern California
Bose-Einstein Condensation of an Atomic Gas

September 26   George Flynn, Columbia University
Desperately Seeking Supercollisions

October 10   Jeffrey Kimble, California Institute of Technology
Quantum Logic and Computation Via Cavity QED

October 17   Roy Glauber, Harvard University
Quantum Behavior of Trapped Ions and Their Interaction with Light

October 24   John Hall, JILA
Using Weak Visible Molecular Overtone Bands: A Big Sensitivity Jump Will Get you Thousands of New Wavelength Standards

October 31   James Childs, MIT
Cavity QED Lineshapes for a Single Intracavity Atom

November 14   Marsha Lester, University of Pennsylvania
Spectroscopy Predissociation Dynamics and Reactions of OH-H$_2$ Complexes

November 21   Wendell Hill, University of Maryland
Atomic Schizophrenia and other Disorders Induced by Intense Laser Fields

December 5   Claudio Cesar, MIT
Laser Studies of Cold Trapped Hydrogen

TUESDAYS, 12:00-1:00, Marlar Lounge (37-252), Ronald E. McNair Building

Refreshments Served Following the Seminar

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AIR, WATER, LASERS AND EARTH

Applications of Lasers in Science, Technology and Medicine

G. R. Harrison Spectroscopy Laboratory
MIT INDEPENDENT ACTIVITIES PERIOD
JANUARY 23, 1996

Coordinators: Drs. Ramachandra R. Dasari and Lev Perelman

The Single Atom Laser: A Quantized Rabi Oscillator
Dr. Kyungwon An - G. R. Harrison Spectroscopy Laboratory, MIT
9:00-9:50 AM, Room 4-153

Holography: The Medium and the Message
Dr. Michael Klug - Media Laboratory, MIT
10:00-10:50 AM, Room 4-153

The Use of Lasers in Medicine: The Routine, The Recent, and The Revolutionary
Dr. Irving Itzkan - Laser Biomedical Research Center, MIT
11:00-11:50 Noon, Room 4-153

Tour of the G. R. Harrison Spectroscopy Laboratory
Dr. Ramachandra Dasari
3:00-4:30 PM Assemble in Room 6-018
Refreschments will follow the tour

✺ PERSONALITIES

IRVING ITZKAN

DR. IRVING ITZKAN was born and raised a Brooklyn Dodger fan in Brooklyn, New York, so immigrating to Boston and becoming a Red Sox fan was a perfectly natural transition. He earned a bachelors degree in Engineering Physics from Cornell University, which qualified him for active service in the US Navy. His time in the Navy was spent entirely at sea, serving on an aircraft carrier, a US destroyer and a Chinese destroyer (where he became proficient in the use of chopsticks). Since then he has maintained a continuing interest in things nautical. He is an avid sailor, which includes instructing students in small boat sailing, extensive coastal cruising on friendly yachts, and several voyages on large schooners.

Irv received a Master of Science in Electrical Engineering from Columbia University and a Ph.D. in Physics from New York University. His thesis title was, "The Clebsch-Gordon Coefficients for the Crystallographic Space Groups". These degrees qualified him for a long and illustrious career in industrial research, starting with research on electron beam tubes for the Sperry Gyroscope Company. At about this time lasers were invented, and he segued into the Sperry Electro-Optics Group as Engineering Section Head for Laser Research, dealing with the physics of lasers, nonlinear optics and optical components, and serving as midwife at the birth of the first laser gyro.

The lure of working with really high power lasers brought him to Boston and the AVCO Everett Research Laboratory, where he operated the first N2 laser pumped dye laser. He then developed narrow band, tunable versions of this dye laser which were used in the first successful laser isotope separation experiment. His position as Chairman of the Optics Research Committee involved him in many other phases of optics research at AVCO. These included laser beam propagation in the atmosphere, the use of lasers for remote sensing, and the interaction of high energy lasers with materials, including metals, dielectrics, and biological tissue.

In 1980 Irv decided to reorient his career towards medical applications of lasers, and helped organize the American Society for Laser Medicine and Surgery, of which he is a Founding Fellow. After taking early retirement from AVCO, he joined the Laser Biomedical Research Center in the Spectroscopy Laboratory at MIT, where he is Senior Scientist and studies problems associated with laser ablation of biological tissue and techniques for the laser diagnosis of disease. He is a member of the Laser Committee at Beth Israel Hospital, and serves as a consultant in lasers and optics to several industrial corporations in the greater Boston area.

Irv and his wife Annette, who is a busy Boston Real Estate broker, occasionally find time to visit their cottage in Chatham on Cape Cod, where they are sometimes joined by their son Seth, who works on educational applications of computers, or their son Harry, who trains people in security techniques.

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ments, the only data needed to perform PLS analysis were the blood spectra themselves and their known glucose concentrations. Analysis was performed via cross-validation, in which each sample's concentration is predicted using the remaining samples as a calibration set. The cross-validation's root mean squared error of prediction (RMSEP) is an estimate of the uncertainty with which glucose could be measured in other, unknown samples, using all of the known samples as a calibration set.

Results

Results from the cross validations were organized in a plot of the PLS-predicted concentrations versus the associated reference concentrations, as shown in Figure 1.

The correlation between the measurements clearly demonstrates that the NIR Raman technique was able to measure blood glucose over the entire concentration range. An optimized RMSEP of 3.6 mM, limited primarily by the spectral noise level, was achieved using six loading vectors and the wavenumber range 785-1760 cm^{-1}.

Because of its general nature, the NIR Raman method can measure the concentration of any Raman-active blood component in the same way that it measures glucose. In order to demonstrate this, we conducted an experiment to measure dissolved bicarbonate in blood. All of the parameters remained the same other than the replacement of glucose with dissolved sodium bicarbonate. The

PLS predictions of bicarbonate in blood are shown versus the reference concentrations in Figure 2. Once again, the ability of the NIR Raman technique to measure the concentration of the analyte in blood was clearly demonstrated. In this case, the RMSEP was 7.4 mM.

Discussion

These experiments indicate the potential for NIR Raman spectroscopy to provide practical measurements of blood analytes such as glucose and bicarbonate in a clinical setting. Using the simple experimental geometry described above, we have achieved RMSEP values in the few mM range for these two analytes. In both cases, this is close to the level of desired clinical uncer-

Figure 1. Cross validation results for measurement of dissolved glucose in samples of whole blood. Each data point represents a sample. PLS prediction of glucose concentration is plotted against reference concentration. Root mean squared error of prediction (RMSEP) is 3.6 mM.

Figure 2. Cross validation results for measurement of dissolved bicarbonate ion in samples of whole blood. RMSEP is 7.4 mM.

tainty. Concentration prediction accuracy in these experiments scales nearly linearly with the signal-to-noise ratio (S/N), which can be improved by increasing the laser power and the collection area. In addition, incorporation of nonimaging optical elements should lead to an order of magnitude improvement in signal collection [Tanaka et al., 1995]. With such improvements, clinically acceptable prediction uncertainties for glucose and bicarbonate should be available from in vitro blood scans in less than one minute.

Conclusion

This study demonstrates that NIR Raman spectroscopy is a promising technique for improved in vitro and novel in vivo measurement of glucose and other blood and tissue analytes. A clear correlation between glucose Raman signal and blood concentration

has been established by PLS analysis, and a similar result has been achieved using bicarbonate. We plan to conduct further experiments investigating other analytes and exploring clinical applications of this spectroscopic method.

Acknowledgments

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