Princeton Instruments

APPLICATION NOTE

Advanced CCD Cameras and Imaging Spectrographs Facilitate Acquisition of Novel Femtosecond Stimulated Raman Spectroscopy Data To Improve SERS Biosensors

Overview

Accurate characterization of surface-enhanced Raman spectroscopy (SERS) biosensors, fluorescent dye molecules that hold great promise for *in vivo* bioanalyte detection, can often be quite difficult as the overwhelming isoenergetic fluorescence signal typically makes it challenging to measure resonance Raman cross-sections for the molecules. To overcome this obstacle, researchers at the University of Minnesota in Minneapolis recently utilized etalon-based femtosecond stimulated Raman spectroscopy (FSRS), a technique designed to acquire a stimulated Raman signal without strong fluorescence or interference from signals resulting from other four-wave mixing pathways.[1]

The Frontiera Research Group, headed by assistant professor of chemistry Dr. Renee R. Frontiera, leverages the latest spectroscopic tools and techniques to probe chemistry at the ultimate limits of space and time. The group investigates fundamental and applied issues in membrane protein biophysics, alternative energy sources, and nanotechnology, determining how local environments affect chemistry. Their highly interdisciplinary research explores current problems at the interface of chemistry, biology, and materials science.[2]

Here, we will focus on the Frontiera Research Group's innovative approach to the determination of resonance Raman cross-sections for use in biological SERS sensing with femtosecond stimulated Raman spectroscopy.

Etalon-Based FSRS Setup

The University of Minnesota researchers report that their novel FSRS data were collected on a newly constructed optical table. A 4.4 W, 1 kHz fundamental output was split to generate the beams needed for stimulated Raman experiments. The 800 nm Raman pump pulse was generated by passing 415 mW through a custom etalon to generate a picosecond pulse that decayed exponentially with time. This pulse was passed through a telescope for beam diameter optimization as well as through a manual retro-reflector stage for time delay measurements. The near-infrared broadband femtosecond probe pulse was created by continuum generation in sapphire, utilizing 2.5 mW of the fundamental output. This beam was passed through a filter to reduce the fundamental intensity and compressed with fused-silica prisms.[1]

Acknowledgment

Princeton Instruments would like to thank Dr. Renee R. Frontiera, University of Minnesota, for her invaluable contributions to this application note.

Both pulses were focused non-collinearly on the sample using a 10 cm focal length lens. Raman pump powers at the sample were varied from 100 µW to 5000 µW, with a 90:10 beam diameter of 150 µm in both dimensions. Probe power was kept constant at 6 µW, with a beam diameter of 40 µm in both dimensions. The probe was collimated after the sample with a 10 cm lens. Both beams were horizontally polarized. No polarization optics were placed before the spectrograph.[1]

The Frontiera Research Group's FSRS system employed a Fabry-Perot etalon so as to minimize contributions from stimulated emission and other four-wave mixing pathways that may occur on resonance when Raman pump photons are incident on the sample prior to the arrival of the probe pulse. This etalon was designed with a reflectivity of 98.5 ± 0.5% and a parallel spacing of 24 µm. The free spectral range was 230 cm^{-1}, resulting in a high-finesse etalon with a spectral bandwidth of ~2.3 cm^{-1}. Because the etalon had zero electric field prior to the arrival of the probe pulse, electronic excitation was minimized. (In FSRS, the Raman amplitude is primarily determined by the Raman pump field amplitude at the time of probe overlap.)[1]

A stimulated Raman signal was generated collinearly with the probe, making alignment straightforward according to the researchers. The probe and signal were directed into a 300 mm Princeton Instruments SpectraPro®:2300i imaging spectrograph, dispersed on a 600 gr/mm grating blazed at 750 nm, and focused onto the photosensitive array of a Princeton Instruments PIXIS:100F CCD camera. The grating was oriented for maximum diffraction efficiency of the horizontally polarized beams. A filter was placed before the spectrograph to compensate for low silicon detector efficiency at long wavelengths.[1]

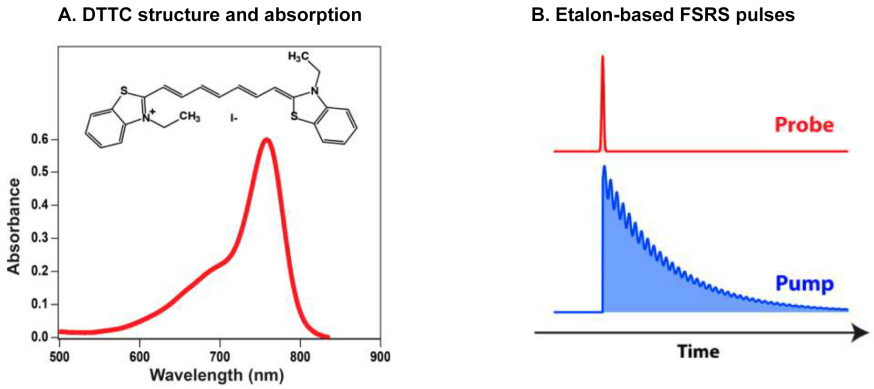
Data collection was enabled by home-written LabVIEW (National Instruments) software, which collected triggered spectra at the 1 kHz repetition rate of the laser. All spectra were presented as Raman gain, in that a single Raman-pump-on spectrum was divided by the subsequent Raman-pump-off spectrum. To this end, a chopper was placed in the Raman pump path and utilized to chop the spectra at half the laser repetition rate, ensuring that the Raman pump pulse was present on the sample for every other spectrum. A home-built flip-flop circuit ensured that the detector received the appropriate triggering signals with respect to the chopper state. Acquisition times for these experiments ranged from 5−10 min per spectrum. As the spectra were intrinsically normalized to the probe pulse power at each wavelength, no detector instrument response function was necessary.[1]

Data & Results

The researchers employed their etalon-based FSRS setup to quantitate the resonance Raman cross-sections for a near-infrared SERS dye commonly used in the biological water window: 3,3′-diethylthiatricarbocyanine (DTTC). The chemical structure and absorption spectrum of DTTC iodide are presented in Figure 1, along with temporal depictions of the two pulses used for the FSRS experiments.[1]

Figure 1.
(A) Structure and absorbance spectrum of DTTC iodide in methanol. (B) Temporal profiles of the two pulses used for FSRS experiments. A stimulated Raman signal was generated through the interaction of the broadband femtosecond probe pulse and the 800 nm picosecond pump pulse. The pump pulse was generated with an etalon, giving rise to the distinctive exponential decay profile.[1]

Data courtesy of Dr. Renee R. Frontiera, University of Minnesota. First published in "Determination of resonance Raman cross-sections for use in biological SERS sensing with femtosecond stimulated Raman spectroscopy," Silva, W.R., Keller, E.L., Frontiera, R.R., Analytical Chemistry, ***2014,*** *86, 7782–7787.*

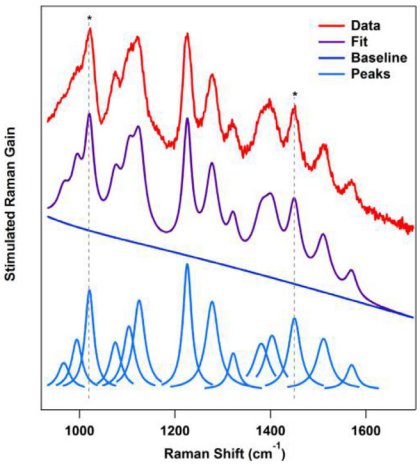


The red edge of the DTTC absorption spectrum was resonant with the 800 nm pump pulse. The researchers note that attempts at measuring the resonance Raman spectrum on a conventional Raman spectrometer resulted in a broad fluorescence background with no discernible Raman peaks, as expected. The temporal depiction of the pulses used in their etalon-based FSRS system consisted of a femtosecond probe pulse with Gaussian temporal shape and a picosecond pump pulse from the etalon. This pulse displayed a sharp rise at time zero, followed by an exponential decay. Very few pump photons interacted with the sample before the probe pulse arrival, leading to minimal excited state generation. Since the picosecond pump pulse was created from a femtosecond pulse repeatedly reflected in the etalon, the researchers did see an oscillatory feature in the pump amplitude corresponding to the pathlength between the mirrors, as depicted in Figure 1B. The researchers report observing periodic modulations of the intensity due to a varying number of reflections of the pulse packet in the etalon.[1]

Figure 2.
FSR spectrum of DTTC in methanol taken with an 800 nm Raman pump pulse. The spectrum (red) can be fit (purple) by a polynomial baseline (dark blue) with Lorentzian peaks (light blue). Solvent peaks are marked with an asterisk.[1]

Data courtesy of Dr. Renee R. Frontiera, University of Minnesota. First published in "Determination of resonance Raman cross-sections for use in biological SERS sensing with femtosecond stimulated Raman spectroscopy," Silva, W.R., Keller, E.L., Frontiera, R.R., Analytical Chemistry, ***2014,*** *86, 7782–7787.*

Figure 2 displays the resonance FSR spectra of DTTC in methanol. Here, it is clear that the stimulated Raman process is sufficiently free from background fluorescence to easily resolve the resonant Raman peaks of DTTC. There is a small but broad background signal from other four-wave mixing pathways.[1]



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Unlike FSR spectra of DTTC taken with Raman pump pulses that are Gaussian in duration, the use of an etalon prevents the appearance of dispersive lineshapes on resonance, and peaks are easily fit by Lorentzian lineshapes. The fit in Figure 2 shows the 12 major peaks of DTTC fit with Lorentzian functions on top of a broad polynomial background. The frequencies, amplitudes, and widths of all peaks were freely varied in the fitting. The oscillatory feature in the etalon temporal profile should have resulted in an oscillatory baseline feature in the spectrum, but this was (fortunately) below the researchers' signal-to-noise ratio and therefore was not detected.[1]

Table 1 lists the values for absolute Raman cross-sections of DTTC. The researchers calculated standard deviations by measuring and fitting spectra from three different DTTC samples. The cross-sections for many of the modes exceeded 10^{-25} cm^2/molecule. These cross-section values indicate that much of the near-infrared SERS signal from DTTC may actually have come from resonance contributions rather than SERS enhancement.[1]

Table 1.
Resonance Raman frequencies, cross-sections, and standard deviations for cross-section measurements in the near-infrared dye DTTC, taken with an 800 nm etalon-based FSR spectrometer.[1]

Data courtesy of Dr. Renee R. Frontiera, University of Minnesota. First published in "Determination of resonance Raman cross-sections for use in biological SERS sensing with femtosecond stimulated Raman spectroscopy," Silva, W.R., Keller, E.L., Frontiera, R.R., Analytical Chemistry, ***2014,*** *86, 7782–7787.*

Raman shift (cm^{-1})	resonance Raman cross-section (cm^2/molecule × 10^{-25})	standard deviation (cm^2/molecule × 10^{-25})
974	2.4	0.9
996	4.6	1.2
1076	6.2	1.2
1106	9.6	1.6
1128	11.5	0.3
1229	14.1	2.4
1281	15.1	2.7
1327	4.4	0.5
1384	7.3	0.9
1406	9.2	0.4
1514	7.1	0.8
1572	2.5	0.4

SERS enhancement factors were determined by first calculating the Raman system response with a straight methanol blank in a matched cuvette. The researchers then determined the DTTC SERS signal by measuring the peak area and using the appropriate cross-section calculated from the FSR data. This was compared to the known DTTC concentration in the SERS sample to obtain the enhancement factor. It should be noted that CW surface-enhanced Raman spectra were acquired utilizing a horizontally polarized 785 nm laser to illuminate the sample through a 20x LWD objective (power at the sample: 30.6 mW). Backscattered Raman light was collected through a 70:30 beamsplitter, focused onto a Princeton Instruments SpectraPro:2500i spectrograph, and dispersed onto the photosensitive array of a Princeton Instruments PIXIS:400BX CCD camera by a 600 gr/mm grating blazed at 750 nm. No polarization optics were used in this SERS setup and the SpectraPro:2500i grating grooves were oriented perpendicular to the excitation beam polarization. The spectra were scaled by a detector response function measured via a standard Princeton Instruments IntelliCal® lamp.[1]

Figure 3 shows an application of the accurate resonance Raman cross-section measurements of DTTC. Here, the researchers examined the resonance SERS behavior of the analyte on aggregated gold colloids in solution. When excited with a resonant 785 nm laser, these simple SERS complexes provided excellent SERS signal magnitudes.[1]

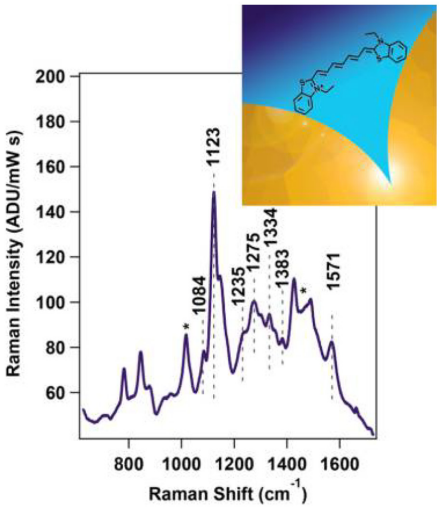

Figure 3.
SERS spectrum of DTTC on aggregated gold colloids (with 785 nm excitation). Raman intensities plotted in terms of analog-to-digital units and normalized by pump power and acquisition time. Strong Raman peaks and significantly reduced fluorescence are seen even with only weakly enhancing SERS substrates. Asterisks indicate solvent peaks.[1]

Data courtesy of Dr. Renee R. Frontiera, University of Minnesota. First published in "Determination of resonance Raman cross-sections for use in biological SERS sensing with femtosecond stimulated Raman spectroscopy," Silva, W.R., Keller, E.L., Frontiera, R.R., Analytical Chemistry, ***2014,*** *86, 7782–7787.*

The researchers assert that the resonance Raman cross-sections determined by their etalon-based femtosecond stimulated Raman spectrometer will significantly impact enhancement factor calculations for SERS substrates optimized for the near-infrared biological imaging window. As resonance Raman cross-sections cannot be measured for fluorescent molecules using standard methods, estimates for these values vary widely. The researchers' novel measurement of the exact cross-sections eliminates this estimation, which should lead to the development of reproducible, highly enhancing, high-signal-magnitude SERS sensors in the biologically relevant near-infrared region.[1]

For more data and calculations, as well as an in-depth discussion of experiment results, please refer to "Determination of resonance Raman cross-sections for use in biological SERS sensing with femtosecond stimulated Raman spectroscopy," Silva, W.R., Keller, E.L., Frontiera, R.R., *Analytical Chemistry,* **2014,** 86, 7782–7787.

Enabling Technology

The important roles of the PIXIS:100 camera and the SpectraPro:2300i imaging spectrograph in this etalon-based FSRS research should by no means be overlooked. "The triggered kHz read rate of the PIXIS is really what enables this kind of spectroscopy," Dr. Frontiera states.

PIXIS cameras (see Figure 4) are fully integrated, low-noise CCD detection systems designed for quantitative scientific imaging and spectroscopy applications performed in the ultraviolet, visible, and near-infrared ranges. Utilizing Princeton Instruments' exclusive XP cooling technology, PIXIS is the one scientific camera platform that offers deep cooling with an all-metal, hermetically sealed design. This innovative cooling technology ensures maintenance-free operation and is backed by the industry's only lifetime vacuum guarantee.

Figure 4.
The PIXIS:100F camera (left) used by the Frontiera Research Group to perform FSRS features a front-illuminated, 1340 x 100 pixel CCD and provides superb sensitivity, triggering, speed, and programmability for myriad spectroscopy applications. The PIXIS:400BX camera (right), which the group used for SERS, utilizes a back-illuminated, 1340 x 400 pixel CCD fabricated using Princeton Instruments' proprietary eXcelon® process to increase detector sensitivity in the blue and near-infrared regions while suppressing etalon interference fringes.

Complete control over all PIXIS hardware features is simple with the latest version of Princeton Instruments' 64-bit LightField® data acquisition software, available as an option. A host of novel functions for easy capture and export of imaging and spectral data are provided via the exceptionally intuitive LightField user interface. LightField also allows seamless integration of hardware controls and direct data acquisition into National Instruments' LabVIEW® and MathWorks' MATLAB®. A built-in math engine analyzes imaging and spectral data in real-time.

The SpectraPro:2300i and SpectraPro:2500i imaging spectrographs (see Figure 5) utilized by the Frontiera Research Group are also easily integrated within practically any conceivable experiment setup via LightField software. For more than a quarter-century, these spectrometers have set the standard for reliable high-performance spectroscopy. Researchers around the world depend on SpectraPro spectrographs and monochromators for a wide variety of applications — Raman, fluorescence, photoluminescence, microspectroscopy, absorption, emission, and more.

Figure 5.
The SpectraPro:2300i (right) and SpectraPro:2500i (left) are high-resolution, high-throughput imaging spectrographs that provide 300 mm and 500 mm focal lengths, respectively.

The advanced instrumentation of the SpectraPro series utilizes computer-optimized optical systems to ensure high spectral resolution, as well as an astigmatism-corrected design to permit multichannel fiber applications. SpectraPro spectrometers afford researchers unrivaled experiment versatility via the use of multiple entrance and exit ports plus the ability to operate either as a spectrograph with an array detector or as a scanning monochromator with an exit slit and single-channel detector. All SpectraPro spectrometers are also fully compatible with Princeton Instruments' patented IntelliCal, the industry's only easy-to-use wavelength and intensity calibration system. IntelliCal includes dual Hg/Ne atomic emission and LED-based, NIST-traceable intensity calibration light sources. Calibration that once took hours, or even days, can now be completed in a matter of minutes with dramatically improved accuracy.

Resources

To learn more about the Frontiera Research Group, including their current work on developing a label-free, super-resolution imaging technique to monitor cells on the nanometer length scale, determining the role of vibrations in driving electron transfer reactions, and using plasmonic nanomaterials to monitor and catalyze chemical reactions, please visit:
http://frontiera.chem.umn.edu/

For further details regarding Princeton Instruments' PIXIS family of CCD cameras and SpectraPro series of spectrometers, please visit:
http://www.princetoninstruments.com/products/PIXIS-CCD
http://www.princetoninstruments.com/products/SpectraPro

References

1. "Determination of resonance Raman cross-sections for use in biological SERS sensing with femtosecond stimulated Raman spectroscopy," Silva, W.R., Keller, E.L., Frontiera, R.R., *Analytical Chemistry,* **2014,** 86, 7782–7787. DOI: 10.1021/ac501701h

2. http://frontiera.chem.umn.edu/ [accessed online in August 2016]