Journal of Visualized Experiments

Ultrafast time-resolved near-IR stimulated Raman measurements of functional π-conjugate systems

--Manuscript Draft--

Article Type:	Invited Methods Article - JoVE Produced Video
Manuscript Number:	JoVE60437R1
Full Title:	Ultrafast time-resolved near-IR stimulated Raman measurements of functional π-conjugate systems
Section/Category:	JoVE Chemistry
Keywords:	Laser Spectroscopy; Near-Infrared; Stimulated Raman Scattering; Femtosecond; Pump-Probe Technique; π-Conjugate; Carotenoid; Vibrational Energy Redistribution
Corresponding Author:	Tomohisa Takaya Gakushuin University Toshima-ku, Tokyo JAPAN
Corresponding Author's Institution:	Gakushuin University
Corresponding Author E-Mail:	tomohisa.takaya@gakushuin.ac.jp
Order of Authors:	Tomohisa Takaya
	Koichi Iwata
Additional Information:	
Question	**Response**
Please indicate whether this article will be Standard Access or Open Access.	Standard Access (US$2,400)
Please indicate the **city, state/province, and country** where this article will be **filmed**. Please do not use abbreviations.	Toshima-ku, Tokyo, Japan

GAKUSHUIN UNIVERSITY

TEL : (03)3986-0221
FAX : (03)5992-1005
CABLE ADDRESS : GAKUSHUDAI TOKYO

1-5-1 Mejiro, Toshima-ku
Tokyo 171-8588, Japan

23 August 2019

Journal of Visualized Experiments
Editorial Office

Dear Dr. Phillip Steindel,

This cover letter accompanies submission of our revised manuscript entitled “Techniques for ultrafast time-resolved near-IR stimulated Raman measurements of functional π-conjugate systems” by Tomohisa Takaya and Koichi Iwata.

We are grateful to you and the reviewers for the important comments and fruitful discussion on our manuscript. In the revised manuscript, we have spelled out the names of the materials in our spectrometer and the journal name in the references, deleted commercial language, and added all the materials in Table of Materials. We have added appropriate references so that readers can learn how the technique of femtosecond time-resolved stimulated Raman spectroscopy is developed. We have made major revisions in order to explain to researchers starting ultrafast spectroscopy how we find and maximize near-IR stimulated Raman signals.

We hope that the revised manuscript is acceptable for publication in Journal of Visualized Experiments.

Sincerely yours,

Tomohisa Takaya

Tomohisa Takaya, Ph. D.
Assistant Professor
Department of Chemistry, Faculty of Science
Gakushuin University
1-5-1 Mejiro, Toshima-ku, Tokyo 171-8588, Japan.
E-mail: tomohisa.takaya@gakushuin.ac.jp
Telephone: +81 3 3986 0221
FAX: +81 3 5992 1029

TITLE:

Ultrafast Time-resolved Near-IR Stimulated Raman Measurements of Functional π-conjugate Systems

AUTHORS AND AFFILIATIONS:

Takaya, Tomohisa[1*], Iwata, Koichi[1*]

[1]Department of Chemistry, Faculty of Science, Gakushuin University, Tokyo, Japan

*The authors contributed equally to this work.

Corresponding Authors:

Takaya, Tomohisa (tomohisa.takaya@gakushuin.ac.jp)
Iwata, Koichi (koichi.iwata@gakushuin.ac.jp)

KEYWORDS:

laser spectroscopy, near-infrared, stimulated Raman scattering, femtosecond, pump–probe technique, π-conjugate, carotenoid, vibrational energy redistribution

SUMMARY:

Details of signal generation and optimization, measurement, data acquisition, and data handling for a femtosecond time-resolved near-IR stimulated Raman spectrometer are described. A near infrared stimulated Raman study on the excited-state dynamics of β-carotene in toluene is shown as a representative application.

ABSTRACT:

Femtosecond time-resolved stimulated Raman spectroscopy is a promising method of observing the structural dynamics of short-lived transients with near infrared (near-IR) transitions, because it can overcome the low sensitivity of spontaneous Raman spectrometers in the near-IR region. Here, we describe technical details of a femtosecond time-resolved near-IR multiplex stimulated Raman spectrometer that we have recently developed. A description of signal generation and optimization, measurement, data acquisition, and calibration and correction of recorded data is provided as well. We present an application of our spectrometer to analyze the excited-state dynamics of β-carotene in toluene solution. A C=C stretch band of β-carotene in the second lowest excited singlet (S_2) state and the lowest excited singlet (S_1) state is clearly observed in the recorded time-resolved stimulated Raman spectra. The femtosecond time-resolved near-IR stimulated Raman spectrometer is applicable to the structural dynamics of π-conjugate systems from simple molecules to complex materials.

INTRODUCTION:

Raman spectroscopy is a powerful and versatile tool for investigating the structures of molecules in a wide variety of samples from simple gases, liquids, and solids to functional materials and biological systems. Raman scattering is significantly enhanced when the photon energy of the excitation light coincides with the electronic transition energy of a molecule. The resonance

Raman effect enables us to selectively observe the Raman spectrum of a species in a sample composed of many kinds of molecules. Near-IR electronic transitions are drawing a lot of attention as a probe for investigating the excited-state dynamics of molecules with large π-conjugated structures. The energy and lifetime of the lowest excited singlet state have been determined for several carotenoids, which have a long one-dimensional polyene chain[1–3]. The dynamics of neutral and charged excitations have been extensively investigated for various photoconductive polymers in films[4–7], nanoparticles[8], and solutions[9–11]. Detailed information on the structures of the transients will be obtainable if time-resolved near-IR Raman spectroscopy is applied to these systems. Only a few studies, however, have been reported on time-resolved near-IR Raman spectroscopy[12–16], because the sensitivity of near-IR Raman spectrometers is extremely low. The low sensitivity principally originates from the low probability of near-IR Raman scattering. The probability of spontaneous Raman scattering is proportional to $\omega_i\omega_s^3$, where ω_i and ω_s are the frequencies of the excitation light and the Raman scattering light, respectively. In addition, commercially available near-IR detectors have much lower sensitivity than CCD detectors functioning in the UV and visible regions.

Femtosecond time-resolved stimulated Raman spectroscopy has emerged as a new method of observing time-dependent changes of Raman active vibrational bands beyond the apparent Fourier-transform limit of a laser pulse[17–33]. Stimulated Raman scattering is generated by irradiation of two laser pulses: the Raman pump and probe pulses. Here it is assumed that the Raman pump pulse has a larger frequency than the probe pulse. When the difference between the frequencies of the Raman pump and probe pulses coincides with the frequency of a Raman active molecular vibration, the vibration is coherently excited for a large number of molecules in the irradiated volume. Nonlinear polarization induced by the coherent molecular vibration enhances the electric field of the probe pulse. This technique is particularly powerful for near-IR Raman spectroscopy, because stimulated Raman scattering can solve the problems of the sensitivity of time-resolved near-IR spontaneous Raman spectrometers. Stimulated Raman scattering is detected as intensity changes of the probe pulse. Even if a near-IR detector has a low sensitivity, stimulated Raman scattering will be detected when the probe intensity is sufficiently increased. The probability of stimulated Raman scattering is proportional to $\omega_{RP}\omega_{SRS}$, where ω_{RP} and ω_{SRS} are the frequencies of the Raman pump pulse and stimulated Raman scattering, respectively[20]. The frequencies for stimulated Raman scattering, ω_{RP} and ω_{SRS}, are equivalent to ω_i and ω_s for spontaneous Raman scattering, respectively. We have recently developed a femtosecond time-resolved near-IR Raman spectrometer using stimulated Raman scattering for investigating the structures and dynamics of short-lived transients photogenerated in π-conjugate systems[2,3,7,10]. In this article, we present the technical details of our femtosecond time-resolved near-IR multiplex stimulated Raman spectrometer. Optical alignment, acquisition of time-resolved stimulated Raman spectra, and calibration and correction of recorded spectra are described. The excited-state dynamics of β-carotene in toluene solution is studied as a representative application of the spectrometer.

PROTOCOL:

1. Startup of electric devices

1.1. Turn on the femtosecond Ti:sapphire laser system according to its operation manual. Wait 2 h for the laser system to warm up.

1.2. Turn on the power switches of the optical chopper, translational stage controllers, spectrograph, InGaAs array detector, and computer while the system is warming up. Fill the detector's Dewar with liquid nitrogen.

2. Optical alignment of spectrometer

2.1. Mirror adjustment (Figure 1B)

2.1.1. Check the position of the support on the mirror mount.

2.1.2. Turn the upper knob of the mount clockwise and counterclockwise to let the reflected laser beam travel down and up in the vertical direction, respectively, if the support is located at the lower part of the mount. Turn the knob in the opposite direction if the support is located at the upper part of the mount.

2.1.3. Turn the knob on the left side of the mount clockwise and counterclockwise to let the reflected laser beam travel right and left in the horizontal direction, respectively, if the support is located on the right side of the mount. Turn the knob in the opposite direction if the support is located at the left side of the mount.

2.2. Lens alignment

2.2.1. Place a business card with a grid behind the lens as a screen.

2.2.2. Remove the lens. Introduce the incident beam and let it hit the screen. Mark the position of the beam spot on the screen with a pen.

2.2.3. Block the beam and place the lens. Introduce the beam and confirm that it hits the mark on the screen exactly. If it does not, adjust the vertical and horizontal positions of the lens.

2.2.4. Prepare a business card with a hole. Let the incident beam pass through the hole in front of the lens and confirm that the specular reflection of the beam by the lens travels in the direction exactly opposite to the incident beam. If it does not, adjust the angle of the lens.

2.3. Laser beam alignment (Figure 1C)

2.3.1. Place a business card behind iris 2 (i2) as a screen.

2.3.2. Let the beam pass through the center of i1 by adjusting mirror 1 (m1) according to section 2.1. Let the beam pass through the center of i2 by adjusting m2 according to section 2.1.

2.3.3. Confirm that the beam passes through the centers of i1 and i2 simultaneously. If the beam does not pass through the center of i1, repeat step 2.3.2 until the beam passes through the centers of both irises.

2.4. **Optical delay line alignment (Figure 1D)**

2.4.1. Remove m3 and m4 on the optical delay line (ODL). Place i1 at the position of m3 at the height of the center of m3.

2.4.2. Move the stage toward m2 as far as it can by placing the direction button of the stage controller. Let the beam pass through the center of i1 by adjusting m1 according to section 2.1.

2.4.3. Move the stage apart from m2 as far as it can by placing the direction button of the stage controller. Let the beam pass through the center of i1 by adjusting m2 according to section 2.1.

2.4.4. Move the stage toward the beam input as far as it can and confirm that the beam passes through the center of i1. If the beam does not pass through the center of i1 after step 2.4.3, repeat steps 2.4.2–2.4.3 until the beam passes through the center of i1 at both ends of the stage.

2.4.5. Remove i1 from the position of m3. Place m3 and m4 on ODL. Let the beam pass through the center of i2 by adjusting m3 and m4 according to steps 2.4.2–2.4.4.

2.4.6. Once steps 2.4.1–2.4.5 are finished, let the beam pass through the center of i2 by adjusting m1 and m2 according to steps 2.4.2–2.4.5.

2.5. **White light continuum generation (Figure 1A)**

2.5.1. Place the variable neutral density filter (VND) VND1 in the incident beam path. Place a business card ~200 mm apart from VND1 as a screen.

2.5.2. Turn VND1 until the incident beam hits the highest optical density position of VND1, where the transmitted beam has the lowest power.

2.5.3. Place the lens (L) L1 (focal length = 100 mm) behind VND1. Place the 3 mm thick sapphire plate (SP) ~105 mm apart from L1, where SP is located slightly behind the focus of the beam, letting the beam pass through SP near the edge.

2.5.4. Set the diameter of I6 to be ~5 mm.

2.5.5. Turn VND1 to gradually increase the power of the transmitted beam until a yellow-white spot is observed on the screen. Turn VND1 further in the same direction very carefully until a purple ring surrounds the yellow-white spot on the screen.

2.6. **Probe beam alignment (Figure 1A)**

2.6.1. Adjust the two pairs of mirrors (M) (M4, M5) and (M7, M8) according to section 2.3. Adjust ODL2 according to section 2.4. Adjust M12 and M13 according to section 2.3.

2.6.2. Generate a white light continuum according to section 2.5.

2.6.3. Remove the color glass filters (F) F1 and F2 and the polarizer (P) P1.

2.6.4. Reflect the white light continuum with the concave mirror (CM). Let the reflected beam pass just beside SP.

2.6.5. Let the beam hit the center of M15 and M16 by adjusting M14 and M15, respectively, according to section 2.1. Remove L2, L3, and L4. Let the beam hit the center of the entrance slit of the spectrograph by adjusting M16.

2.6.6. Measure the diameter of the white light continuum beam at CM and the entrance slit using grid paper. If the diameters are significantly altered between the two positions, adjust the position of CM parallel with the beam using a micrometer on the base plate of CM until the diameters become almost identical. Conduct steps 2.6.4–2.6.5 after the adjustment.

2.6.7. Place L2, L3, and L4 according to section 2.2 and then place F1, F2, and P1.

2.7. **Raman pump beam alignment (Figure 1A)**

2.7.1. Place the volume-grating reflective bandpass filter (BPF) in the output beam path of the optical parametric amplifier (OPA) OPA1. Adjust BPF and M17 according to section 2.3. Use a near-IR phosphor card for observing the beam spot.

2.7.2. Set the angle of the half-wave plate (HWP) HWP2 at 45° in order to set the Raman pump polarization to vertical. Remove L5, L6, and L7.

2.7.3. Let the beam hit the center of M19, M20, and M21 by adjusting M18, M19, and M20, respectively, according to section 2.1. Use a near-IR phosphor card to observe the beam spot.

2.7.4. Place L5, L6, and L7 according to section 2.2 using a near-IR phosphor card as a screen.

2.8. **Actinic pump beam alignment (Figure 1A)**

2.8.1. Remove L8 and L9. Let the output beam from OPA2 pass through the center of the iris (I) I12 by adjusting M22 according to section 2.1.

2.8.2. Adjust M24 and M25 according to section 2.3. Place L8 and L9 according to section 2.2. Adjust ODL1 according to section 2.4.

2.8.3. Measure the diameter of the actinic pump beam at M24 and M32 using grid paper. If the diameters are significantly different between the two positions, adjust the position of L9 parallel with the beam using a micrometer on the base plate of L9 until the diameters become almost identical.

2.8.4. Remove L10 and M32. Adjust M30 and M31 according to section 2.3.

2.8.5. Place P2 at the position of M32. Place a business card behind P2 as a screen.

2.8.6. Set P2 at the angle that allows the pulse to be polarized at 35.3° with respect to the vertical axis to pass through P2. Rotate HWP3 until the beam spot on the screen fully disappears. Conduct this protocol for eliminating the effect of molecular reorientation on time-resolved measurements.

2.8.7. Remove P2. Place M32 and reflect the beam toward the flow cell (FC). Place L10 according to section 2.2.

2.9. **Flow cell startup (Figure 1E)**

2.9.1. Attach a 2 mm quartz flow cell to the mount. Connect each end of the flow cell to a polyfluoroacetate (PFA) tube (length = ~500 mm; outer diameter = 1/8 inch) with an elastomer tube (length = ~10 mm).

2.9.2. Insert the tube from the bottom of the flow cell to a reservoir filled with a sample solution. Attach the tube from the top of the flow cell to the inlet of the magnet gear pump.

2.9.3. Attach a PFA tube (length = ~500 mm; outer diameter = 1/8 inch) to the outlet of the magnet gear pump and insert the other end to the reservoir.

2.9.4. Place the flow cell mount at the focus of the probe beam.

2.9.5. Turn on the magnetic gear pump. Adjust the flow rate to ~20 mL/min using the voltage control of the pump in order to replace the sample in the illuminated volume before every actinic pump pulse reaches FC.

3. Software operation

3.1. **Detector setup**

3.1.1. Open the **Detector** pane. Click the **Initialize** button. Wait until the **Detector Initialized** indicator is lit.

3.1.2. Enter 40 in the **Exposure time (ms)** box.

3.1.3. Select **IGA Lo Gain** and **IGA 280 kHz** from the **A/D gain** and **A/D rate** drop-down menus, respectively. IGA and A/D stand for InGaAs and the analog-to-digital converter, respectively.

3.1.4. Click the **Set** button below the **Detector Set Up** indicator. Confirm that the indicator light is on.

3.1.5. Set the **Trigger** switch to **External** from the **Trigger Event** drop-down menu. Select **Each – For Each Acq** and **TTL Rising Edge** from the **Trigger Edge** drop-down menu. TTL stands for transistor-transistor logic.

3.1.6. Click the **Set** button below the **Trigger Se** indicator. Confirm that the indicator light is on.

3.1.7. Click the **Read** button at the bottom of the pane. Confirm that the **Detector Temperature (K)** box displays a value below 170 K. If not, wait until the temperature decreases below 170 K.

3.2. **Spectrograph setup**

3.2.1. Open the **Spectrograph** pane. Click the **Initialize** button. Wait until the **Spectrograph Initialized** indicator light is on.

3.2.2. Select **1. Grooves 300 g/mm, Blaze wavelength 2000 nm** from the **Grating** drop-down menu. Click the **Set** button on the right-hand side of the **Grating** drop-down menu.

3.2.3. Enter the center wavelength of the spectrograph in the **Move To** box and click the **Go** button. The center wavelength is typically located between 1,380 and 1,430 nm when the spectrograph covers the fingerprint region of the stimulated Raman spectrum.

3.2.4. Enter an entrance slit width in the **Set Entrance** box and click the **Set** button on the right-hand side of the box. The entrance slit width is typically set at 0.3 mm.

3.3. **Stage position control**

3.3.1. Open the **Preview** pane. Enter a value of the ODL1 position in micrometers in the **SK Stage Position (µm)** box. The box accepts values from 0 to 200,000 (µm). Click the **Go** button on the right-hand side of the box.

3.3.2. Enter a value of the ODL2 position in 0.1 µm in the **FA stage position (1/10 µm)** box. The box accepts values from -250,000 to 250,000 (x 1/10 µm). Click the **Go** button on the right-hand side of the box.

3.4. **Single measurement**

3.4.1. Enter the number of accumulations for a single measurement of a spectrum in the

Accumulation box. The box accepts values from 1 to 999.

3.4.2. Close the entrance of the spectrograph by pushing the diaphragm bar to the right as far as it can move. Click the **Store Dark** button. Open the entrance of the spectrograph by pulling the diaphragm bar to the left as far as it can move.

3.4.3. Check the **Average** box to preview only an averaged result.

3.4.4. Select **Acquire Light Spectrum** and **Check Transient Absorption** from the **Operation Mode** drop-down list for measuring probe intensities and measuring stimulated Raman or transient absorption spectra, respectively.

3.4.5. Click the **Acquire** button.

3.4.6. To automatically repeat measurements, check the **Continuous** box and click the **Acquire** button. Uncheck the **Continuous** box to stop the continuous measurements.

3.4.7. Open the file dialog by clicking the folder icon. Double-click a folder for saving data. Enter a file name with the extension ".txt" and click **OK**. Click the **Save** button.

3.5. **Time-resolved measurement**

3.5.1. Open the **Experiment** pane. Enter a name within 20 characters that briefly describes an experiment (e.g., names of samples, conditions) in the **Experiment Name** box.

3.5.2. Open the file dialog by clicking the folder icon. Double-click a folder for saving data and click **OK**.

3.5.3. Enter the number of translational stage scans in the **Number of Scans** box.

3.5.4. Select the translational stage scanned in the experiment in the **Stage for Scan** drop-down menu.

3.5.5. Enter a stage position where the scan starts in the **From** box of **Range A**. The unit and range of the acceptable values depend on the stage (see section 3.3).

3.5.6. Enter an interval between two successive stage positions in the **Step** box of **Range A**. The interval of 1 µm in the stage position corresponds to the interval of 6.7 fs in the time delay between the actinic (or Raman) pump and probe pulses.

3.5.7. Enter the number of stage positions in a scan in the **Points** box of **Range A**.

3.5.8. If more than one interval is required in a single scan, check the **Range B** box and repeat steps 3.5.5–3.5.7 for **Range B**. Three intervals can be set using **Range A**, **B**, and **C**.

3.5.9. Start the scans by clicking the **Run** button. The **Experiment Running** indicator light will turn on. Wait until the indicator light turns off.

4. Optimization of probe spectrum

4.1. Place beam dumps in the paths of the actinic and Raman pump beams. Set P1 at the angle that allows the vertically polarized pulse to pass through P1.

4.2. Set the number of accumulations to be 10 according to step 3.4.1. Store the dark signal according to steps 3.4.2. Select **Acquire Light Spectrum** according to step 3.4.4.

4.3. Run continuous measurements according to step 3.4.6 for previewing data. Maximize detector counts on the display by gradually rotating HWP1.

4.4. Gradually increase the intensity of the incident pulse by rotating VND1 until the maximal and minimal detector counts reach around 30,000 and 4,000, respectively. If a large oscillatory pattern starts to be observed, rotate VND1 in the opposite direction until the pattern disappears.

4.5. Stop the continuous measurements according to step 3.4.6.

5. Measurement of stationary stimulated Raman spectra

5.1. Spatial overlap of Raman pump and probe pulses

5.1.1. Remove the beam dump in the Raman pump beam path. Place the optical chopper (OC) in the Raman pump beam path.

5.1.2. Place a near-IR phosphor card at the sample position. Adjust the direction of the Raman pump beam by adjusting M21 according to section 2.1 until the spots of the Raman pump and probe beams fully overlap with each other. Remove the phosphor card.

5.2. Temporal overlap of Raman pump and probe pulses

5.2.1. Place an InGaAs PIN photodiode at the sample position where the Raman pump and probe beams spatially overlap with each other as a result of section 5.1.

5.2.2. Connect the signal output of the photodiode to a 500 MHz, 5 GS/s digital oscilloscope in order to monitor when the Raman pump and probe pulses arrive at the sample position.

5.2.3. Set the horizontal scale of the oscilloscope to be 1 ns/div.

5.2.4. Read the peak time of the signal intensity for the Raman pump and probe pulses blocking the other pulse.

5.2.5. If a difference in the peak time is observed for the two pulses, adjust the position of ODL2 according to section 3.3 until the difference becomes smaller than 200 ps.

5.3. **Adjustment of optical chopper rotational phase**

5.3.1. Add 40 mL of cyclohexane to the reservoir. Start flowing cyclohexane according to step 2.9.5.

5.3.2. Set the center wavelength of the spectrograph to be 1,190 nm according to step 3.2.3 to observe Rayleigh scattering of the Raman pump pulse.

5.3.3. Set the number of accumulations to 10 according to step 3.4.1. Store the dark signal according to step 3.4.2.

5.3.4. Select **Check Transient Absorption** according to step 3.4.4.

5.3.5. Run continuous measurements according to step 3.4.6.

5.3.6. Maximize the amplitude of the apparent transient absorption signal with the negative sign at the Raman pump wavelength, which originates from the presence and absence of the scattered Raman pump pulse due to chopping, by adjusting the rotational phase of OC from -180°−170° on the front panel of the controller.

5.3.7. Stop the continuous measurements according to step 3.4.6.

5.4. **Signal maximization**

5.4.1. Set the center wavelength of the spectrograph to be 1,410 nm according to step 3.2.3 for observing stimulated Raman spectra.

5.4.2. Run continuous measurements according to step 3.4.6 and check if stimulated Raman bands of cyclohexane are observed in the display. The strongest band of cyclohexane appears at the 55th–58th pixels when the center wavelength is set at 1,410 nm.

5.4.3. If the stimulated Raman bands are not observed, try to change the position of ODL2 by ±15,000 µm at 150 µm intervals according to section 3.3 and see if the stimulated Raman bands are observed.

5.4.4. If the stimulated Raman bands are not observed after step 5.4.3 is conducted, retry step 5.1.2 to obtain the spatial overlap between the Raman pump and probe beams and conduct step 5.4.2 again.

5.4.5. Once the stimulated Raman bands are detected, maximize the band intensities in the display by iteratively readjusting M21, the rotational phase of OC, and the position of ODL2.

5.4.6. Stop the continuous measurements according to step 3.4.6.

5.5. **Measurement**

5.5.1. Set the number of accumulations to be 500 according to step 3.4.1. Store the dark signal according to step 3.4.2.

5.5.2. Run a single measurement according to step 3.4.5. Save the spectrum according to step 3.4.7. Repeat the measurement at least 4x.

5.5.3. Remove the FC inlet tube from the reservoir and wait until the flow is interrupted by the air. Minimize the voltage of the magnetic gear pump.

5.5.4. Replace the content of the reservoir with the one filled with 100 mL of fresh acetone.

5.5.5. Set the inlet and outlet tubes into the reservoir and empty flask, respectively. Start the magnetic gear pump according to step 2.9.5 and let toluene flow through the FC.

5.5.6. Wait until the flow is interrupted by the air. Minimize the voltage of the magnetic gear pump.

5.5.7. Repeat steps 5.5.4–5.5.6 at least 2x.

5.5.8. Add 40 mL of acetone to the reservoir. Start flowing acetone according to step 2.9.5.

5.5.9. Record the stimulated Raman spectrum of acetone according to step 5.5.2.

5.5.10. Remove acetone from the FC according to step 5.5.3.

5.5.11. Repeat steps 5.5.4–5.5.10 using toluene instead of acetone.

6. **Measurement of time-resolved absorption spectra**

6.1. Empty the reservoir and add 25 mL of toluene solution of β-carotene with a concentration of 1 x 10^{-4} mol dm^{-3}. Start flowing the sample solution according to step 2.9.5.

6.2. Place the OC in the actinic pump beam path.

6.3. Move the beam dump from the path of the actinic pump beam to that of the Raman pump beam.

6.4. Spatially overlap the actinic pump and probe beams at the sample position according to step

5.1.2 using a business card instead of the near-IR phosphor card.

6.5. Temporally overlap the two beams at the sample position according to section 5.2 using a Si PIN photodiode instead of the InGaAs PIN photodiode.

6.6. Set the number of accumulations to be 10 according to step 3.4.1. Store the dark signal according to step 3.4.2.

6.7. Select **Check Transient Absorption** according to step 3.4.4.

6.8. Run continuous measurements according to step 3.4.6 and check if the transient absorption of β-carotene is observed in the display. The absorption band appears with a shape decreasing monotonically towards longer wavelengths (the second lowest excited singlet state, S_2) or with three peaks at around the 35th, 220th, and 485th pixels (the lowest excited singlet state, S_1).

6.9. If the transient absorption is not observed, try to change the position of ODL1 by ±15,000 µm at 150 µm intervals according to section 3.3.

6.10. If no absorption band is observed after step 6.9 is conducted, retry step 6.4 to obtain the spatial overlap between the actinic pump and probe beams.

6.11. Maximize the absorption intensity by readjusting M32 once the transient absorption band is detected.

6.12. Stop the continuous measurements according to step 3.4.6.

6.13. Decrease the position of ODL1 according to section 3.3 until the transient absorption fully disappears.

7. Measurement of time-resolved stimulated Raman spectra

7.1. Place the OC in the Raman pump beam path. Remove the beam dump from the Raman pump beam path.

7.2. Set the number of accumulations to 200 according to step 3.4.1. Store the dark signal according to step 3.4.2.

7.3. Run a time-resolved experiment according to section 3.5. In step 3.5.4, select **SK stage**. Set the **Start** value of **Range A** to be smaller by around 50 µm than the position where the transient absorption signal disappeared in step 6.13.

8. Raman shift calibration

8.1. Calculate the average of the four stimulated Raman spectra for cyclohexane, acetone, and toluene recorded in section 5 using data analysis software of your choice.

8.2. Plot the averaged stimulated Raman spectra of the solvents against the pixel number of the InGaAs array detector.

8.3. Estimate the peak positions of the stimulated Raman bands of the solvents by least-squares fitting analysis with the Lorentz function. If the Lorentz function is not available, use polynomial functions instead.

8.4. Plot the peak wavenumbers of the Raman bands of the solvents in a reference book (e.g., Hamaguchi and Iwata[34]) against the estimated peak positions in the pixel number.

8.5. Obtain a calibration function between the Raman shift and the pixel number by the least-squares fitting analysis with a second- or third-degree polynomial function.

REPRESENTATIVE RESULTS:

Femtosecond time-resolved near-IR stimulated Raman spectroscopy was applied to β-carotene in toluene solution. The concentration of the sample was 1 x 10^{-4} mol dm^{-3}. The sample was photoexcited by the actinic pump pulse at 480 nm with a pulse energy of 1 μJ. Time-resolved stimulated Raman spectra of β-carotene in toluene are shown in **Figure 2A**. The raw spectra contained strong Raman bands of the solvent toluene and a weak Raman band of β-carotene in the ground state as well as Raman bands of photoexcited β-carotene. They were subtracted using the stimulated Raman spectrum of the same solution at 1 ps before photoexcitation. The spectra after the subtraction (**Figure 2B**) showed distorted baselines that are caused by absorption of photoexcited β-carotene and/or other nonlinear optical processes. The baselines became flat after they were corrected with polynomial functions (**Figure 2C**).

The time-resolved stimulated Raman spectra of β-carotene showed two strong bands in the 1,400–1,800 cm^{-1} region (**Figure 2C**). A broad stimulated Raman band at 0 ps was assigned to the in-phase C=C stretch vibration of S_2 β-carotene. Its peak position was estimated to be 1,556 cm^{-1}. The in-phase C=C stretch band of S_1 β-carotene appeared as the S_2 C=C stretch band decays. The peak position of the S_1 C=C stretch band was upshifted by 8 cm^{-1} from 0.12 to 5 ps (**Figure 2D**). The time constant of the upshift was estimated to be 0.9 ps. The upshift originates from vibrational energy redistribution in S_1 β-carotene[2,3].

Figure Legends:

Figure 1: Instrument diagrams. (**A**) The block diagram of a femtosecond time-resolved near-IR stimulated Raman spectrometer. Ti:S = Modelocked Ti:sapphire laser system; BS = Beamsplitter; OPA = Optical parametric amplifier; BBO = β-Barium borate crystal; OC = Optical chopper; ODL = Optical delay line; BPF = Volume-grating reflective bandpass filter; SP = Sapphire plate; FC = Flow cell; M = Mirror; CM = Concave mirror; L = Lens; I = Iris; P = Polarizer; HWP = Half-wave plate; F = Color glass filter; VND = Variable optical density filter. The figure is adapted from Takaya[11] with permission from the PCCP Owner Societies. (**B**) Four configurations of a mirror mount. V, H, and S represent the vertical adjustment knob, horizontal adjustment knob, and support, respectively.

See section 2.1 for details. (**C**) A schematic diagram of laser beam alignment. m = Mirror; i = Iris. See section 2.3 for details. (**D**) A schematic diagram of optical delay line alignment. m = Mirror; i = Iris. See section 2.4 for details. (**E**) Structure of a flow cell mount. See section 2.9 for details.

Figure 2: Femtosecond time-resolved near-IR stimulated Raman spectra. (**A**) Femtosecond time-resolved near-IR stimulated Raman spectra of β-carotene in toluene with the actinic pump wavelength at 480 nm. Raman bands of toluene and β-carotene in the ground state are denoted with circles and a triangle, respectively. (**B**) Femtosecond time-resolved near-IR stimulated Raman spectra of β-carotene in toluene after the Raman bands of toluene and β-carotene in the ground state are subtracted. The baselines of the spectra were fitted with polynomial functions (broken traces). (**C**) Femtosecond time-resolved near-IR stimulated Raman spectra of β-carotene in toluene after the baseline correction. (**D**) The peak positions of the in-phase C=C stretch band in the S_1 state plotted against the time delay. The C=C stretch bands were fitted with a Gaussian function for estimating their peak positions. The best fitted curve for the shift of the S_1 C=C stretch band (a solid trace) was obtained by the least-squares fitting analysis with an exponential function.

DISCUSSION:

Crucial factors in femtosecond time-resolved near-IR multiplex stimulated Raman measurement

To obtain time-resolved near-IR stimulated Raman spectra with a high signal-to-noise ratio, the probe spectrum should ideally have uniform intensity in the whole wavelength range. White-light continuum generation (section 2.5) is, therefore, one of the most crucial parts of time-resolved near-IR stimulated Raman experiments. In general, the probe spectrum becomes broad and flat as the intensity of the incident beam increases. A high beam intensity, however, easily produces undesirable nonlinear optical effects other than white light continuum generation. In a worst-case scenario, the nonlinear effects provide the probe spectrum with a large intensity fluctuation and an oscillatory pattern that significantly lowers the signal-to-noise ratio of stimulated Raman spectra. **Figure 2C** shows how the oscillatory pattern affects the spectra. It shows oscillatory patterns from -0.30 to 4 ps, but the patterns appear only weakly, with a peak-to-peak amplitude of 1×10^{-4}, as white light generation is carefully optimized. Another undesirable effect on the probe spectrum can be provided by water vapor in the air[2,11]. The effect of water vapor might be avoided if part of the spectrometer, including the white light generation optics, sample, and spectrograph, is set in a chamber filled with dry nitrogen.

Accuracy of Raman shift calibration

As described in section 8, we calibrate the Raman shift axis by the least-squares fitting analysis of the peak positions of the solvent bands in Raman shift against those in the pixel number of the detector with a polynomial function. We think this protocol works well as long as the Raman pump wavelength cannot be determined with high accuracy. It is the case for our spectrometer because each pixel of our detector covers as large as 3.5 cm^{-1} at around the wavenumber of the Raman pump pulse. However, the solvents must be chosen so that all the transient stimulated Raman bands of the sample appear between the highest and lowest wavenumbers of the solvent bands (section 8). The Raman shift calibration curve loses its accuracy beyond the range of the solvent bands. In **Figure 2**, a Raman band of S_1 β-carotene in toluene, at 1,785 cm^{-1}, appears beyond the highest wavenumber of the solvent bands, 1,710 cm^{-1}. We have confirmed that the peak position agrees well with that in spectra of benzene determined by picosecond time-resolved spontaneous Raman spectroscopy[35].

Effectiveness and perspective of femtosecond time-resolved near-IR multiplex stimulated Raman spectrometer

It has been demonstrated that the femtosecond time-resolved near-IR multiplex stimulated Raman spectrometer can observe stimulated Raman spectra, which provides information almost equivalent to spontaneous Raman spectra of short-lived species with near-IR transitions. Small differences in the peak position of a band can be detected with the spectrometer because of its sufficiently high sensitivity. The spectrometer will be applicable to a wide variety of π-conjugate systems from simple aromatic molecules to photoconductive polymers. Stationary near-IR multiplex stimulated Raman spectroscopy is also a powerful tool for observing molecular vibrations without fluorescence interference from the sample, because the energy of near-IR photons is generally much lower than the electronic transition energy of molecules from the lowest excited singlet state to the ground state. The spectrometer will be applicable to in vivo observation of the structural dynamics in biological systems.

ACKNOWLEDGMENTS:

This work was supported by JSPS KAKENHI Grant Numbers JP24750023, JP24350012, MEXT KAKENHI Grant Numbers JP26104534, JP16H00850, JP26102541, JP16H00782, and MEXT-Supported Program for the Strategic Research Foundation at Private Universities, 2015–2019.

DISCLOSURES:

The authors have nothing to disclose.

REFERENCES:

1. Polívka, T., Herek, J. L., Zigmantas, D., Åkerlund, H.-E., Sundström, V. Direct Observation of the (Forbidden) S_1 State in Carotenoids. *Proceedings of the National Academy of Sciences of the United States of America*. **96** (9), 4914–4917 (1999).
2. Takaya, T., Iwata, K. Relaxation Mechanism of β-Carotene from S_2 ($1B_u^+$) State to S_1 ($2A_g^-$) State: Femtosecond Time-Resolved Near-IR Absorption and Stimulated Resonance Raman Studies in 900–1550 nm Region. *Journal of Physical Chemistry A*. **118** (23), 4071 – 4078 (2014).
3. Takaya, T., Anan, M., Iwata, K. Vibrational Relaxation Dynamics of β-Carotene and Its Derivatives with Substituents on Terminal Rings in Electronically Excited States as Studied by Femtosecond Time-Resolved Stimulated Raman Spectroscopy in the Near-IR Region. *Physical Chemistry Chemical Physics*. **20** (5), 3320–3327 (2017).
4. Guo, J., Ohkita, H., Benten, H., Ito, S. Near-IR Femtosecond Transient Absorption Spectroscopy of Ultrafast Polaron and Triplet Exciton Formation in Polythiophene Films with Different Regioregularities. *Journal of the American Chemical Society*. **131** (46) (2009).

5. Hwang, I.-W. et al. Carrier Generation and Transport in Bulk Heterojunction Films Processed with 1,8-Octanedithiol as a Processing Additive. *Journal of Applied Physics*. **104** (3), 033706 (2008).

6. Yonezawa, K., Kamioka, H., Yasuda, T., Han, L., Morimoto, Y. Fast Carrier Formation from Acceptor Exciton in Low-Gap Organic Photovoltaic. *Applied Physics Express*. **5** (4), 042302 (2012).

7. Takaya, T., Enokida, I., Furukawa, Y., Iwata, K. Direct Observation of Structure and Dynamics of Photogenerated Charge Carriers in Poly(3-hexylthiophene) Films by Femtosecond Time-Resolved Near-IR Inverse Raman Spectroscopy. *Molecules*. **24** (3), 431 (2019).

8. Clafton, S. N., Huang, D. M., Massey, W. R., Kee, T. W. Femtosecond Dynamics of Excitons and Hole-Polarons in Composite P3HT/PCBM Nanoparticles. *Journal of Physical Chemistry B*. **117** (16), 4626–4633 (2013).

9. Cook, S., Furube, A., Katoh, R. Analysis of the Excited States of Regioregular Polythiophene P3HT. *Energy & Environmental Science*. **1** (2), 294–299 (2008).

10. Okino, S., Takaya, T., Iwata, K. Femtosecond Time-Resolved Near-Infrared Spectroscopy of Oligothiophenes and Polythiophene: Energy Location and Effective Conjugation Length of Their Low-Lying Excited States. *Chemistry Letters*. **44** (8), 1059–1061 (2015).

11. Takaya, T., Iwata, K. Development of a Femtosecond Time-Resolved Near-IR Multiplex Stimulated Raman Spectrometer in Resonance with Transitions in the 900–1550 nm Region. *Analyst*. **141** (14), 4283–4292 (2016).

12. Jas, G. S., Wan, C., Johnson, C. K. Picosecond Time-Resolved Fourier Transform Raman Spectroscopy of 9,10-Diphenylanthracene in the Excited Singlet State. *Applied Spectroscopy*. **49** (5), 645–649 (1995).

13. Jas, G. S., Wan, C., Kuczera, K., Johnson, C. K. Picosecond Time-Resolved Fourier-Transform Raman Spectroscopy and Normal-Mode Analysis of the Ground State and Singlet Excited State of Anthracene. *Journal of Physical Chemistry*. **100** (29), 11857–11862 (1996).

14. Sakamoto, A., Okamoto, H., Tasumi, M. Observation of Picosecond Transient Raman Spectra by Asynchronous Fourier Transform Raman Spectroscopy. *Applied Spectroscopy*. **52** (1), 76–81 (1998).

15. Sakamoto, A., Matsuno, S., Tasumi, M. Construction of Picosecond Time-Resolved Raman Spectrometers with Near-Infrared Excitation. *Journal of Raman Spectroscopy*. **37** (1-3), 429–435 (2006).

16. Sakamoto, A., Matsuno, S., Tasumi, M. Picosecond Near-Infrared Excited Transient Raman Spectra of β-Carotene in the Excited S_2 State: Solvent Effects on the in-Phase C=C Stretching Band and Vibronic Coupling. *Journal of Molecular Structure*. **976** (1-3), 310–313 (2010).

17. Yoshizawa, M., Kurosawa, M. Femtosecond Time-Resolved Raman Spectroscopy Using Stimulated Raman Scattering. *Physical Review A*. **61** (1), 013808 (2000).

18. Yoshizawa, M., Kubo, M., Kurosawa, M. Ultrafast Photoisomerization in DCM Dye Observed by New Femtosecond Raman Spectroscopy. *Journal of Luminescence*. **87-89**, 739–741 (2000).

19. Yoshizawa, M., Aoki, H., Hashimoto, H. Vibrational Relaxation of the $2A_g^-$ Excited State in All-*Trans*-β-Carotene Obtained by Femtosecond Time-Resolved Raman Spectroscopy. *Physical Review B*. **63** (18), 180301 (2001).

20. McCamant, D. W., Kukura, P., Mathies, R. A. Femtosecond Broadband Stimulated Raman: A New Approach for High-Performance Vibrational Spectroscopy. *Applied Spectroscopy*. **57** (11), 1317–1323 (2003).

21. McCamant, D. W., Kukura, P., Yoon, S., Mathies, R. A. Femtosecond Broadband Stimulated Raman Spectroscopy: Apparatus and Methods. *Review of Scientific Instruments*. **75** (11), 4971–4980 (2004).

22. Kukura, P., McCamant, D. W., Mathies, R. A. Femtosecond Stimulated Raman Spectroscopy. *Annual Review of Physical Chemistry*. **58**, 461–488 (2007).

23. Laimgruber, S., Schachenmayr, H., Schmidt, B. Zinth, W., Gilch, P. A Femtosecond Stimulated Raman Spectrograph for the Near Ultraviolet. *Applied Physics B*. **85** (4), 557–564 (2006).

24. Umapathy, S., Lakshmanna, A., Mallick, B. Ultrafast Raman Loss Spectroscopy. *Journal of Raman Spectroscopy*. **40** (3), 235–237 (2009).

25. Mallick, B., Lakshmanna, A., Umapathy, S. Ultrafast Raman Loss Spectroscopy (URLS): Instrumentation and Principle. *Journal of Raman Spectroscopy*. **42** (10), 1883–1890 (2011).

26. Kloz, M., van Grondelle, R., Kennis, J. T. M. Wavelength-Modulated Femtosecond Stimulated Raman Spectroscopy–Approach towards Automatic Data Processing. *Physical Chemistry Chemical Physics*. **13** (40), 18123–18133 (2011).

27. Kloz, M., Weißenborn, J., Polívka, T., Frank, H. A., Kennis, J. T. M. Spectral Watermarking in Femtosecond Stimulated Raman Spectroscopy: Resolving the Nature of the Carotenoid S* State. *Physical Chemistry Chemical Physics*. **18** (21), 14619–14628 (2016).

28. Kuramochi, H., Takeuchi, S., Tahara, T. Ultrafast Structural Evolution of Photoactive Yellow Protein Chromophore Revealed by Ultraviolet Resonance Femtosecond Stimulated Raman Spectroscopy. *Journal of Physical Chemistry Letters*. **3** (15), 2025–2029 (2012).

29. Wang, S. et al. Dynamic High Pressure Induced Strong and Weak Hydrogen Bonds Enhanced by Pre-Resonance Stimulated Raman Scattering in Liquid Water. *Optics Express*, **25** (25), 31670–31677 (2017).

30. Ashner, M. N., Tisdale, W. A. High Repetition-Rate Femtosecond Stimulated Raman Spectroscopy with Fast Acquisition. *Optics Express*, **26** (14), 18331–18340 (2018).

31. Quincy, T. J., Barclay, M. S., Caricato, M., Elles, C. G. Probing Dynamics in Higher-Lying Electronic States with Resonance-Enhanced Femtosecond Stimulated Raman Spectroscopy. *Journal of Physical Chemistry A*. **122** (42), 8308–8319 (2018).

32. Taylor, M. A. et al. Delayed Vibrational Modulation of the Solvated GFP Chromophore into a Conical Intersection. *Physical Chemistry Chemical Physics*. **21** (19), 9728–9739 (2019).

33. Cassabaum, A. A., Silva, W. R., Rich, C. C., Frontiera, R. R. Orientation and Polarization Dependence of Ground- and Excited-State FSRS in Crystalline Betaine-30. *Journal of Physical Chemistry C*. **123** (20), 12563–12572 (2019).

34. Hamaguchi, H., Iwata, K. eds. *Raman Spectroscopy (The Spectroscopical Society of Japan, Spectroscopy Series 1)*. Kodansha, Tokyo, Japan (2015).

35. Noguchi, T., Hayashi, H., Tasumi, M., Atkinson, G. H. Solvent Effects on the a_g C=C Stretching Mode in the $2^1A_g^-$ Excited State of β-Carotene and Two Derivatives: Picosecond Time-Resolved Resonance Raman Spectroscopy. *Journal of Physical Chemistry*. **95** (8), 3167–3172 (1991).

Figure 1

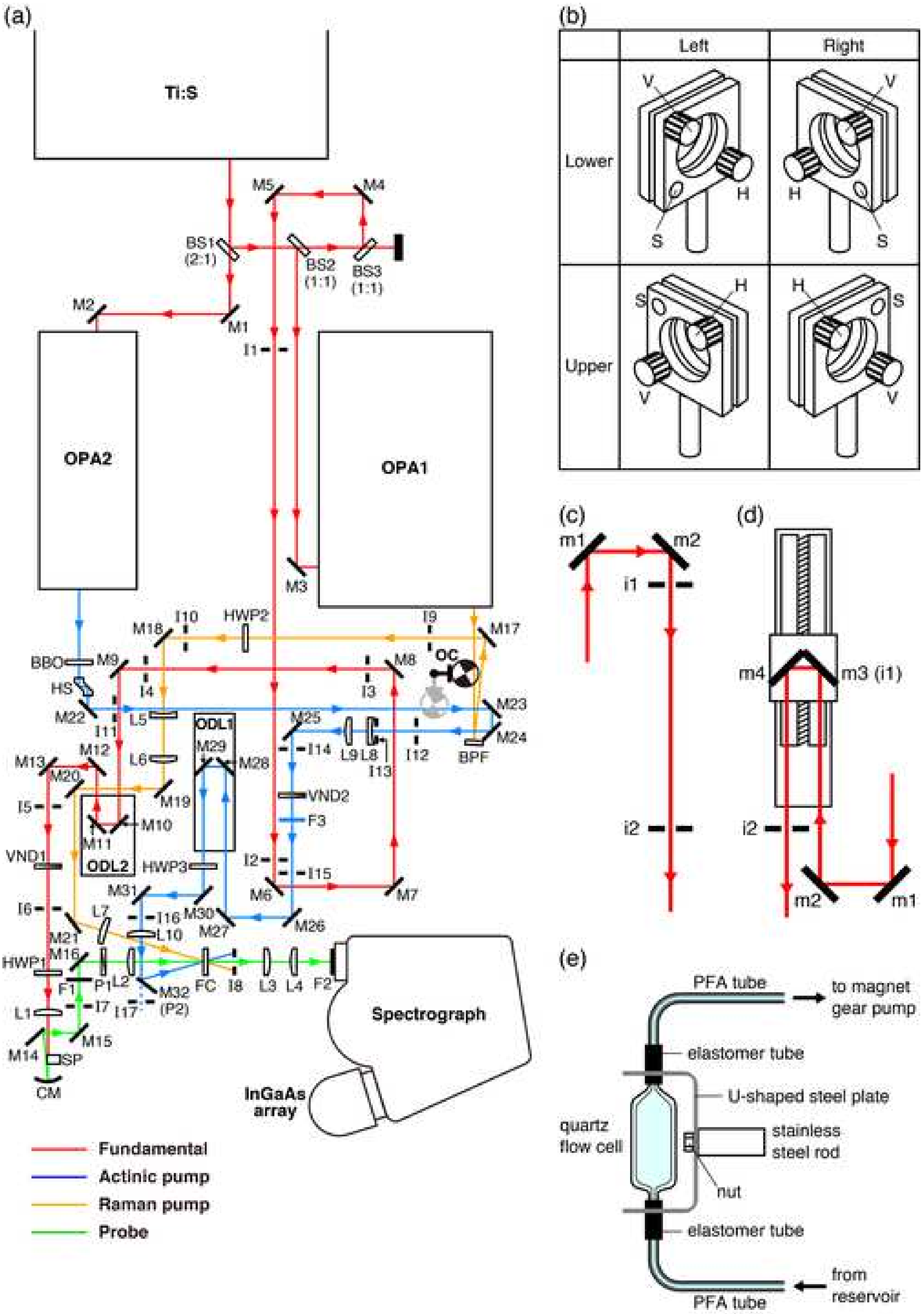

(a)
Ti:S
M5
M4
BS1 (2:1)
BS2 (1:1)
BS3 (1:1)
M2
M1
I1
OPA2
OPA1
M3
M18
I10
HWP2
I9
M17
BBO
M9
OC
M8
HS
I4
I3
M22
I11
L5
M23
ODL1
M25
M24
M13
M12
M29
M28
I14
L9
L8
I12
BPF
M20
L6
I13
I5
M19
VND2
M10
F3
M11
VND1
ODL2
HWP3
I2
I15
M31
M6
M7
I6
L7
I16
M30
M26
M21
L10
M27
M16
HWP1
P1
L2
FC
I8
L3
L4
F2
F1
M32 (P2)
L1
I7
I17
Spectrograph
M15
M14
SP
CM
InGaAs array
Fundamental
Actinic pump
Raman pump
Probe
(b)
Left
Right
Lower
Upper
V
H
S
(c)
m1
m2
i1
i2
(d)
m4
m3 (i1)
i2
m2
m1
(e)
PFA tube
to magnet gear pump
elastomer tube
U-shaped steel plate
quartz flow cell
stainless steel rod
nut
elastomer tube
from reservoir
PFA tube

FIgure 2

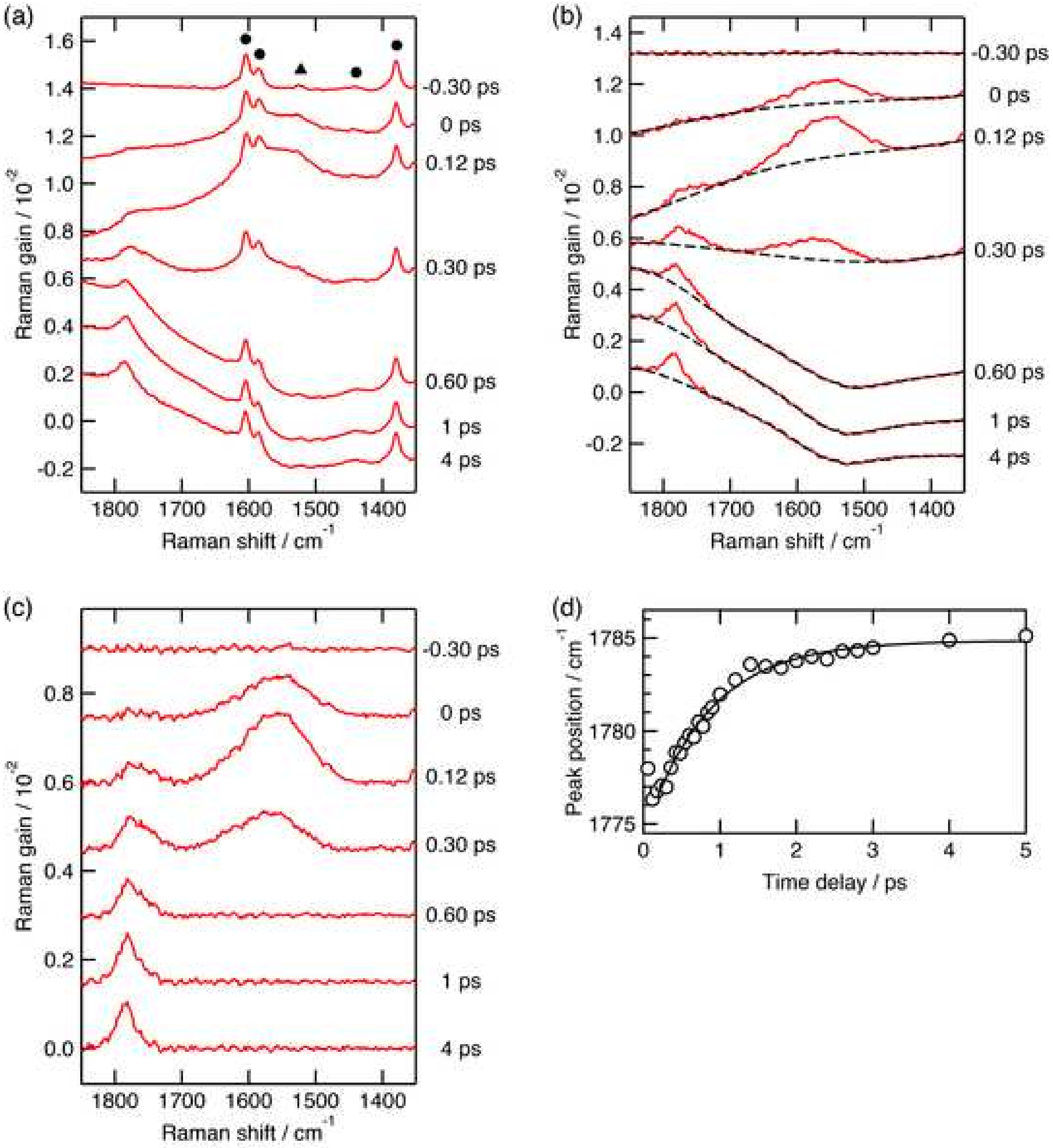

(a)
Raman gain / 10^{-2}
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
●
●
▲
●
●
-0.30 ps
0 ps
0.12 ps
0.30 ps
0.60 ps
1 ps
4 ps
1800
1700
1600
1500
1400
Raman shift / cm^{-1}
(b)
Raman gain / 10^{-2}
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
-0.30 ps
0 ps
0.12 ps
0.30 ps
0.60 ps
1 ps
4 ps
1800
1700
1600
1500
1400
Raman shift / cm^{-1}
(c)
Raman gain / 10^{-2}
0.8
0.6
0.4
0.2
0.0
-0.30 ps
0 ps
0.12 ps
0.30 ps
0.60 ps
1 ps
4 ps
1800
1700
1600
1500
1400
Raman shift / cm^{-1}
(d)
Peak position / cm^{-1}
1785
1780
1775
0
1
2
3
4
5
Time delay / ps

Table of Materials

Name of Material/ Equipment	Company	Catalog Number
1-Axis Translational Stage	OptSigma	TSD-401S
20-cm Optical Delay Line	OptSigma	SGSP26-200
3-Axis Translational Stage	OptSigma	TSD-405SL
3-Axis Translational Stage	Suruga Seiki	B72-40C
5-cm Optical Delay Line	PMT	HRS-0050
Al Concave Mirror	Thorlabs	CM254-050-G01
Base Plate	Suruga Seiki	A21-6
BBO Crystal	EKSMA Optics	-
BK7 Plano-Concave Lens	OptSigma	SLB-25.4-50NIR2
BK7 Plano-Convex Lens	OptSigma	SLB-25.4-150PIR2
BK7 Plano-Convex Lens	OptSigma	SLB-25.4-100PIR2
BK7 Plano-Convex Lens	OptSigma	SLB-25.4-200PIR2
Broadband Dielectric Mirror	OptSigma	TFMS-25.4C05-2/7
Broadband Dielectric Mirror	Precision Photonics (Advanced Thin Films)	-
Broadband Half-Wave Plate	CryLight	-
Color Glass Filter	HOYA	IR85
Color Glass Filter	HOYA	RM100
Color Glass Filter	Schott	BG39
Computer	Dell	Vostro 200 Mini Tower
Cyclohexane	Kanto Kagaku	07547-1B
Data Analysis Software	Wavemetrics	Igor Pro 8
Dielectric Beamsplitter	LAYERTEC	-
Dielectric Beamsplitter	LAYERTEC	-
Dielectric Mirror	Precision Photonics (Advanced Thin Films)	-
Digital Oscilloscope	Tektronix	TDS3054B
Elastomer Tube	-	-

Femtosecond Ti:sapphire Oscillator	Coherent	Vitesse 800-2
Femtosecond Ti:sapphire Regenerative Amplifier	Coherent	Legend-Elite-F-HE
Film Polarizer	OptSigma	SPFN-30C-26
Glan-Taylor Prism	OptSigma	GYPB-10-10SN-3/7
Gold Mirror	OptSigma	TFG-25C05-10
Half-Wave Plate	OptSigma	WPQ-7800-2M
Harmonic Separator	Coherent	TOPAS-C HRs 410-540 nm
InGaAs Array Detector	Horiba	Symphony-IGA-512X1-50-1700-1LS
InGaAs PIN Photodiode	Hamamatsu Photonics	G10899-01K
IR Half-Wave Plate	OptiSource	-
Iris	Suruga Seiki	F74-3N
Lens Holder	OptSigma	LHF-25.4S
Magnetic Gear Pump	Micropump	184-415
Mirror Mount	Siskiyou	IM100.C2M6R
near-IR phosphor card	Thorlabs	VRC2
Nut	-	-
Optical Chopper	New Focus	3501
Optical Parametric Amplifier	Coherent	OPerA-F
Optical Parametric Amplifier	Coherent	TOPAS-C
Polarizer Holder	OptSigma	PH-30-ARS
Polyfluoroacetate Tube	-	-
Post Holder	OptSigma	BRS-12-80
Quartz Flow Cell	Tosoh Quartz	T-70-UV-2
Quartz Plano-Concave Lens	OptSigma	SLSQ-25-50N
Quartz Plano-Convex Lens	OptSigma	SLSQ-25-100P

Quartz Plano-Convex Lens	OptSigma	SLSQ-25-220P
Sapphire Plate	Pier Optics	-
Si PIN Photodiode	Hamamatsu Photonics	S3883
Single Spectrograph	Horiba Jobin Yvon	iHR320
Stainless Steel Rod	Suruga Seiki	A41-100
Stainless Steel Rod	Newport	J-SP-2
Toluene	Kanto Kagaku	40180-1B
U-Shaped Steel Plate	-	-
Variable Neutral Density Filter (with a holder)	OptSigma	NDHN-100
Variable Neutral Density Filter (with a holder)	OptSigma	NDHN-U100
Visual Programming Language	National Instruments	LabVIEW 2009
Volume-Grating Bandpass Filter	OptiGrate	BPF-1190
β-Carotene	Wako Pure Chemical Industries	035-05531

Comments/Description
Products equivalent to this are used as well; for M22, L9, and CM in Figure 1A
ODL1 in Figure 1A
For L8 in Figure 1A
For FC in Figure 1A
ODL2 in Figure 1A
Focal length: 50 mm; CM in Figure 1A
Products equivalent to this are used as well; for M1-M32, BS1-BS3, L1-L10, I1-I17, P1-P2, HWP1-3, F1-F3, VND1-VND2, OC, BPF, HS, BBO, SP, CM, and FC in Figure 1A
Type 1, θ = 23.2 deg; BBO in Figure 1A
Focal length: 50 mm; IR anti-reflection coating; L6 in Figure 1A
Focal length: 150 mm; IR anti-reflection coating; L2, L3, L5 in Figure 1A
Focal length: 100 mm; IR anti-reflection coating; L4 in Figure 1A
Focal length: 200 mm; IR anti-reflection coating; L7 in Figure 1A
M22-M25, M28, M29 in Figure 1A
M26, M27, M30-M32 in Figure 1A
HWP3 in Figure 1A
F1 in Figure 1A
F2 in Figure 1A
F3 in Figure 1A
OS: Windows XP
HPLC grade
Reflection : Transmission = 2 : 1; BS1 in Figure 1A
Reflection : Transmission = 1 : 1; BS2, BS3 in Figure 1A
M1-M8 in Figure 1A
500 MHz, 5 GS/s
Figure 1E

Wavelength: 800 nm, pulse duration: 100 fs, average power: 280 mW, repetition rate: 80 MHz; included in Ti:S in Figure 1A
Wavelength: 800 nm, pulse duration: 100 fs, pulse energy: 3.5 mJ, repetition rate: 1 kHz; included in Ti:S in Figure 1A
P1 in Figure 1A
P2 in Figure 1A
M9-M21 in Figure 1A
HWP1 in Figure 1A
HS in Figure 1A

512 ch, Liquid nitrogen cooled

HWP2 in Figure 1A
Products equivalent to this are used as well; I1-I17 in Figure 1A
Products equivalent to this are used as well; for L1-L10 in Figure 1A

Products equivalent to this are used as well; for M1-M32, BS1-BS3, BBO, CM in Figure 1A

Figure 1E, M4; purchased from a DIY store
OC in Figure 1A
OPA1 in Figure 1A
OPA2 in Figure 1A
Products equivalent to this are used as well; for P1-P2 and HWP1-3 In Figure 1A
Figure 1E
Products equivalent to this are used as well; for M1-M32, BS1-BS3, L1-L10, I1-I17, P1-P2, HWP1-3, F1-F3, VND1-VND2, OC, BPF, HS, BBO, SP, CM, and FC in Figure 1A
FC in Figure 1A
Focal length: 50 mm; L8 in Figure 1A
Focal length: 100 mm; L1, L9 in Figure 1A

Focal length: 220 mm; L10 in Figure 1A
3 mm thick; SP in Figure 1A

Focal length: 32 cm
Products equivalent to this are used as well; for M1-M32, BS1-BS3, L1-L10, I1-I17, P1-P2, HWP1-3, F1-F3, VND1-VND2, OC, BPF, HS, BBO, SP, CM, and FC in Figure 1A
Figure 1E
HPLC grade
Figure 1E; purchased from a DIY store

VND1 in Figure 1A

VND2 in Figure 1A

The control software in this study is programmed in LabVIEW 2009

BPF in Figure 1A

JOURNAL OF VISUALIZED EXPERIMENTS
1 Alewife Center #200
Cambridge, MA 02140
tel. 617.945.9051
www.jove.com

ARTICLE AND VIDEO LICENSE AGREEMENT

Title of Article: Techniques for ultrafast time-resolved near-IR stimulated Raman measurements of functional π-conjugate systems

Author(s): Tomohisa Takaya, Koichi Iwata

Item 1 (check one box): The Author elects to have the Materials be made available (as described at http://www.jove.com/publish) via: ☑ Standard Access ☐ Open Access

Item 2 (check one box):

☑ The Author is NOT a United States government employee.

☐ The Author is a United States government employee and the Materials were prepared in the course of his or her duties as a United States government employee.

☐ The Author is a United States government employee but the Materials were NOT prepared in the course of his or her duties as a United States government employee.

ARTICLE AND VIDEO LICENSE AGREEMENT

1. Defined Terms. As used in this Article and Video License Agreement, the following terms shall have the following meanings: "**Agreement**" means this Article and Video License Agreement; "**Article**" means the article specified on the last page of this Agreement, including any associated materials such as texts, figures, tables, artwork, abstracts, or summaries contained therein; "**Author**" means the author who is a signatory to this Agreement; "**Collective Work**" means a work, such as a periodical issue, anthology or encyclopedia, in which the Materials in their entirety in unmodified form, along with a number of other contributions, constituting separate and independent works in themselves, are assembled into a collective whole; "**CRC License**" means the Creative Commons Attribution-Non Commercial-No Derivs 3.0 Unported Agreement, the terms and conditions of which can be found at: http://creativecommons.org/licenses/by-nc-nd/3.0/legalcode; "**Derivative Work**" means a work based upon the Materials or upon the Materials and other pre-existing works, such as a translation, musical arrangement, dramatization, fictionalization, motion picture version, sound recording, art reproduction, abridgment, condensation, or any other form in which the Materials may be recast, transformed, or adapted; "**Institution**" means the institution, listed on the last page of this Agreement, by which the Author was employed at the time of the creation of the Materials; "**JoVE**" means MyJove Corporation, a Massachusetts corporation and the publisher of *The Journal of Visualized Experiments;* "**Materials**" means the Article and / or the Video; "**Parties**" means the Author and JoVE; "**Video**" means any video(s) made by the Author, alone or in conjunction with any other parties, or by JoVE or its affiliates or agents, individually or in collaboration with the Author or any other parties, incorporating all or any portion of the Article, and in which the Author may or may not appear.

2. Background. The Author, who is the author of the Article, in order to ensure the dissemination and protection of the Article, desires to have the JoVE publish the Article and create and transmit videos based on the Article. In furtherance of such goals, the Parties desire to memorialize in this Agreement the respective rights of each Party in and to the Article and the Video.

3. Grant of Rights in Article. In consideration of JoVE agreeing to publish the Article, the Author hereby grants to JoVE, subject to **Sections 4** and **7** below, the exclusive, royalty-free, perpetual (for the full term of copyright in the Article, including any extensions thereto) license (a) to publish, reproduce, distribute, display and store the Article in all forms, formats and media whether now known or hereafter developed (including without limitation in print, digital and electronic form) throughout the world, (b) to translate the Article into other languages, create adaptations, summaries or extracts of the Article or other Derivative Works (including, without limitation, the Video) or Collective Works based on all or any portion of the Article and exercise all of the rights set forth in (a) above in such translations, adaptations, summaries, extracts, Derivative Works or Collective Works and (c) to license others to do any or all of the above. The foregoing rights may be exercised in all media and formats, whether now known or hereafter devised, and include the right to make such modifications as are technically necessary to exercise the rights in other media and formats. If the "Open Access" box has been checked in **Item 1** above, JoVE and the Author hereby grant to the public all such rights in the Article as provided in, but subject to all limitations and requirements set forth in, the CRC License.

4. Retention of Rights in Article. Notwithstanding the exclusive license granted to JoVE in **Section 3** above, the Author shall, with respect to the Article, retain the non-exclusive right to use all or part of the Article for the non-commercial purpose of giving lectures, presentations or teaching classes, and to post a copy of the Article on the Institution's website or the Author's personal website, in each case provided that a link to the Article on the JoVE website is provided and notice of JoVE's copyright in the Article is included. All non-copyright intellectual property rights in and to the Article, such as patent rights, shall remain with the Author.

5. Grant of Rights in Video – Standard Access. This **Section 5** applies if the "Standard Access" box has been checked in **Item 1** above or if no box has been checked in **Item 1** above. In consideration of JoVE agreeing to produce, display or otherwise assist with the Video, the Author hereby acknowledges and agrees that, Subject to **Section 7** below, JoVE is and shall be the sole and exclusive owner of all rights of any nature, including, without limitation, all copyrights, in and to the Video. To the extent that, by law, the Author is deemed, now or at any time in the future, to have any rights of any nature in or to the Video, the Author hereby disclaims all such rights and transfers all such rights to JoVE.

6. Grant of Rights in Video – Open Access. This **Section 6** applies only if the "Open Access" box has been checked in **Item 1** above. In consideration of JoVE agreeing to produce, display or otherwise assist with the Video, the Author hereby grants to JoVE, subject to **Section 7** below, the exclusive, royalty-free, perpetual (for the full term of copyright in the Article, including any extensions thereto) license (a) to publish, reproduce, distribute, display and store the Video in all forms, formats and media whether now known or hereafter developed (including without limitation in print, digital and electronic form) throughout the world, (b) to translate the Video into other languages, create adaptations, summaries or extracts of the Video or other Derivative Works or Collective Works based on all or any portion of the Video and exercise all of the rights set forth in (a) above in such translations, adaptations, summaries, extracts, Derivative Works or Collective Works and (c) to license others to do any or all of the above. The foregoing rights may be exercised in all media and formats, whether now known or hereafter devised, and include the right to make such modifications as are technically necessary to exercise the rights in other media and formats. For any Video to which this Section 6 is applicable, JoVE and the Author hereby grant to the public all such rights in the Video as provided in, but subject to all limitations and requirements set forth in, the CRC License.

7. Government Employees. If the Author is a United States government employee and the Article was prepared in the course of his or her duties as a United States government employee, as indicated in **Item 2** above, and any of the licenses or grants granted by the Author hereunder exceed the scope of the 17 U.S.C. 403, then the rights granted hereunder shall be limited to the maximum rights permitted under such statute. In such case, all provisions contained herein that are not in conflict with such statute shall remain in full force and effect, and all provisions contained herein that do so conflict shall be deemed to be amended so as to provide to JoVE the maximum rights permissible within such statute.

8. Likeness, Privacy, Personality. The Author hereby grants JoVE the right to use the Author's name, voice, likeness, picture, photograph, image, biography and performance in any way, commercial or otherwise, in connection with the Materials and the sale, promotion and distribution thereof. The Author hereby waives any and all rights he or she may have, relating to his or her appearance in the Video or otherwise relating to the Materials, under all applicable privacy, likeness, personality or similar laws.

9. Author Warranties. The Author represents and warrants that the Article is original, that it has not been published, that the copyright interest is owned by the Author (or, if more than one author is listed at the beginning of this Agreement, by such authors collectively) and has not been assigned, licensed, or otherwise transferred to any other party. The Author represents and warrants that the author(s) listed at the top of this Agreement are the only authors of the Materials. If more than one author is listed at the top of this Agreement and if any such author has not entered into a separate Article and Video License Agreement with JoVE relating to the Materials, the Author represents and warrants that the Author has been authorized by each of the other such authors to execute this Agreement on his or her behalf and to bind him or her with respect to the terms of this Agreement as if each of them had been a party hereto as an Author. The Author warrants that the use, reproduction, distribution, public or private performance or display, and/or modification of all or any portion of the Materials does not and will not violate, infringe and/or misappropriate the patent, trademark, intellectual property or other rights of any third party. The Author represents and warrants that it has and will continue to comply with all government, institutional and other regulations, including, without limitation all institutional, laboratory, hospital, ethical, human and animal treatment, privacy, and all other rules, regulations, laws, procedures or guidelines, applicable to the Materials, and that all research involving human and animal subjects has been approved by the Author's relevant institutional review board.

10. JoVE Discretion. If the Author requests the assistance of JoVE in producing the Video in the Author's facility, the Author shall ensure that the presence of JoVE employees, agents or independent contractors is in accordance with the relevant regulations of the Author's institution. If more than one author is listed at the beginning of this Agreement, JoVE may, in its sole discretion, elect not take any action with respect to the Article until such time as it has received complete, executed Article and Video License Agreements from each such author. JoVE reserves the right, in its absolute and sole discretion and without giving any reason therefore, to accept or decline any work submitted to JoVE. JoVE and its employees, agents and independent contractors shall have

full, unfettered access to the facilities of the Author or of the Author's institution as necessary to make the Video, whether actually published or not. JoVE has sole discretion as to the method of making and publishing the Materials, including, without limitation, to all decisions regarding editing, lighting, filming, timing of publication, if any, length, quality, content and the like.

11. Indemnification. The Author agrees to indemnify JoVE and/or its successors and assigns from and against any and all claims, costs, and expenses, including attorney's fees, arising out of any breach of any warranty or other representations contained herein. The Author further agrees to indemnify and hold harmless JoVE from and against any and all claims, costs, and expenses, including attorney's fees, resulting from the breach by the Author of any representation or warranty contained herein or from allegations or instances of violation of intellectual property rights, damage to the Author's or the Author's institution's facilities, fraud, libel, defamation, research, equipment, experiments, property damage, personal injury, violations of institutional, laboratory, hospital, ethical, human and animal treatment, privacy or other rules, regulations, laws, procedures or guidelines, liabilities and other losses or damages related in any way to the submission of work to JoVE, making of videos by JoVE, or publication in *JoVE* or elsewhere by JoVE. The Author shall be responsible for, and shall hold JoVE harmless from, damages caused by lack of sterilization, lack of cleanliness or by contamination due to the making of a video by JoVE its employees, agents or independent contractors. All sterilization, cleanliness or decontamination procedures shall be solely the responsibility of the Author and shall be undertaken at the Author's expense. All indemnifications provided herein shall include JoVE's attorney's fees and costs related to said losses or damages. Such indemnification and holding harmless shall include such losses or damages incurred by, or in connection with, acts or omissions of JoVE, its employees, agents or independent contractors.

12. Fees. To cover the cost incurred for publication, JoVE must receive payment before production and publication the Materials. Payment is due in 21 days of invoice. Should the Materials not be published due to an editorial or production decision, these funds will be returned to the Author. Withdrawal by the Author of any submitted Materials after final peer review approval will result in a US$1,200 fee to cover pre-production expenses incurred by JoVE. If payment is not received by the completion of filming, production and publication of the Materials will be suspended until payment is received.

13. Transfer, Governing Law. This Agreement may be assigned by JoVE and shall inure to the benefits of any of JoVE's successors and assignees. This Agreement shall be governed and construed by the internal laws of the Commonwealth of Massachusetts without giving effect to any conflict of law provision thereunder. This Agreement may be executed in counterparts, each of which shall be deemed an original, but all of which together shall be deemed to me one and the same agreement. A signed copy of this Agreement delivered by facsimile, e-mail or other means of electronic transmission shall be deemed to have the same legal effect as delivery of an original signed copy of this Agreement.

A signed copy of this document must be sent with all new submissions. Only one Agreement required per submission.

CORRESPONDING AUTHOR:

Name: Tomohisa Takaya
Department: Department of Chemistry, Faculty of Science
Institution: Gakushuin University
Article Title: Techniques for ultrafast time-resolved near-IR stimulated Raman measurements of functional π-conjugate systems
Signature: Tomohisa Takaya
Date: 06-17-2019

Please submit a signed and dated copy of this license by one of the following three methods:

1) Upload a scanned copy of the document as a pfd on the JoVE submission site;
2) Fax the document to +1.866.381.2236;
3) Mail the document to JoVE / Attn: JoVE Editorial / 1 Alewife Center #200 / Cambridge, MA 02139

For questions, please email submissions@jove.com or call +1.617.945.9051

Reply to Comments

Reply to Editorial comments:

General:

1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues.

We have thoroughly read through the manuscript in order to make sure that there is no problem on spelling or grammar.

2. Please define all abbreviations before use; e.g. VND, L, m/M, ODL, HWP, etc.

We have defined all abbreviations in the manuscript before the first use.

3. JoVE cannot publish manuscripts containing commercial language. This includes trademark symbols (TM), registered symbols (®), and company names before an instrument or reagent. Please limit the use of commercial language from your manuscript and use generic terms instead. All commercial products should be sufficiently referenced in the Table of Materials and Reagents.
For example: Labview

We have deleted "LabVIEW" from the manuscript. LabVIEW has already been referenced in Table of Materials and Reagents.

Protocol:

1. For each protocol step/substep, please ensure you answer the “how” question, i.e., how is the step performed? Alternatively, add references to published material specifying how to perform the protocol action. If revisions cause a step to have more than 2-3 actions and 4 sentences per step, please split into separate steps or substeps.

We have revised the manuscript so that we ensure we answer the "how" questions. We have checked that every step is composed of 3 actions or less and 4 sentences or less.

Figures:

1. Please obtain explicit copyright permission to reuse any figures from a previous publication. Explicit permission can be expressed in the form of a letter from the editor or a link to the editorial policy that allows re-prints. Please upload this information as a .doc or .docx file to your Editorial Manager account.

We have prepared a Word document containing information on copyright permission provided by Physical Chemistry Chemical Physics Owner Societies. Please find the document uploaded to the Editorial Manager account.

References:

1. Please do not abbreviate journal titles.

We have spelled out all the journal abbreviations.

Table of Materials:

1. Please ensure the Table of Materials has information on all materials and equipment used, especially those mentioned in the Protocol.

We have revised the Table of Materials. We have added all the optics and optical mounts to the table.

Reply to Reviewer #1's comments:

We highly appreciate Reviewer #1 for giving the positive evaluation to our manuscript. As Reviewer #1 mentions, we hope that the manuscript will be helpful for staffs and students in laboratories to newly construct a femtosecond time-resolved Raman spectrometer. Below is the reply to the minor concern of Reviewer #1.

Minor Concerns:
References in the Introduction completely ignore some recent reports using fs-Raman technique, which is somehow unfair. I understand that the experiment described here uses probe spectrum shifted even more to near-IR than in other experiments, but for example the lab of John Kennis in Amsterdam successfully used this technique and even to study excited state properties of carotenoids (e.g. Kloz et al. Phys. Chem. Chem. Phys. 18, 2016, 14619, or Hontani et al. J. Phys. Chem. Lett. 9, 2018, 1788).

We thank Reviewer #1 for pointing out the problem on the lack of the references. We have added several references on the development and recent advances of femtosecond time-resolved stimulated Raman spectroscopy in the revised manuscript.

Reply to Reviewer #2's comments:

We are grateful to Reviewer #2 for giving the positive evaluation to our manuscript and for the important comments on our protocol. According to the comments of Reviewer #2, we have made major revisions on our protocol, in particular Protocol 5, in order to answer how to find and maximize stimulated Raman signals. Below are the replies to the individual comments.

Minor Concerns:
While the overall structure of the article, or proposed video really, is good, I felt that the more complicated techniques required a more expansive discussion. As it is, it seemed like more complicated procedures like "try to change the position of ODL2 according to Protocol 3.4..." receive only as much discussion as very rudimentary procedures like adjusting lens positions (2.2.4). In general, finding the spatial and temporal overlap of femtosecond laser pulses at the sample is still quite a challenge experimentally, and so this article would provide a great service to the field if it spent some time discussing how to establish these overlaps. This is particularly challenging in the near infrared, where the authors laser runs.

We thank Reviewer #2 for the comment on the protocol of finding the spatial and temporal overlap of the three pulses. We have made a major revision for Chapter 5 in the manuscript in order to explain how to obtain the spatial and temporal overlap with a high certainty, which we actually performed when we constructed the spectrometer from scratch.

(1) p. 3 of the pdf, line 76: In noting the frequency dependence of SRS versus traditional Raman spectroscopy, the authors should not use different notation than the prior paragraph for the two relevant frequencies or they should note that w(i) is equivalent to w(RP) and w(s) is equivalent to w(SRS).

We thank Reviewer #2 for pointing out the problem on the notation which may confuse readers. We have added the following note in the revised manuscript: "The frequencies, ω_{RP} and ω_{SRS}, for stimulated Raman scattering are equivalent to ω_i and ω_s for spontaneous Raman scattering, respectively."

(2) In 2.5, White-light generation, the authors don't mention the variable iris i6. They should describe how changing both VND1 and the diameter of the beam via i6 can improve the white-light spectrum. This comes up again in 4.7.

We thank Reviewer #2 for pointing out the role of the iris I6. We have added the following protocol in Section 2.5: " Set the diameter of I6 to be around 5 mm." The diameter of I6 is fixed at around 5 mm in our spectrometer regardless of the intensity of the incident beam.

(3) In 2.6.10, I believe they mean "white light continuum beam" in line 210 not "actinic pump beam".

We thank Reviewer #2 for pointing out the mistake. We have corrected it.

(4) Line 213, step 2.6.10, how can one conduct protocols 2.6.5-8 when those involve removing optics that are already removed?

We have revised Step 2.6.10 as "Protocols 2.6.6–2.6.8".

(5) 2.7.1, line 219, the characteristics of this BPF are quite important. Can the authors describe what is necessary here? In the diagram it looks like the BPF is used in a reflective geometry, does that indicate that it is actually a reflective "notch filter"?

We have spelled out "BPF" in Step 2.7.1 as "volume-grating reflective bandpass filter". It works as a notch filter for the transmitted beam.

(6) In section 2.9.7, line 279, I wonder if these directions could be improved by providing better guiding principles instead of just directions. For instance here, the authors should state "Adjust the flow rate to ~20 ml/min so that the illuminated volume in the FC is replaced every 1 ms." Similarly, in step 2.8.10, some mention of choosing to pump the sample at magic angle polarization to eliminate rotational diffusion artifacts should be made. Without these ideas mentioned when they are relevant, the directions appear too arbitrary, even though there are very important reasons to do it this way.

We have added guiding principles for a few protocols, including those which Reviewer #2 pointed out (Steps 2.8.10 and 2.9.7), in the revised manuscript within the page limit of the manuscript.

(7) 3.1.1: Will this lab view software be made available to the readers? If not, then these protocols should be adjusted to a general audience using their own software.

We have deleted Section 3.1 in the former manuscript, which contains the word "LabView", because Editor has pointed out that the manuscript should not contain commercial language.

(8) 3.3.5. It seems that there is no exit slit in this system, so why must we set the exit slit to 0 mm? Although I know that this is just an artifact of the software, some explanation is necessary here, or else this makes no sense. In general, sections 3.2 (detector) and 3.3 (spectrograph) have a lot of random commands issued without any explanation. I think this section would benefit from explanations, so that people not using this specific model of detector and spectrograph could still follow along.

We have deleted the statements on the exit slit, which appears in Section 3.3.5 in the former manuscript (Section 3.2.5 in the revised one). They are applicable for our spectrograph, which is equipped with a side exit port.

(9) 3.4 (Stage position control) It is not clear what is happening in this section. ODL positions need to be set according to the relative path lengths of the different beams. Shouldn't this be the spot where finding the temporal overlap of the pulses is described?

We just describe how we operate our software to drive translational stages in Section 3.4. We have rewritten Chapter 5 for explaining detailed protocol of finding temporal overlap for the three pulses.

(10) 5.1.11. Directions are needed on HOW to find the cyclohexane stimulated Raman signal. How should the scan of overlap, via M21, and delay, via ODL2 be performed. What is the user looking for? How much signal should they see when it is "good"? This is necessary down in section 6.9 also for transient absorption.

We thank Reviewer #2 for pointing out that we need to explain how to find stimulated Raman bands of cyclohexane in details. We have rewritten Chapter 5 with sections on how to find spatial overlap of the pulses, how to find their temporal overlap, how to adjust the rotational phase of the optical chopper, and at which pixels the strongest Raman band of cyclohexane appears for explaining the details. We are unable, however, to describe how much signals will appear, because the signal intensity is sensitively affected by the pulse energy, the pulse duration, the spot diameter at the sample point, and the intensity distribution in the laser spots, as well as the quality of the beam alignment.

(11) 8.4: The authors' Raman shift calibration method is incorrect, although it is clearly effective. The Raman shifts should be converted to wavelength using the known wavelength of the Raman pump. In wavelength vs pixel, the calibration curve will be linear, allowing extrapolation to frequencies beyond those of the solvent. A third-order polynomial fitting of Raman shift vs pixel is likely to veer off in

odd directions beyond the regions of the solvent peaks, providing a poor calibration of modes around 1600-1700 cm-1.

We thank Reviewer #2 for discussing the manner of Raman shift calibration. As Reviewer #2 points out, almost linear regression will be obtained if the peak positions of the Raman bands are analyzed in the wavelength axis. The accurate conversion in wavelength, however, is possible only when we know the exact wavelength of the Raman pump pulse. It is not the case for our spectrometer, because it has spectral resolution of around 0.5 nm, or 3.5 cm^{-1}, at around the Raman pump wavelength. This is the reason why we conduct the "practical" calibration by analyzing the Raman bands in Raman shift. We agree with Reviewer #2 that the accuracy of our calibration will be rapidly lost as the Raman shift goes apart from the range of the solvent peaks. We have added the above discussion in the revised manuscript so that readers can choose the calibration method suitable for their spectrometers.

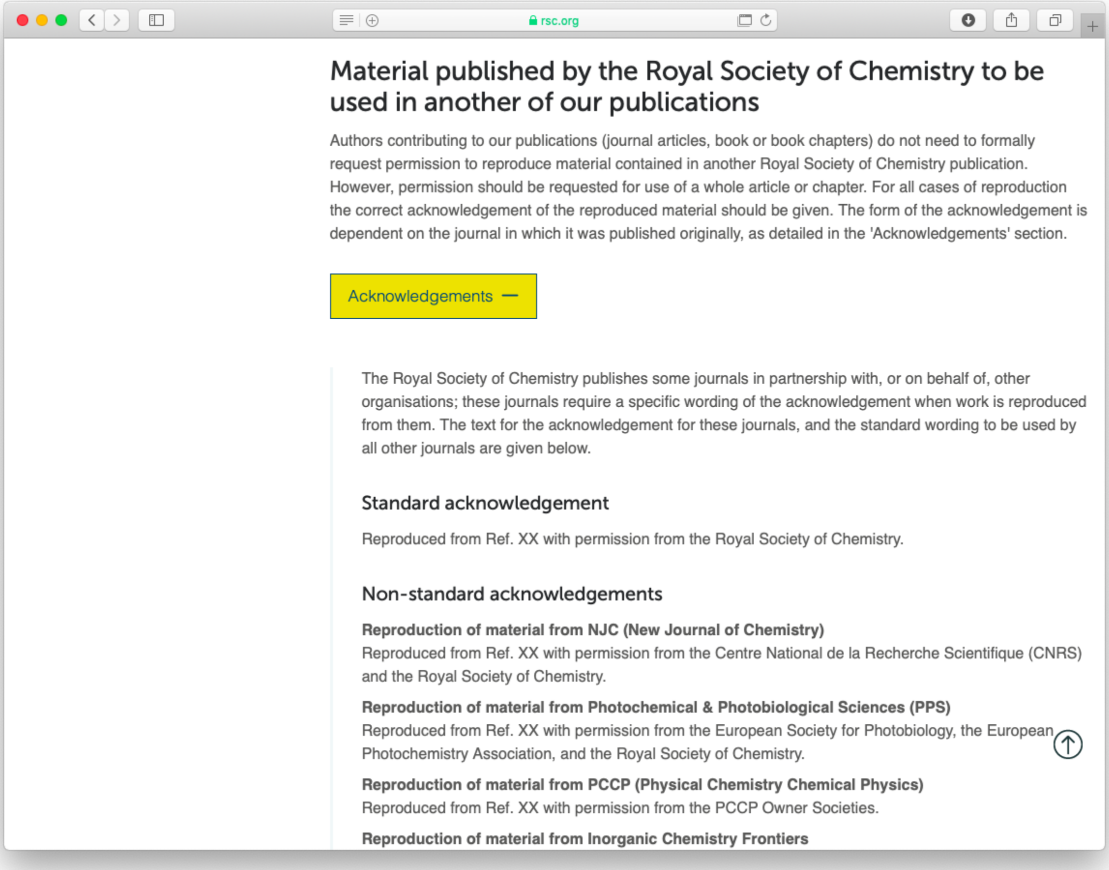
August 7, 2019

Dear Dr. Phillip Steindel,

The explicit permission with respect to Figure 1 in the manuscript is available from the following URL:

https://www.rsc.org/journals-books-databases/journal-authors-reviewers/licences-copyright-permissions/#reuse-permission-requests

I attach a screenshot of the above website with this letter.



Material published by the Royal Society of Chemistry to be used in another of our publications

Authors contributing to our publications (journal articles, book or book chapters) do not need to formally request permission to reproduce material contained in another Royal Society of Chemistry publication. However, permission should be requested for use of a whole article or chapter. For all cases of reproduction the correct acknowledgement of the reproduced material should be given. The form of the acknowledgement is dependent on the journal in which it was published originally, as detailed in the 'Acknowledgements' section.

The Royal Society of Chemistry publishes some journals in partnership with, or on behalf of, other organisations; these journals require a specific wording of the acknowledgement when work is reproduced from them. The text for the acknowledgement for these journals, and the standard wording to be used by all other journals are given below.

Standard acknowledgement

Reproduced from Ref. XX with permission from the Royal Society of Chemistry.

Non-standard acknowledgements

Reproduction of material from NJC (New Journal of Chemistry)
Reproduced from Ref. XX with permission from the Centre National de la Recherche Scientifique (CNRS) and the Royal Society of Chemistry.

Reproduction of material from Photochemical & Photobiological Sciences (PPS)
Reproduced from Ref. XX with permission from the European Society for Photobiology, the European Photochemistry Association, and the Royal Society of Chemistry.

Reproduction of material from PCCP (Physical Chemistry Chemical Physics)
Reproduced from Ref. XX with permission from the PCCP Owner Societies.

Reproduction of material from Inorganic Chemistry Frontiers

Sincerely yours,

Tomohisa Takaya

Tomohisa Takaya
Department of Chemistry, Faculty of Science
Gakushuin University
1-5-1 Mejiro, Toshima-ku, Tokyo 171-8588
Japan