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RPPR Final Report
as of 14-Mar-2023

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Proposal Number: 78038RTREP **Agreement Number: W911NF-21-1-0137**
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Report Date: 31-Jul-2022 Date Received: 05-Oct-2022
Final Report for Period Beginning 01-May-2021 and Ending 30-Apr-2022

Title: Photochemical-Photophysical Laboratory for the Study of Enhancer-Free Photo-Generated Singlet Oxygen
Begin Performance Period: 01-May-2021 **End Performance Period:** 30-Apr-2022
Report Term: 0-Other

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STEM Degrees: 5 **STEM Participants:** 5

Major Goals: Goal 1 - Enhance the research capabilities of the university and strength its STEM education programs by developing a non-previously existing laboratory on Photophysics and Photochemistry.
Goal 2 - Apply the new facility to research on enhancer-free photoactivation of singlet oxygen using visible light as a field of interest to DoD programs.
Goal 3 - Attract students to STEM careers, including students from underrepresented minorities (URM).

Accomplishments: The accomplished under the Goals has bee uploaded in pdf document.

Training Opportunities: The acquired equipment under this Award provides opportunities in training in laser spectroscopy, Raman, fluorescence, phosphorescence, absorption, and photothermal spectroscopy.
It program also provides opportunities in training of Photophysics and Photochemistry of singlet oxygen including its photogeneration and its detection.

Results Dissemination: We reported the scientific findings in the peer-reviewed manuscript:
Aristides Marcano Olaizola, David Kinsgely, Robinson Kuis, and Anthony Johnson, “Stimulated Raman Generation of Aqueous Singlet Oxygen without Photosensitizers,” Journal of Photochemistry and Photobiology B: Biology, online September 8, Vol 235 (2022) 112562.
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RPPR Final Report
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Project Contribution:
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Project Contribution:
National Academy Member: N

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Project Contribution:
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RPPR Final Report
as of 14-Mar-2023

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Participant Type: Graduate Student (research assistant)
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Article Title: Stimulated Raman generation of aqueous singlet oxygen without photosensitizers
Authors: Aristides Marcano Olaizola, Robinson Kuis, Anthony Johnson, David Kingsley
Keywords: Singlet oxygen, Raman spectroscopy, Raman transitions
Abstract: Singlet oxygen is traditionally produced via photosensitizer molecules such as methylene blue, which function as catalysts. Here we investigate stimulated Raman generation of singlet oxygen from dissolved oxygen in both water (H2O) and heavy water (D2O) using nanosecond-pulsed visible blue laser light in the 400 - 440 nm spectral region without singlet oxygen photosensitizers. We report an oxygen-dependent Stokes peak in the red spectrum (600 - 670 nm) that is identical when produced in H2O and D2O. These red Stokes photons are not detected when an oxygen quencher is present. Temporal photodepletion of the uric acid absorbance peak at 294 nm is consistent with singlet oxygen generation. We postulate that a two-photon stimulated Raman process produces singlet oxygen from O2 dissolved within the solvents. We note that the energy difference between input and output photons of 0.97 eV is precisely the energy needed to excite O2 to its singlet state.
Distribution Statement: 1-Approved for public release; distribution is unlimited.
Acknowledged Federal Support: **Y**

RPPR Final Report
as of 14-Mar-2023

Partners

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I certify that the information in the report is complete and accurate:
Signature: Aristides Marcano Olaizola
Signature Date: 10/5/22 3:18PM

Final Report

Award W911NF2110137

Photochemical-Photophysical Laboratory for the Study of Enhancer-Free Photogenerated Singlet Oxygen

PI: Aristides Marcano

Abstract

The program develops a state-of-the-art Photochemical and Photophysical laboratory at Delaware State University, an HBCU land-grant university in Dover, Delaware. The primary purpose is to conduct research and train new generations of scientists and professionals in the photodetection and photogeneration of singlet oxygen. Singlet oxygen is an electronic excited level of the molecule of oxygen, which plays a crucial role in photochemical reactions that regulate the process of life and death in living systems. The molecule is highly electrophilic able to break covalent bonds of organic compounds. Singlet oxygen has been identified as the main factor in the combat of pathogens, viruses, bacteria, or any kind microparasites, invading a healthy organism. The role of singlet oxygen in photodynamic therapy for the destruction of cancer tumors or damaged cells is also well-established. Despite its significant importance, the ways of photogeneration of singlet oxygen are not fully understood. For example, we have reported that the illumination of water samples containing viruses using blue light leads to the inactivation of the virus. We experiment without the use of any photosensitizer or photo-enhancement compounds generally applied in the generation of singlet oxygen. We have established that the production of singlet oxygen in water is the cause of the effect. We propose different hypotheses of the generation of singlet oxygen from the oxygen dissolved in water, including the possibility of a Raman process. Understanding how light generates singlet oxygen in natural waters will lead to the creation of more efficient systems for the control and destruction of viruses, improving the disinfection of food, blood, and other pharmaceutical products. The program includes the acquisition of a tunable nanosecond high-intensity laser and a high-sensitivity fluorescence/phosphorescence spectrometer with accessories specially designed to detect singlet oxygen. The new facility will significantly improve our university's research infrastructure, impacting different programs in the Department of Physics, Engineering, Mathematics, and Computer Science, the Department of Biological Sciences, Chemistry, Agriculture, and Human Ecology. Upon installation of the laboratory, we will train a significant number of undergraduate and graduate students, mostly from underrepresented minorities taking advantage of the fact that more than 75% of our students are minorities. Students from any STEM discipline will be able to join the program. The new facilities will promote the studies in Photophysics, Photochemistry, Photobiology, and Photomedicine as fundamentally cross-disciplinary programs with crucial applications in food pharmaceutical disinfection technology, virus and bacteriological containment, and phototherapy applications for the treatment of cancer and other diseases.

Objectives for the entire research project

- Enhance the research capabilities of the university and strength its STEM education programs by developing a non-previously existing laboratory on Photophysics and Photochemistry.
- Apply the new facility to research on enhancer-free photoactivation of singlet oxygen using visible light as a field of interest to DoD programs.
- Attract students to STEM careers, including students from underrepresented minorities (URM).

Findings

Objective 1 - Enhance the research capabilities of the university and strength its STEM education programs by developing a non-previously existing laboratory on Photophysics and Photochemistry

We have enhanced the research capabilities of the university though the acquisition of a high sensitivity fluorescence-phosphorescence spectrophotometer (Fluo-Time 300 system from PicoQuant), a Radiant 355 tunable nanosecond high intensity laser source (Opotek), and an optical table (Newport) where the new spectrophotometer has been installed. The new state-of-the-art spectrophotometer includes picosecond fixed wavelength laser sources, an infrared photomultiplier, a visible high sensitivity photomultiplier, entrance and exit monochromators, and other minor complementary equipment. The new equipment constitutes the basis of a new Laboratory on Photophysics and Photochemistry. Figure 1 shows a photograph of the new laser facility which is a nanosecond optical parametric oscillator tunable from 200 to 2000 nm. The device will allow the conduction of Raman experiments in a variety of samples. Figure 2 shows a photograph of the new spectrophotometer Fluo-Time 300.

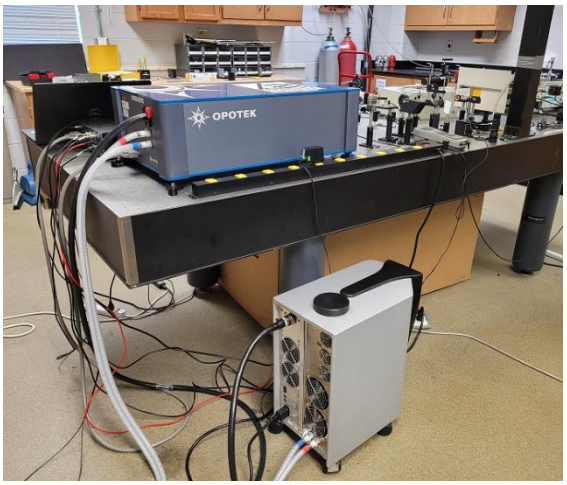


Figure 1. Optical parametric oscillator (Radiant 355, Opotek) installed in the DSU Laboratory.

Figure 2. The new high sensitivity fluorescence-phosphorescence spectrophotometer (Fluo-Time 300, Picoquant).

Objective 2 - Apply the new facility to research on enhancer-free photoactivation of singlet oxygen using visible light as a field of interest to DoD programs.

Following the installation of the new laser facility, we started the research to demonstrate that singlet oxygen can be generated via a stimulated Raman process without using photosensitizers or any other chemical enhancer. Raman is a well-known spectroscopic method for investigating the properties of matter. Raman is a two-photon process where the frequency ν of a pumping photon mixes with the energy modes of the molecule Ω to produce a Stokes photon of less energy and frequency ν_S. The original molecule is excited from the ground to an excited mode at energy $h\Omega$ according to the equation:

$$h\nu - h\nu_S = h\Omega \ , \qquad (1)$$

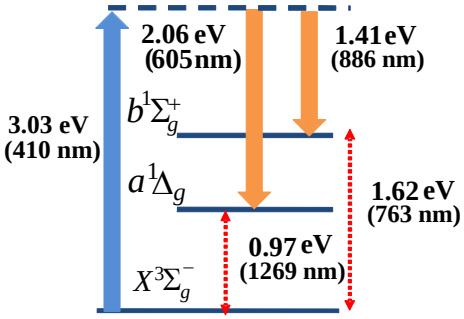


Figure 1. Raman path for singlet oxygen generation.

where h is the Planck constant. The Stokes signal provides information about the internal frequencies of the system Ω. The Quantum Mechanical selection rules from Raman transitions substantially differ from the one-photon ones. In general, the golden rule applies - whenever one-photon direct dipole transition is forbidden, the Raman transition occurs with high probability. Quantum Mechanics prohibits the direct transition of the oxygen molecule from ground to the singlet oxygen state ($^1\Delta_g$). **According to the golden rule of Raman Spectroscopy, the two-photon Raman transition should occur with high probability**. The symmetry property of the states favors the Raman transitions instead of the

one-photon excitation. Quantum Mechanics predicts an increased likelihood of the Raman transitions between the lowest electronic states of the oxygen molecule. Figure 1 shows the expected Raman transitions from ground to $^1\Delta_g$ or $b^1\Sigma_g^+$ levels when using blue light at 410 nm. The 410-nm photon produces a Stokes photon at 610 nm, and the transition from the ground to the $^1\Delta_g$ level through the virtual state (dash-line). It can also create a NIR Stokes photon at 886 nm and excitation of the second electronic excited level $b^1\Sigma^+_g$ from where the oxygen relaxes fast toward $^1\Delta$. Using a traditional spectrometer, Stokes signals in the VI and NIR must be observable. The different rotational-vibrational sublevels of the molecular oxygen electronic levels may generate a set of Stokes lines. The interaction of oxygen with surrounding solvent molecules must substantially broaden the spectral lines. Any detected spectral lines with wavelengths defined by the inequalities

$$\frac{1}{\lambda} > \frac{1}{410\,nm} - \frac{1}{1269\,nm} \quad \text{or} \quad \frac{1}{\lambda} > \frac{1}{410\,nm} - \frac{1}{763\,nm}\ , \tag{2}$$

corresponding to the excitation of the $^1\Delta_g$ level or the level $b^1\Sigma_g^+$, respectively, may represent a Raman line response.

The use of intense laser pulses opens the possibility of $^1\Delta_g$ generation in a stimulated coherent Raman configuration. Figure 2 shows the stimulated Raman transition. The effect is a nonlinear optical process of the third order, where two photons of the incoming excitation light of frequency ν produce Stokes and anti-Stokes photons of frequencies ν_s and ν_a.

The Raman Experiment

We conduct stimulated Raman excitation of oxygen dissolved in water (H_2O) and heavy water (D_2O) at room temperature and one-atmosphere pressure using nanosecond pulses from an optical parametric oscillator (OPO) pumped by an Nd-YAG laser. The experiments identify peaks that represent the contribution from the stretching modes of the solvent molecules and, most importantly, a smaller red spectrum peak that we identify as the Raman response of $^1\Delta$ - oxygen molecules dissolved in the water samples.

Figure 2 shows a schematic of the Raman experiment. The pump source is an optical parametric oscillator (OPO Radiant QX 4130, OPOTEK) tunable in the visible region (410-700 nm). In the device, an Nd-YAG laser at 1064 pumps the OPO crystal. The system generated 10-ns pulses at a 10 Hz rate. The average energy per pulse is about 30 mJ. In the experiments discussed below, the OPO radiation is used at 410, 420, 430, and 440 nm. A beam-splitter (B) redirects a small portion of the light to a reference detector (Ref). A 40-cm focal length lens (L_1) focused the OPO light onto the sample. The samples were doubled distilled H_2O and D_2O (99.9 atom % D, Sigma Aldrich) in 30-cm and 10-cm length glass cuvettes. The long-pass cuvette has two purposes. First, to provide enough path length of the sample, and second, to avoid damage to the cuvette's windows, ensuring that the light intensity at the entrance and exit of the cuvette is small enough. The focused light generates stimulated Raman signals in slightly different directions than the original pumping beam. Most of the light is in the beam center, which also contains the pumping light. The stimulated Raman also generates a ring of light centered in the direction of the original pumping beam. A blocker (D) eliminated the beam's central spot. The procedure depletes most of the pumping light. A long-pass filter (LPF) blocks the remnant

excitation light. Lens L_2 collimates the light from the ring. A mirror (M) redirects part of the light of the ring structure into a monochromator (Cornerstone 260, Newport). Rotation of the mirror allowed the selection of different parts of the ring structure. A diode detector (DET36A2, Thorlabs) collects the light from the monochromator sending the generated signal toward a current pre-amplifier (SR570 Stanford Research). The amplified signal is redirected to a digital oscilloscope (TDS3052, Tektronix) for averaging and recording.

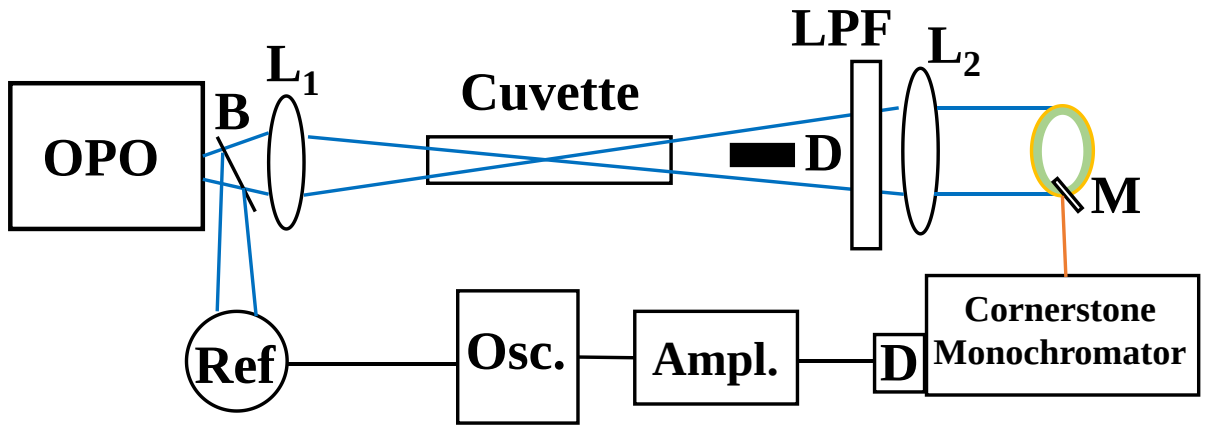


Figure 2. Raman scattering experimental set-up consisting of an optical parametric oscillator (OPO), a beam splitter (B), a reference diode detector (Ref), a focusing lens (L_1), a long-path glass cuvette, a beam blocker (D), a long-pass filter (LPF), a collimating lens (L_2), a mirror, a monochromator, a current amplifier and a digital oscilloscope (Osc).

1.
2.

3. Main Results

Figure 3-left shows the stimulated Raman spectra collected for distilled H_2O (open blue squares) and D_2O (open red stars) when pumping at 410 nm. Figure 3-right exhibits the spectra obtained when pumping at 440 nm. We observed two peaks that shift according to the pumping wavelength changes, confirming their Raman character. The first Stokes peak for H_2O occurs at 475 nm and 515 nm when pumping at 410 nm and 440 nm. The corresponding peaks for D_2O occur at 454 nm and 492 nm, respectively. The Stokes frequency shift of the first peak averages (3340±20) cm^{-1} for H_2O and (2250± 20) cm^{-1} for D_2O. The values correspond to reported stretching mode frequencies for both solvents. The H_2O's second peak occurs at 567 nm and 630 nm when pumping at 410 nm and 440 nm light, respectively. We observe similar additional peaks for D_2O at 507 nm and 556 nm, respectively, when pumping with 410 and 440 nm light. The frequency shifts of the second peak average (6743 ± 110) cm^{-1} and (4770 ± 60) cm^{-1} for H_2O and D_2O, respectively. Because the value of the second peak is near twice the shift of the first peak, we interpret the signal as resulting from a second-order cascade Raman effect with the Stokes light corresponding to the first peak that produces this secondary Raman emission.

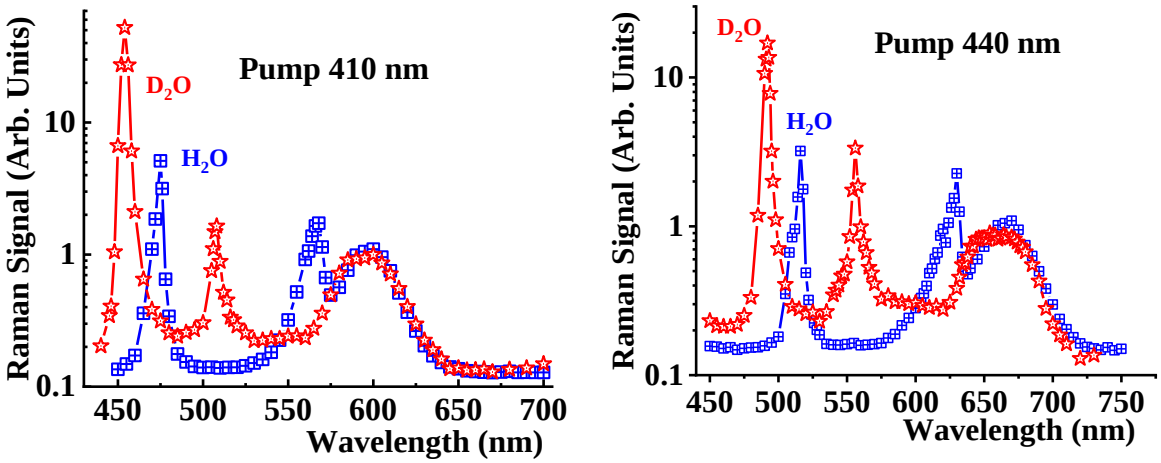


Figure 3. Left -Stokes peaks of the Raman spectra from water (blue crossed squares) and heavy water (open red stars) when pumping at 410 nm. The first two peaks correspond to the stretching modes of the solvent molecule (H_2O or D_2O). The third peak, indicated by the green star, is associated with the generation of the $^1\Delta$ state. Right - Stokes peaks of the Raman spectra from water (blue crossed squares) and heavy water (open red stars) when pumping at 440 nm. The first two peaks correspond to the stretching modes of the solvent molecules. The third peak, indicated by the green star, is associated with the generation of the $^1\Delta$ state.

In contrast to other observed peaks, a third "red peak," exhibits the same shape and frequency shift for H_2O and D_2O and is much broader than the previous two peaks. For both solvents, peak detection occurs at (600 ± 25) nm and (670 ± 25) nm when pumping at 410 nm and 440 nm, respectively. This frequency shift is (7856 ± 240) cm^{-1}, and does not coincide with possible water molecule vibrations. The Stokes frequency shift of the "red peak" in both solvents corresponds to the energy of (0.97 ± 0.3) eV in agreement with the transition to the $^1\Delta$-level. We are left to interpret the third "red peak" as generated from the oxygen molecules dissolved within the water. The corresponding wavelength is (1272 ± 44) nm, which agrees with the predicted $^1\Delta_g$ -phosphorescence photoemission. Experiments pumping at 420 nm and 430 nm are consistent with those observed at 410 and 440 nm, with the first two shifted peaks present and a solvent-independent "red peak" with H_2O and D_2O.

To further test that the red region's peaks are oxygen-dependent, we use H_2O solutions of sodium bisulfite ($NaHSO_3$). As discussed above, Sodium bisulfite is a well-known oxygen quencher. We use a long-pass filter blocking any light below 590 nm to deplete the Stokes peaks from the stretching modes. Figure 4-left shows the Stokes peaks in the red region for H_2O (blue line) and solutions of sodium bisulfite ($NaHSO_3$) in H_2O at concentrations of 40 mg/ml (red line). The solution with $NaHSO_3$ exhibits a significantly reduced "red peak." Figure 4-right shows similar results for the solution of $NaHSO_3$ in D_2O. The oxygen quencher depletes the oxygen concentration, reducing the "red peak."

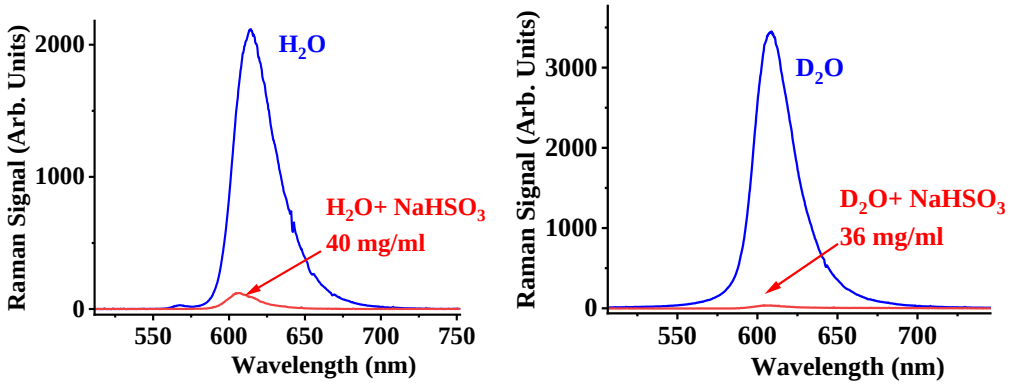


Figure 4. Left - Stokes red peak in H_2O obtained using a long-pass filter >590 nm in front of the monochromator (blue line) when pumping at 410 nm. When adding the oxygen quencher $NaHSO_3$ at a 40 mg/ml concentration, the peak substantially decreases. Right - The same Stokes red peak measured in D_2O. Again, the addition of $NaHSO_3$ depletes the signal.

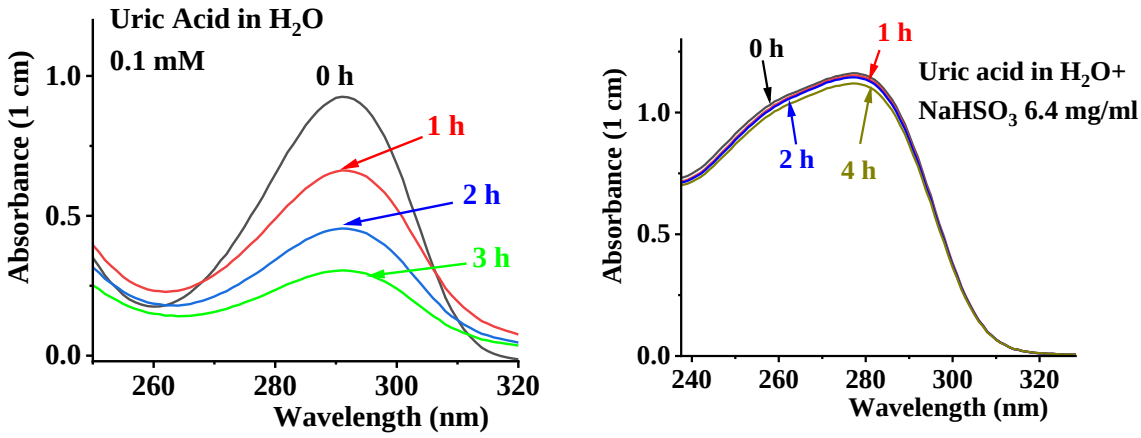


Figure 5. Left - UV peak at 294 nm of a 0.1 mM uric acid water solution before irradiation (black line). Irradiation of the sample for one hour (red line), two hours (blue line), and three hours induces the photobleaching of the peak as evidence of $^1\Delta$ generation. Right - The same 294 nm peak of the uric acid water solution when adding the oxygen quencher $NaHSO_3$. Almost no photobleaching is observed due to the depleted oxygen concentration in the solvent.

As additional evidence of the generation of the $^1\Delta$-state, we measure the temporal destruction of uric acid as a measure of singlet oxygen production. We determine the UV spectrum of a diluted uric acid solution in distilled water irradiated at 410 nm at different exposure times. The UV absorbance peak at 294 nm exhibits photodepletion upon irradiation due to a lytic reaction between the $^1\Delta$ and uric acid molecules. After irradiation, we measure the corresponding UV spectra. Figure 5-left shows the UV spectra of a 0.1 mM uric acid solution in H_2O. The non-irradiated sample has a clear peak at 294 nm (black line). The peak consistently decreases upon

irradiation over half of an hour (red line), one hour (blue line), and two hours (green line). As generally accepted, this is an indication that $^1\Delta_g$ is being produced. We have also used uric acid solutions in D_2O, yielding similar results. Adding the oxygen quencher $NaHSO_3$ to the solution substantially reduces the photobleaching of the uric acid UV peak. Figure 5-right shows the UV spectra of a 0.1 mM uric acid water solution with $NaHSO_3$ added at a 6.4 mg/ml concentration. Irradiating by a 410 nm laser light for one hour (red line), two hours (blue line), and four hours (olive line) does not significantly affect the signal. We observe peak reductions of less than 4%, confirming that the destruction of uric acid is oxygen-dependent.

In conclusion, we have completed a study of the Raman response of H_2O and D_2O upon nanosecond illumination in the blue region of the spectrum 410-440 nm. We report evidence of singlet oxygen formation within aqueous solvents in the absence of enhancers based on the presence of peaks in the 600-660 nm region, which we postulate correspond to the Raman transition of ground oxygen dissolved in the solvents to generate the $^1\Delta$-state. Experiments using sodium bisulfite as an oxygen quencher and UV spectroscopy of uric acid solutions confirm the $^1\Delta_g$ generation.

The results discussed here were recently published in the peer-reviewed publication Journal of Photochemistry and Photobiology B: Biology, Vol 235 (2022) 112562. https://doi.org/10.1016/j.jphotobiol.2022.112562

We have demonstrated that singlet oxygen can be photogenerated in water environments without the need of enhancers through a simulated Raman process. We will continue the research taking advantages of the new Photochemical and Photophysical laboratory created.

Objective 3 - Attract students to STEM careers, including students from underrepresented minorities (URM).

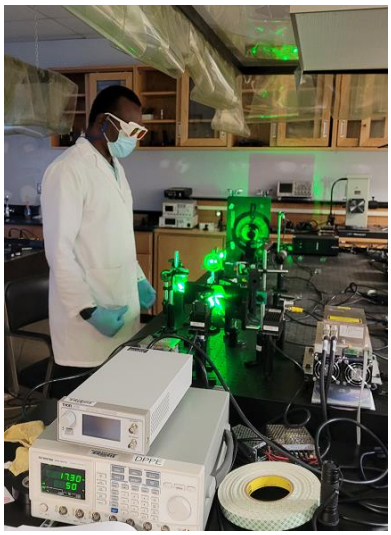
During the last year we trained four undergraduate students and one graduate student in different projects of the laboratory of Quantum Optics at DSU, despite the restrictions imposed by the Covid 19 pandemics. In February 2022, students have the opportunity to attend a training offered by the company PicoQuant provided of a new high sensitivity spectrophotometer based on photon counting technology. Below we provide a brief introduction of the students.

Milia Johnson (US citizen) – DSU undergraduate student majoring Engineering Physics. Ms. Johnson worked under that mentorship of DR. A. Marcano on a project about Raman cascade effect in water and heavy water. She reported the findings in an oral presentation during the Research Day in April 2022.

Portia Wiggins (US citizen) - DSU undergraduate student majoring Engineering Physics. Ms. Wiggins worked under that mentorship of DR. A. Marcano on a project about photogeneration of singlet oxygen without photosensitizers. She reported the findings in an oral presentation during the Research Day in April 2022.

Ms. Wiggins working in the laboratory (March 2022).

Daniel Orefuwa (US permanent resident) – DSU Ph. D. graduate student majoring Applied Chemistry. Mr. Orefuwa works under the mentorship of DR. Bizuneh Workie (Department of Chemistry) and co-mentorship of Dr. A. Marcano on photothermal characterization of films. He completed a presentation for the Research Day of April 2022.

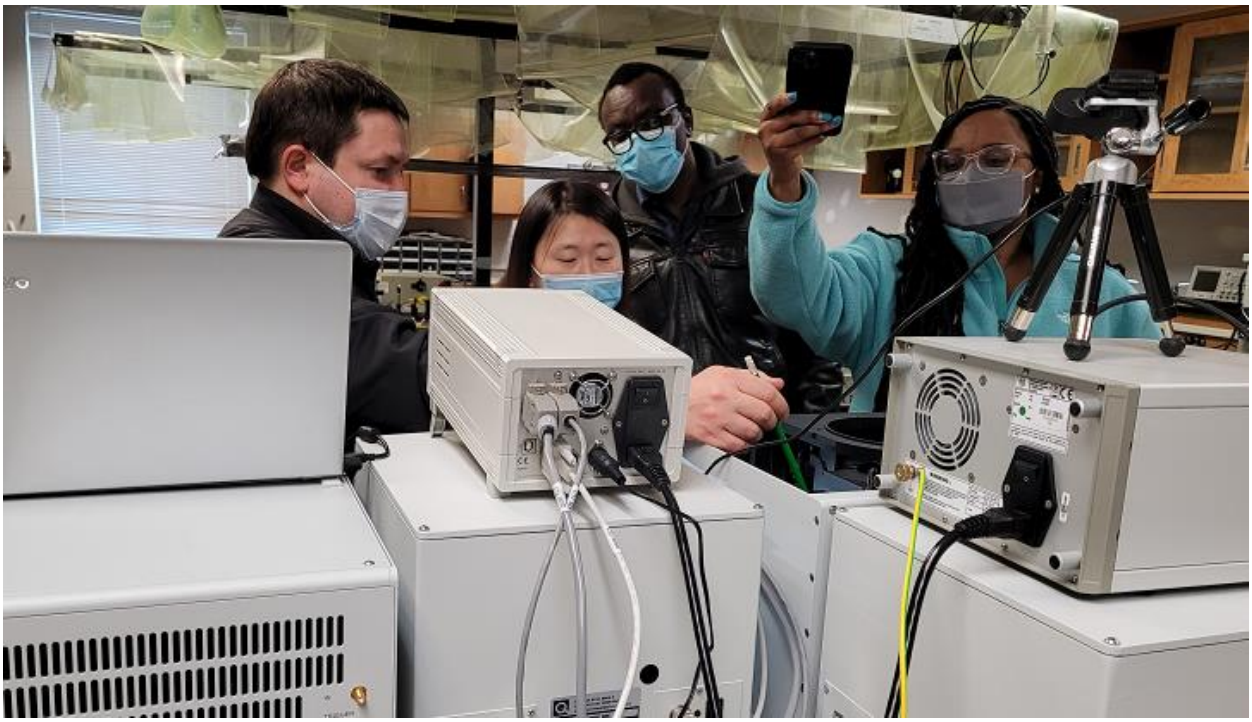


Mr. Orefuwa conducting experiments (May 2022).

Berl-Eddlie Brillant (US citizen) – DSU undergraduate student majoring Engineering Physics. Mr. Brillant worked during the summer of 2022 on photothermal spectroscopy of semiconductors under the mentorship of Dr. A. Marcano. He reported his findings in the Summer Workshop of July 2022. His poster received an award as the best poster in the category of "Physics." He currently continues to work in the laboratory taking advantage of the new facilities

Ameen Zerrad (US citizen) – DSU undergraduate student majoring Engineering Physics. He is currently working under the mentorship o Dr. A. Marcano on stimulated Raman investigation of water.

Mr. Zerrad conducting experiment on stimulated Raman scattering on water (September 2022).



Students being trained in the use of high sensitivity spectrophotometer (February 2022).