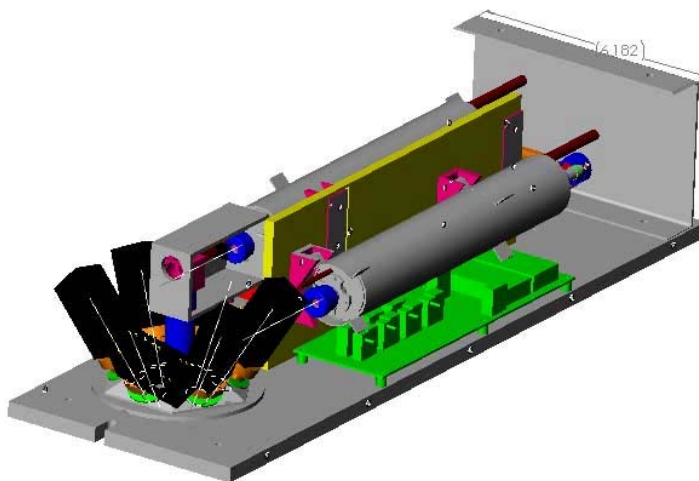


□ **WasteWater** **Targeted Ultraviolet** **Chemical Sensor**

Targeted Ultraviolet Chemical Sensors (TUCS) are in-situ, non-contact, non-invasive, non-destructive sensors that require no sample handling or preparation. They employ UV laser induced native fluorescence and resonance Raman spectroscopy to detect and classify unknown molecules and molecular structures. By employing deep UV excitation wavelengths the need for tagging target materials with dyes is eliminated. Dye tags are a significant source of error in many measurements and can obscure the chemistry being measured. Using excitation wavelengths below 250nm, native or endogenous fluorescence and UV Raman emission of target molecules can be measured simultaneously without mutual interference. *The potential for using UV Raman spectroscopy to measure nitrate and nitrite in wastewater treatment systems has been demonstrated down to the 100ppb range and will be discussed below. Other wastewater materials call also be identified using the same techniques described below.*



Fluorescence Limit of Detection of this sensor is as low as one microorganism at a working distance of 45cm or 100 TRP molecules at a distance of 4cm. Other organic molecules such as BTEX have sensitivity in the part per trillion range. Resonance Raman sensitivity is about 1000 times lower. Specificity will be discussed later.

Sensitivity and Specificity

Fluorescence Limit of Detection of this sensor is as low as one microorganism at a working distance of 45cm or 100 TRP molecules at a distance of 4cm. Other organic molecules such as BTEX have sensitivity in the part per trillion range. Resonance Raman sensitivity is about 1000 times lower. Specificity will be discussed later.

General Applications:

TUCS are designed to be employed as sensitive, in situ, autonomous, industrial or environmental monitors. The sensors developed by Photon Systems operate in the deep ultraviolet to enhance their sensitivity and specificity in identifying certain molecular structures such as organic molecules and well as other molecules that are optimally excited in the UV to emit fluorescence and Raman.

Typical applications for TUCS instruments include in situ, real time measurement and characterization:

- Homeland security detectors for biological and chemical weapons materials;
- Ultrasensitive organic surface contamination detection in high quality optical fabrication, operating room surface inspection, etc.
- Cleaning validation for ultra-sensitive reagent applications
- Water quality in waste treatment plants where the content of nitrates, nitrites, carbonates, aluminates, and other water quality contaminants need to be measured on a regular basis;
- Well water or ocean water quality: sea farming, well water testing, domestic water quality;
- Chemical and microbial detection and identification in water, air and or surfaces
- Quality measurement of synthetic chemical vapor deposited diamond and diamond like carbon films in hard disc drive, super abrasives manufacturing, and other synthetic diamond applications;
- Quality measurement of epitaxial films employed in microelectronics manufacturing; substrate surface quality.
- Quality measurement of the surface condition, chemistry and quality of other elements of microelectronic devices;
- General measurement of targeted chemical compositions in quality control measurements in manufacturing such as adhesives, plastics, organic chemicals, etc

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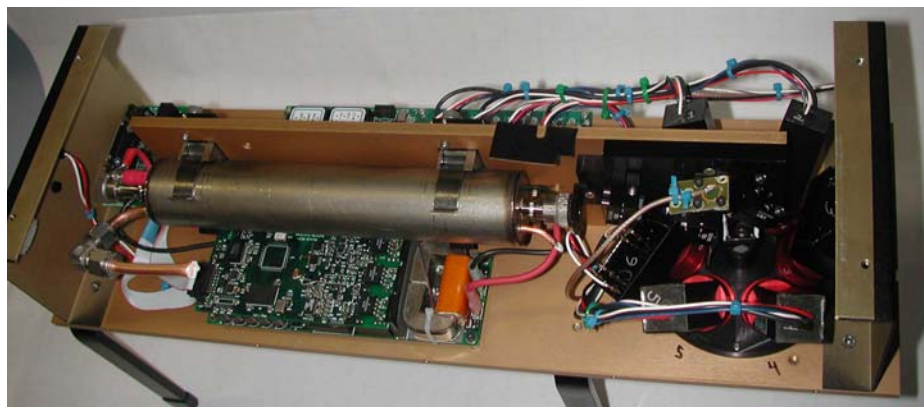
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Instrument Specification:

Self-contained deep UV Chemical Sensor containing:

- High power deep UV laser with power supply and digital controller capable of data sampling rates to 30 Hz.
- Spectral isolation filter for excitation wavelength
- Laser excitation power reference detector
- 6 high gain miniature PMT detection channels used to simultaneously detect a combination of laser induced native fluorescence and Raman emissions.
- A choice of number of fluorescence detection channels with selectable band center, bandwidth and slope matched to the chemical environment of the sensor.
- 7 channel PMT controller with digital gated boxcar integrator and averager containing 64MIP microprocessor with 256K RAM and 256K flash memory.
- Power: 5W standby, 6W full power – can be operated by battery or solar cell array.
- Voltage: 24VDC from battery or line source
- Size: 6” x 4” x 14”
- Weight: 5 lbs
- Control: Ethernet, IEEE485, or RF
- Software: Labview VI operated from PDA or other computer



Photos of Targeted UV Chemical Sensor

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Background on UV laser induced resonance Raman and resonance fluorescence

High levels of chemical specificity can be obtained using Raman spectroscopy without sample preparation, contact, or destruction¹. Raman scattering is, in general, a very inefficient process. Normal Raman scatter cross-sections are about 10^{-26} cm² for a major Raman line. Normal Raman occurs when the excitation wavelength is far from an electronic absorption band of the material. If the excitation wavelength is within a major electronic absorption band associated with the Raman band, the scattering signal is “resonance” enhanced by as much as eight (8) orders of magnitude, such that the scatter cross-section improves to about 10^{-18} cm². In contrast, maximum resonance (native) fluorescence cross-sections, measured over a 30nm wide bandwidth near the peak of fluorescence, are about 10^{-11} cm². This is a factor of 10^7 improvement over resonance Raman and clearly demonstrates the sensitivity of resonance fluorescence compared to resonance Raman. However, much higher levels of specificity can be obtained with Raman.

Resonance bands for nucleic and aromatic amino acids occur in the deep UV between about 220nm and 280nm. When excited at wavelengths less than 250nm, Raman scattering occurs within about 20nm to 30nm above the excitation wavelength, corresponding to about 4000 cm⁻¹. Fluorescence occurs only above about 280nm, independent of excitation wavelength. Between the excitation wavelength at about 280nm, there exists a fluorescence-free region in which to observe the weak Raman scattering signal. A Raman shift of 4000 cm⁻¹ corresponds to a wavelength of 247nm when excited at 225nm, 278nm when excited at 250nm and 298nm when excited at 266nm. It is therefore ideal to combine UV resonance fluorescence and resonance Raman spectroscopy to form an integrated tool for both detection and identification of biological agents since they offer a great combination of sensitivity and specificity that do not share overlapping observation wavebands.

Although resonance fluorescence is not the specific subject of this program, it is an integral part of the overall detection method and is closely tied to the excitation wavelengths used for resonance Raman excitation. Therefore we will include a brief discussion of resonance fluorescence here also.

¹ Cary, P.R. (1982) *Biological applications of Raman and resonance Raman spectroscopies*, Academic Press, New York.

Background on nitrate/nitrite removal

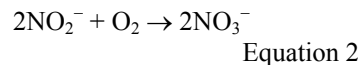
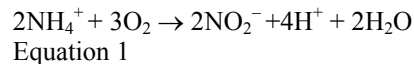
The most common, well accepted and economical approach to accomplish nitrogen removal is biological nitrification and denitrification. Nitrogen enters wastewater treatment plants in the form of organic and ammonium nitrogen, and during nitrification, the nitrogen is oxidized by autotrophic bacteria to nitrate nitrogen. During biological denitrification, biological reactors are operated without oxygen addition so that the bacteria use nitrate as an electron acceptor for their respiration. Various designs are used, and one of the common approaches employs an anoxic tank (anoxic meaning respiration using nitrate in the absence of oxygen) ahead of aeration where recycled nitrate is contacted with influent wastewater to promote its reduction to nitrogen gas.

In recent years a great deal of interest has been focused on treatment plant designs and control strategies which achieve simultaneous nitrification and denitrification in one reactor. To reliably achieve SNdN, the dissolved oxygen levels must be tightly controlled at very low levels. Advantages which can be attributed to SNdN as compared to more conventional nitrogen removal processes include:

1. Reduced tankage requirements (lower capital costs and smaller footprint).
2. Internal recycle not required (lower capital costs and simpler operation).
3. Reduced energy consumption due to improved oxygen transfer driving force.

The major challenge involved in the design and operation of a system to reliably achieve SNdN is the development of an instrumentation and control system which can maintain the required low levels of dissolved oxygen even as influent loading and other operating conditions vary. If too little oxygen is present, then nitrification will be inhibited and the effluent ammonia will increase. If the dissolved oxygen levels are too high then denitrification will be inhibited resulting in reduced energy efficiency and increased effluent nitrate nitrogen. As discussed below, various methods have been employed to control dissolved oxygen levels within the range needed to achieve SNdN, however each of these has significant limitations. The control method that we propose here will dramatically improve reliability and cost effectiveness of SNdN treatment processes by reducing energy consumption as well as the potential for both ammonia bleed through and excess effluent nitrate.

The generally accepted energy yielding two-step oxidation of ammonia to nitrate is as follows by *Nitrosomonas* (Equation 1) and *Nitrobacter* (Equation 2). (Randall et al., 1992):



The total reaction is:



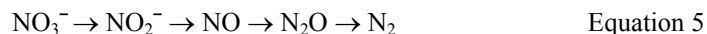
The total reaction shows that 4.57 g O₂ is required per g-NH₄⁺-N oxidized, but when the nitrogen used for cell syntheses is included, the oxygen requirement is 4.3 g O₂/g-NH₄⁺-N oxidized to nitrate (Randall et al. 1992). Temperature, dissolved oxygen (DO) concentration, and ammonia nitrogen concentration all affect nitrification rates. Nitrification kinetics are normally based on ammonia concentrations since nitrite is oxidized rapidly under fully aerobic conditions. The actual growth rate of nitrifiers (μ_N) can be expressed as a function of ammonia and DO by a Monod kinetic equation:

$$\mu_N = \mu_{N_{\max}} \times [(\text{NH}_4^+ - \text{N}) / (\text{K}_N + \text{NH}_4^+ - \text{N})] \times [(\text{DO}) / (\text{K}_O + \text{DO})]$$

Equation 4

At very low DO levels the term [(DO)/(K_O + DO)] approaches zero and no nitrification will occur. Under long periods of very low DO conditions the nitrifiers will be lost from the system.

Nitrate reduction in wastewater systems occurs through assimilation and denitrification. In assimilatory nitrate reduction, nitrate is reduced to ammonia and assimilated for cell synthesis. In denitrification bacteria use nitrate as an electron acceptor in the absence of oxygen to oxidize an organic or inorganic electron donor. Nitrate is reduced to nitrite, to nitric oxide, to nitrous oxide, and to nitrogen in a four-step process (Payne 1981):



The rate of denitrification (R_{DN}) is dependent on temperature and DO concentration, where K is the temperature correction coefficient, which is commonly assumed to be 1.09.

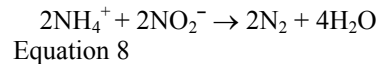
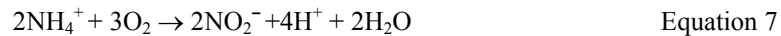
$$R_{DN(T)} = R_{DN(20)} \times K^{(T-20)} \times (1 - DO) \quad \text{Equation 6}$$

Equation 6 shows that the rate of denitrification decreases linearly from 0 to 1 mg/l of DO. At DO levels of 1 mg/l and above the rate of denitrification becomes negligible. From equations 4 and 6, it is apparent that in order to have conditions which support SNdN it is necessary to maintain the DO concentration within a very narrow range. Aeration control methods based on a DO measurement feedback loop have proven to be inadequate to achieve reliable and consistent SNdN operating conditions. The problems with DO measurement alone are twofold. The first problem is that DO instrumentation has historically been unable to provide the necessary accuracy at the very low DO levels required to achieve SNdN (typically in the range of 0 to 0.3 mg/l). A second and more fundamental problem is that the DO measurement alone is not sufficient to define the nitrification and denitrification metabolic conditions within the reactor.

Nitrification and denitrification rates are a function of DO, temperature, pH, solids retention time (SRT), microbial population dynamics, and other factors. For example, one system under a given set of conditions may lose nitrification at a DO level of 0.3 mg/L while another system may continue to nitrify when the measured DO level in the bulk solution is very near 0 mg/L.

Researchers using laboratory bench scale reactors (Kuai and Verstraete, 1998) have demonstrated that the nitrite oxidizers responsible for conversion of nitrite to nitrate are strongly inhibited by low DO concentrations. In this work $[NO_2^-]/[NO_2^-+NO_3^-]$ ratios were found to be in the range of 0.9 to 1.0 under oxygen limiting conditions. Under fully aerobic conditions nitrite concentrations are typically very low and the corresponding ratio of $[NO_2^-]/[NO_2^-+NO_3^-]$ ratio will also be very low (<0.1).

Other researchers have also observed that at very low DO concentrations a “Nitrite Shunt” may be occurring (O’Neill and Huren, 1995) in which nitrite is produced from nitrification without nitrate formation due to inhibition of the nitrite-oxidizing bacteria by the low DO concentration. The removal of NH_4^+ under these conditions is assumed to occur in a 2-step process as follows:



With the overall reaction being:



The partial oxidation of ammonia to nitrite as described by Equation 7 has been demonstrated using the SHARON process (Mulder et al., 2001). The SHARON (Single reactor High activity Ammonia removal Over Nitrite) process accomplishes the partial oxidation to nitrite by operating the reactor at elevated temperatures (>30 °C) to inhibit oxidation of nitrite to nitrate by *Nitrobacter* bacteria. The metabolic reaction described in Equation 8 has also been called anaerobic ammonia oxidation or “Anammox” (Jetten et al., 2001). Based on the above stoichiometry, the removal of ammonium nitrogen via the nitrite shunt pathway results in a 63% energy savings versus conventional nitrification processes (Kuai and Verstraete, 1998), and nearly 40% versus conventional nitrification-denitrification processes.

Regardless of whether SNdN is a result of the formation of anoxic regions within an otherwise aerobic environment due to incomplete mixing, a result of diffusional limitations on the oxygen transfer into the floc (causing an anoxic region within the floc itself), distinct metabolic processes (such as the “anammox” and “nitrite shunt” pathways), or a combination of these factors, the measurement and control system that we propose is ideally suited to maintaining conditions in a reactor to maximize the potential for reliably and efficiently achieving SNdN. An aeration system control algorithm which we will be possible using our proposed UVRN nitrate/nitrite monitoring system will utilize high and low setpoints for the ratio of nitrite to nitrate in order to maintain aeration supply rates within the very narrow range necessary to reliably achieve SNdN and possibly promote the nitrite shunt pathway at ambient temperatures to further enhance the potential for energy savings.

Commercial Opportunities



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According to the U.S. Environmental Protection Agency, 25% of all U.S. households and 33% of new development are served by onsite and decentralized treatment systems. In some areas of the country, onsite system failure rates are high resulting in water quality and public health concerns. However, for many communities, it can be cost prohibitive to transport wastewater to a centralized municipal treatment facility. Furthermore, as water supplies become scarcer and costs increase, many communities are looking for wastewater treatment solutions that enable recycle and reuse. The high cost of piping reclaimed wastewater through miles of distribution lines from a centralized wastewater reclamation plant back to where it is needed also stands in the way of implementing cost effective reuse strategies. A technology which is receiving increasing interest for wastewater treatment and reuse for small communities and decentralized systems is the membrane bioreactor (MBR) treatment process. In order to comply with reclaimed water reuse standards it is necessary to meet strict effluent limits for nitrogen. In order to be cost effective, small decentralized wastewater reclamation systems must be capable of reliable performance while at the same time being easy to operate. Improved instrumentation and automated control systems are essential to achieving these objectives and in order for decentralized MBR systems to gain wide acceptance. The instrumentation and control system that we propose based on UVRR for monitoring of nitrate and nitrite will be ideally suited to achieving reliable SNdN and energy savings with minimal operator attention using MBRs.

Another significant commercial opportunity for our proposed monitoring and control system is its application to the retrofitting of existing treatment plants for improved nitrogen removal efficiency and reductions in energy requirements. As we illustrate by examples presented in Section 9 of this proposal, the energy savings that will be possible using our more robust instrumentation system will be very substantial. Our system could also be used with MBRs, similar to those described above, for treatment of high nitrogen side streams in larger treatment plants. The treatment of these sidestreams would be of particular interest for the implementation of operation and control strategies aimed at achieving the nitrite shunt metabolic pathway using our nitrate/nitrite monitoring system.

Follow-on research and commercialization efforts will include development of other special purpose low cost UVRR instruments for detection of compounds of interest in water supplies such as algal biotoxins, disinfection by-products, and other toxic materials which could be accidentally or deliberately introduced.

UV Raman Prototype Instrument

Members of our team have previously demonstrated, nitrate and nitrite have strong UV resonance Raman shift lines due to the symmetrical N-O stretch vibrational modes at 1044 cm^{-1} and 1325 cm^{-1} respectively (Ianou, Coleman, and Asher, 2002). Raman spectra for nitrate in water over a range of concentrations are shown in Figure 1. Similar spectra for nitrite in water are shown in Figure 2. As can be seen in Figure 1, nitrate also has a weaker Raman shift band between 1300 and 1400 cm^{-1} . While this band overlaps the strong nitrite band at 1325 cm^{-1} , it will be proportionate to the nitrate band at 1044 cm^{-1} . Therefore, its contribution to the intensity of the band at 1325 cm^{-1} can be subtracted in order to determine the sample's nitrite concentration.

The Raman shift bands for nitrate and nitrite in a filtered wastewater sample matrix in the low concentration ranges are shown in Figure 3 and Figure 4, respectively. As can be seen in these figures, the detection limits for both nitrate and nitrite were approximately 0.2 ppm under the experimental conditions used in our previous research. In these experiments we used an intracavity frequency doubled argon ion laser with 10 mW output at 229 nm and a very high quality spectrometer coupled to a liquid nitrogen cooled intensified CCD detector. This kind of instrument proved excellent for producing UV Raman spectra, however it has a serious drawback in terms of its cost and power consumption. The above laser cost is nearly \$100,000, requires 15 KW of input power, and a constant supply of cooling water. The CCD detector costs \$50,000.

Clearly, a different approach must be taken if we are to develop a low cost on-line or portable UVRR instrument for real time monitoring of nitrate and nitrite in wastewater systems or other environmental sample matrices. Instead of generating entire spectra using a multi-channel CCD detector, the instrument that we propose to develop and assemble under this task will utilize dielectrically coated, ultra-narrow-



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band, angle tuned filter technology, developed by Photon Systems, and ultra-sensitive PMT detectors to measure the intensities of only the discrete spectral bands needed to measure the concentrations of nitrate and nitrite (1044 cm^{-1} and 1325 cm^{-1} , respectively). The filtered output of the PMT detector will be integrated in a digital gated boxcar integrator developed by Photon Systems. A software algorithm will be developed to adjust for any contribution that nitrate may have in the spectral band for nitrite at 1325 cm^{-1} .

A Photon Systems, 224nm, HeAg laser will be used as the excitation source. The HeAg laser has lower average but similar quasi-cw peak power output compared to a frequency doubled argon ion laser used in the previous research described above. The doubled argon ion laser had a 10 mW CW output at 229 nm, and we used a 10 minute accumulation time on a CCD array detector.

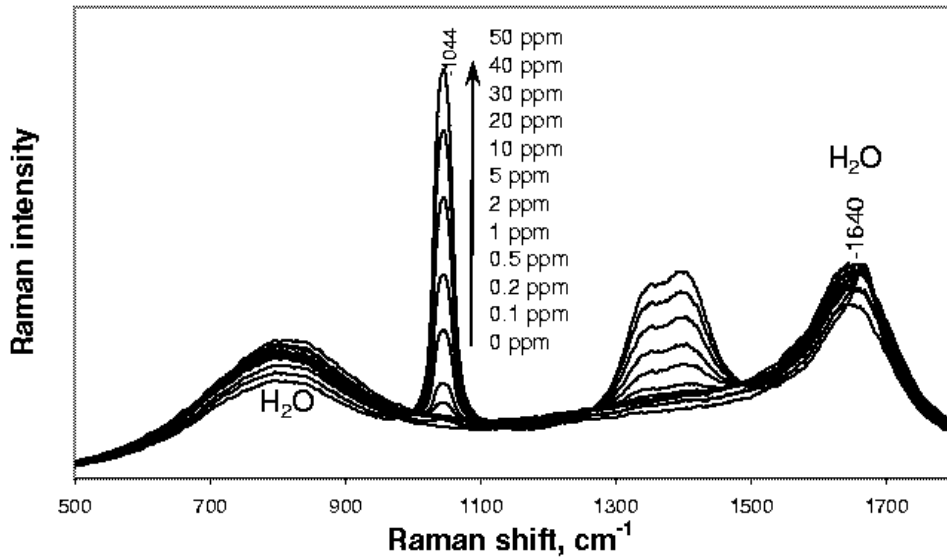


Figure 1 UV resonance Raman spectra of NO_3^- in pure water for a range NO_3^- concentrations as N (from Ianoul, Coleman, and Asher, 2002).

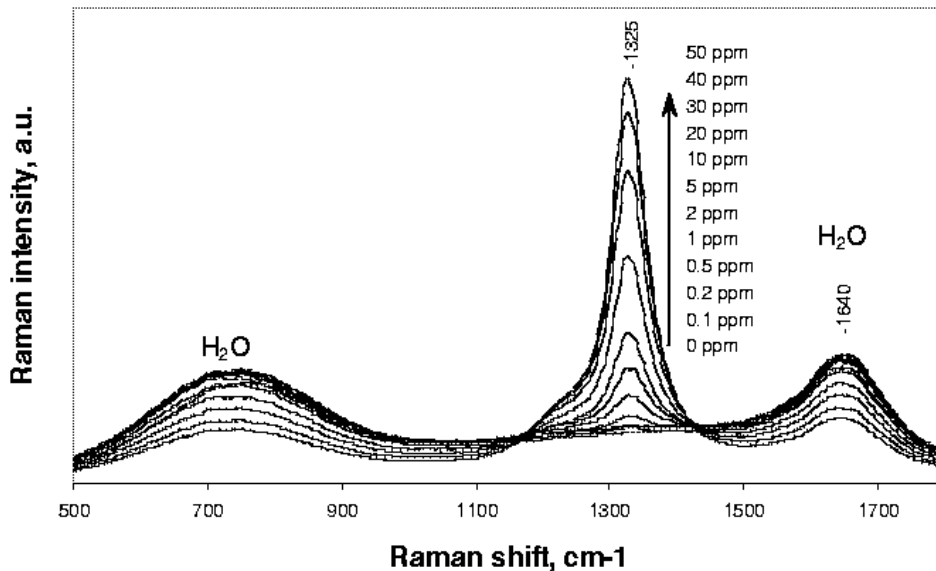


Figure 2 UV resonance Raman spectra of NO_2^- in pure water for a range NO_2^- concentrations as N (from Ianoul, Coleman, and Asher, 2002).

to achieve 200 ppb detection limits for nitrate or nitrite. This corresponds to excitation energy of 0.6 joules. In the proposed nitrate/nitrite monitoring instrument we plan to use the 40mW quasi-cw output of a HeAg laser with a 100 microsecond PMT output integration time. A 10^7 gain PMT is sampled by a digital gated boxcar integrator to detect the Raman emission.

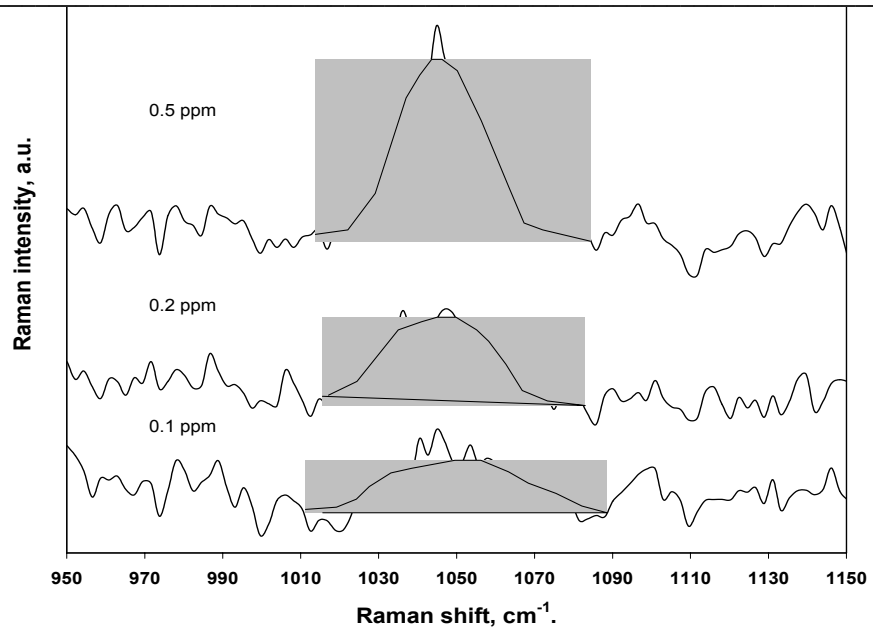


Figure 3 NO_3^- detection limits in wastewater sample matrix using UVRR (from Ianoul, Coleman, and Asher, 2002)

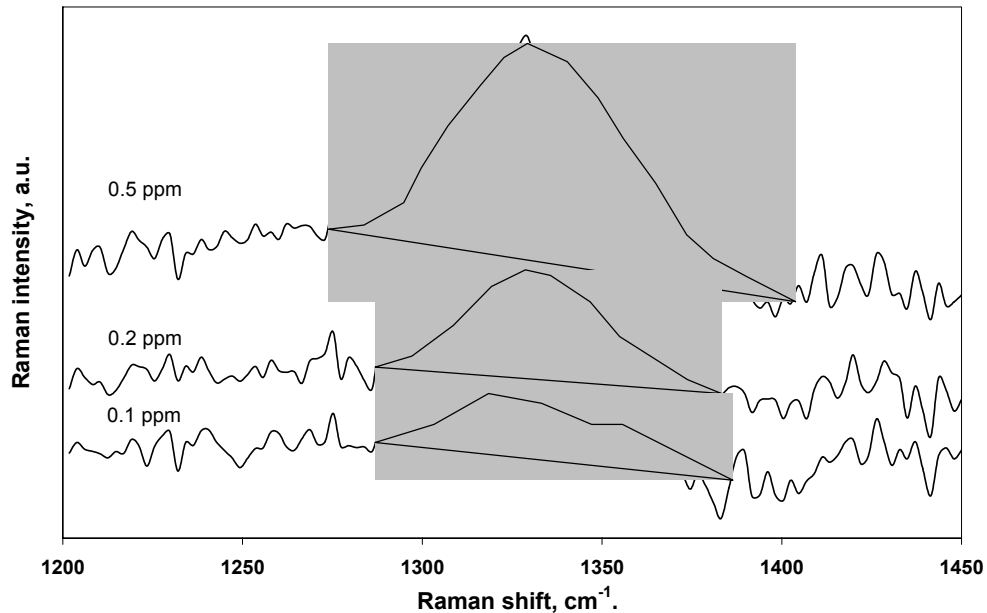


Figure 4 NO_2^- detection limits in wastewater sample matrix using UVRR (from Ianoul, Coleman, and Asher, 2002)

The ultimate detection limit should be at least 30 times lower than that of the frequency doubled argon ion laser system. With digital averaging of 10 pulses within a 1 second period, the detection limit can be

improved to over 100 times lower than the previous results. If necessary to further improve the detection limits, the accumulation time can be increased up to 5 or 10 seconds without adversely affecting the ability to achieve real time monitoring and control of the S_NdN nitrogen removal process. A schematic diagram of the proposed low cost UVRR instrument is shown in Figure 5, below.

Other materials that this TUCS instrument can

In addition to UV Raman, this TUCS instrument utilizes fluorescence of materials for identification. Although fluorescence is not known for its chemical specificity, by choice of the number of spectral detection bands and their bandwidth and band location, a significant level of chemical specificity can be achieved. Below, in Fig. 5, six detection bands are shown that are used in our TUCS instrument. We can alter these bands but the following selection is a good example of what level of specificity is achievable.

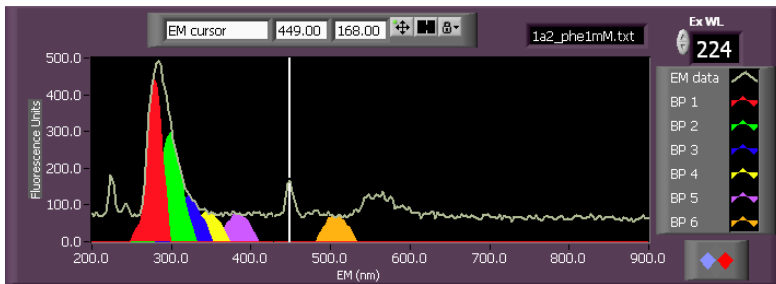


Figure 5. Illustration of six resonance fluorescence detection bands

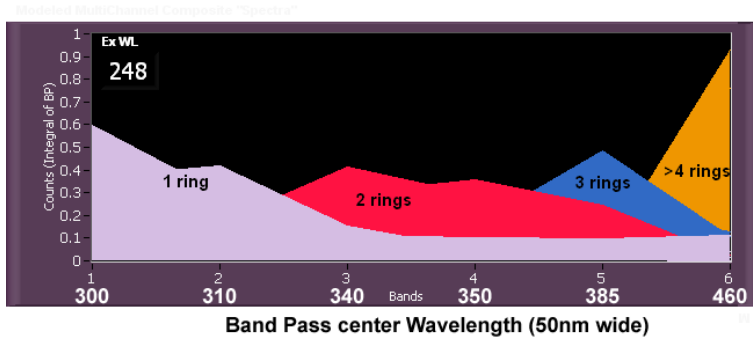
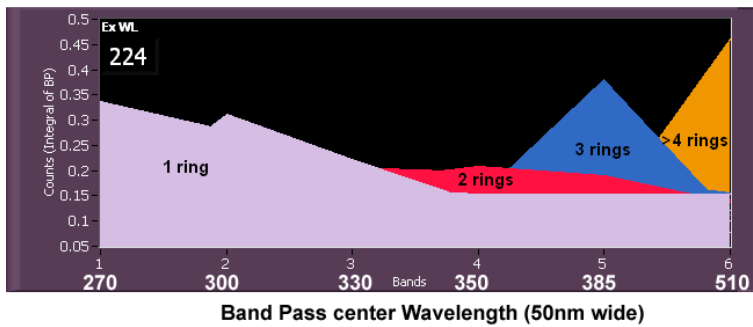


Figure 6. Resonance fluorescence for 1 through 4 ring chemical structures with excitation at 224nm and 248nm. (Courtesy of Pan Conrad and Rohit Bhartia, Caltech/JPL)

The following figure shows the ability to distinguish multi-ring polycyclic aromatic hydrocarbons using resonance fluorescence measured in six bands.

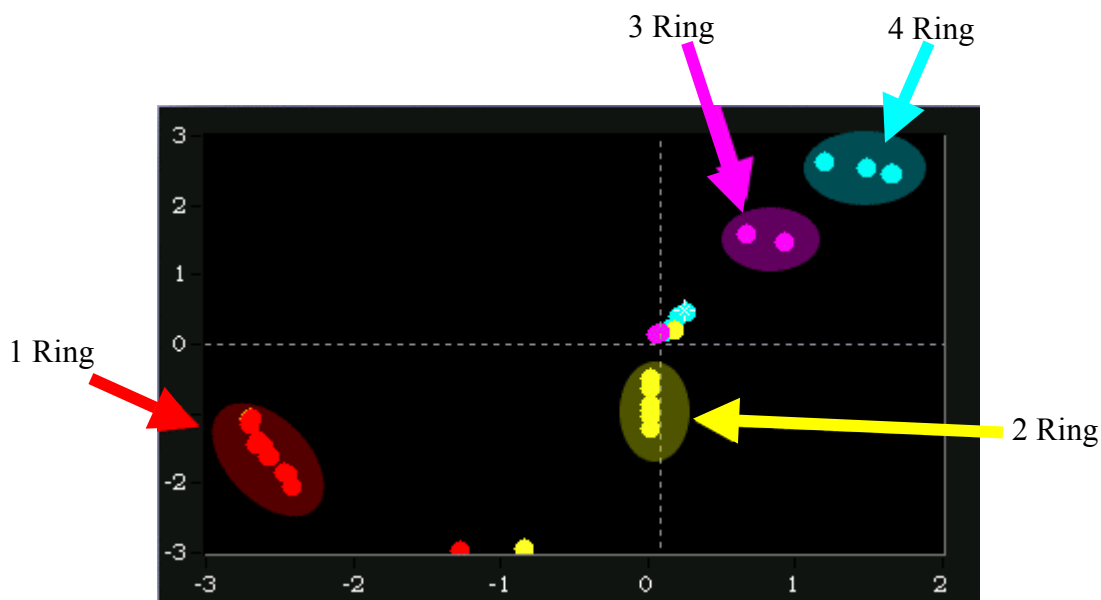


Figure 7. Six-band resonance fluorescence detection and classification of polycyclic aromatic hydrocarbons using simple band differencing algorithm. (Courtesy of Pan Conrad and Rohit Bhartia, Caltech/JPL)

Forty three (43) different PAH's are compared in the above illustration. Variances within each Ring Group are due to functional groups (-methyl, dimethyl, etc.). Outliers are a result of addition of conjugated bonds (C=C) or non-aromatic rings.

Although we are far from optimizing the performance of this instrument, Fig. 7 demonstrates a considerable level of specificity, while at the same time having sensitivity better than one femtomole ($1/10^{15}$) and perhaps as high as one attomole ($1/10^{18}$).

Within each Ring Group we are able to further differentiate molecules such as aromatic amino acids from single benzene-ring structures. We are able to clearly differentiate between spores, vegetative cells and virus?. And these can be clearly differentiated from minerals and other background materials.