DIRECTLY MODULATED DIODE LASER FREQUENCY DOUBLED IN A KTP WAVEGUIDE AS AN EXCITATION SOURCE FOR CO₂ AND O₂ PHASE FLUOROMETRIC SENSORS

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ABSTRACT
A directly modulated infrared diode laser was frequency doubled in an intracavity KTP quasi-phase-matched waveguide, producing modulated laser light at 425 nm, which was subsequently employed for phase fluorometric detection of CO₂ and O₂ gases. The frequency-doubled source was coupled to a single optical fiber to deliver the excitation light to the sensor mounted at the fiber tip. Fluorescence from the sensor returned through the same fiber to a photodetector. This compact, low-power source provided continuously tunable modulation frequencies up to 200 MHz. In the detection of CO₂, the doubled infrared diode laser produced 425-nm light that was modulated to a depth of 27% at a modulation frequency of 95 MHz.

Key Words waveguide; frequency doubling; phase fluorescence detection; oxygen sensors; carbon dioxide sensors; fiber optic sensors.

1 INTRODUCTION
Optical sensors, based on fluorescence intensities or lifetimes, have been developed for oxygen and carbon dioxide as an alternative to electrode methodologies. The rapid and accurate determination of physiological concentrations of biologically important gases like O₂ and CO₂ has recently been demonstrated using the technique of phase-modulation fluorometry. In phase fluorometry, the phase of the modulated fluorescence of the sensor molecule is detected relative to the phase of the intensity-modulated light used for excitation. The presence of the analyte gas results in a change of the quenching of the fluorescence, which causes a change in fluorescence lifetime and hence a change in the relative phase of the modulated fluorescence. The change in phase is measured and calibrated relative to the concentration of the analyte gas. The phase change is insensitive to large changes in optical intensity, making phase fluorometry a promising technique for biosensors. The main emphasis in this paper is on the demonstration of a novel and practical optical source for phase fluorometric sensing of O₂ and CO₂. Although we demonstrate this source with O₂ and CO₂ sensors, it should be noted that the source could be used with any sensing fluorophore requiring blue excitation, high modulation frequencies, or coupling into a single fiber optic.

To make this optical sensor technique practical in a wide variety of settings requires a compact, efficient, reliable, and low-cost instrument. For use in clinical settings, small size and low power requirements are especially important, as bedside space is limited, and must be shared with other apparatus. In addition, for measurement of arterial blood gases, the sensor should fit into a 20-gauge arterial catheter, requiring excitation through single optical fibers. A small sensor size is also useful for measuring local concentrations, and usually requires a single optical fiber. These constraints, coupled with those imposed by the sensor fluorophores and the application, will place restrictions on the optical source. The absorbance spectrum of the sensor fluorophores will determine the wavelength of the source. Although some fluorophores have been de-
veloped that absorb toward the red end of the optical spectrum, most currently available fluorophores absorb in the blue and green portions of the optical spectrum and emit at longer wavelengths. If the excitation is not at the maximum of the absorbance, using light of a shorter wavelength will still produce fluorescence, but less than that obtainable with excitation at the absorbance maximum. Although excitation of the fluorophore is possible to the red of the absorbance maximum, it will result in much less fluorescence compared with blue excitation since the absorbance is usually very low to the red of the absorption maximum. In addition, the red excitation wavelength will then be close to the wavelength of the fluorescence maximum, and expensive optics will be required to exclude the excitation from the emission detector.

Optical fibers are useful for delivering light to regions with small access areas (such as needles), to remote sites, and to sites which may contain electromagnetic interference. Introducing significant amounts of optical power into a fiber requires that the optical source be focusable, or the use of fiber optic bundles. Fiber bundles are orders of magnitude more expensive than single fibers, much more bulky, and cannot be used in applications requiring a small sampling area. Further, one can envision using multiple fiber sensors fed by a common optical source.

If multiple sensors are excited simultaneously, significant optical power is required from the source. This is especially true if low-cost, low-voltage photodiode detectors are used instead of more expensive photomultiplier tubes, which require high voltage supplies. The modulation of the optical source should be simple and efficient for modulation frequencies up to 100 to 200 MHz. In certain sensing applications, it would be desirable to make a sensor for O_2 and CO_2 using one optical source that could easily be modulated at two frequencies, one for the O_2 sensor and the other for the CO_2 sensor.

Semiconductor optical sources, such as light-emitting diodes (LEDs) and laser diodes (LDs), satisfy many of these requirements and are potentially the most promising optical sources for these types of sensing instruments. In fact, the synthesis of molecules with a large quantum yield, long lifetimes, and sensitivity to O_2 has permitted use of blue LEDs as an excitation source for phase fluorometric detection of O_2. However, the coupling of the output of a high-power LED into a fiber is poor due to mode mismatch problems, making it difficult to develop a single-fiber-based O_2 sensor. A chemical system sensitive to CO_2 has been developed which requires excitation wavelengths in the blue or green portion of the spectrum, but the lifetime of the molecule used in the detection of CO_2 is much shorter, requiring modulation frequencies outside the range provided by conventional blue LEDs. Thus it would appear that laser diodes are ideal sources for such sensors because by modulating the driving current to the laser diode, one can easily modulate the optical output of the laser to greater than 1 GHz. Laser diodes are compact and emit high optical power in diffraction-limited beams that can be efficiently coupled into single-mode optical fibers.

Mass production has led to the availability of reliable laser diodes for under $100. Blue-emitting diode lasers based on II–VI materials, in particular ZnSe and its dopants have been demonstrated. While this approach is promising, there are significant technical issues that must be overcome before it can become commercially practical. First, extended defects that grow from the substrate severely limit the lifetimes of these devices. The longest lifetimes that have been achieved to date are less than 1 hour. High contact impedance at the p-type layer is a related problem, which results in significant power dissipation and limits modulation frequencies to less than 1 kHz. Infrared laser diode technology had to overcome similar difficulties to become commercially viable, but it is fair to say that the long-term commercial viability of blue-green laser diodes remains uncertain at this point. We note that extensive research has been carried out with the goal of making blue emitters from GaN. Although this has resulted in the blue light-emitting diodes, as yet no current injected laser diodes have been reported, and these efforts are considerably behind the II–VI materials research.

### 2 Frequency-Doubled Infrared Diode Laser Sources

We have extended a very successful approach which consists of frequency doubling of infrared laser diodes to optical frequencies in the blue portion of the spectrum. The advantages of this approach are that infrared laser diodes have desirable attributes including reliability, high output power, focusability, compactness, ease of modulation, and low production costs. The frequency-doubled light is automatically modulated by directly modulating the laser diode. In general, the frequency doubling efficiency of the nonlinear optical materials used for this purpose is low. A near-infrared diode laser was frequency doubled by focusing in a potassium dihydrogen phosphate (KDP) crystal, with the 50 nW of doubled light used to excite an intensity-based oxygen sensor. A frequency-doubled source has been previously used for phase fluorometric detection; however, it produced only 220 nW of average power. To increase the efficiency of the optical frequency doubling process while retaining the compact size of the optical source and ease of modulation, we have used frequency doubling optical waveguides made from the nonlinear optical material potassium titanyl phosphate, KTiOPO_4 (KTP). Among other factors, the efficiency of the optical frequency doubling process is proportional to the
optical intensity in the doubling material. Since waveguides can confine the optical field for extended lengths in much smaller dimensions (on the order of a few wavelengths of the light) than can bulk material, an optical waveguide has the potential of being more efficient than bulk material. This implies that one must be able to couple the fundamental (infrared) light efficiently into the optical waveguide.

However, a more difficult condition to meet is matching the phase velocity of the infrared (fundamental) wave and the blue (doubled) wave in the nonlinear material. In general the optical field at the doubled frequency increases linearly with the length of interaction up to the coherence length, \( l_c = \frac{\lambda_f}{2\Delta n} \), where \( \lambda_f \) is the wavelength of the fundamental in vacuum and \( \Delta n \) is the difference between the index of refraction of the nonlinear material at the fundamental and the doubled frequencies. For most materials, the coherence length is only a few micrometers at near-infrared wavelengths, severely limiting the conversion efficiency. For some birefringent nonlinear materials, one can choose different axes along which to propagate the fundamental and second harmonic to achieve an effective coherence length, \( l_{\text{eff}} \), of millimeters or even centimeters depending on the material and specific implementation.\(^{18}\) This leads to efficient second harmonic conversion. Waveguide modes have very different phase velocities than the bulk material and the mode velocities are highly dependent on the polarization of the optical field, destroying the phase match that one can achieve in the bulk.\(^{19}\) However, for waveguides, the technique of quasi-phase matching (QPM) has been used in a variety of materials including LiNbO\(_3\) and KTP.\(^{12,13}\) QPM is a technique whereby the direction (sign) of the optical nonlinearity is periodically reversed.

To understand the technique of QPM, the equation \( l_c = \frac{\lambda_f}{2\Delta n} \) is still valid but \( \Delta n \) is now the difference in the effective index for the waveguide mode at the fundamental frequency and that at the second harmonic. Making the period of domain reversal \( \Lambda = m l_c \) (where \( m \) is an integer) is equivalent to phase velocity matching. This technique has been perfected in KTP by Bierlein and co-workers at DuPont, leading to effective coherence lengths \( (l_{\text{eff}}) \) of 4 to 5 mm, resulting in up to 3 mW of doubled output from a commercially available infrared laser diode.\(^{14}\) In KTP, the method of achieving this domain reversal involves using standard photolithographic techniques to periodically mask areas along the surface of a KTP crystal, resulting in alternating masked and exposed sections on the KTP surface. The width of these sections is approximately 4 \( \mu \text{m} \), chosen to provide a large waveguide mode for ease of coupling in the 800-nm region. The period \( \Lambda \) of the sections is also about 4 \( \mu \text{m} \) and is chosen to satisfy \( \Lambda = m l_c \) for efficient frequency doubling. The K in the crystal is then replaced with Rb/Ba in an ion exchange process that produces the domain reversal and changes the index of refraction for waveguide confinement.\(^{15}\)

As shown schematically in Figure 1, the laser diode is coupled to the KTP waveguide. By using a laser diode with an antireflection coated front facet, the waveguide becomes part of an extended laser cavity. Some mechanism is then required to provide feedback. Figure 1 shows a bulk grating that provides feedback, causing the laser to oscillate at the frequency determined by the grating. In this way the grating can tune the laser wavelength to the quasi-phase matched wavelength (determined by \( \Lambda = m l_c / (2\Delta n) \)). By placing the waveguide inside the cavity, more blue light can be generated due to the larger optical fields existing inside the laser cavity. A second refinement of this scheme is made possible by adding a second section to the waveguide.

The ion exchange process also produces a change in index of refraction between the masked and unmasked regions. The periodicity will then cause high reflectivity at wavelengths that are integer multiples of the half-wavelengths of the light in the material. This can be expressed as the Bragg condition where the periodicity in the second section, \( B \), is such that \( B = m' \lambda_f / (2n) \) where \( m' \) is an integer (different from \( m \)) and \( n \) is the effective index of the waveguide mode. Thus one can adjust \( B \) (by lithography) independently of \( \Lambda \) and satisfy both quasi-phase matching and high reflectivity at the same wavelength \( \lambda_f \). This second section provides the feedback mechanism for the laser diode, leading to a much more compact device, as shown schemati-
cally in Figure 2. We will demonstrate both configurations in our experiments described in the following sections. Recently T. Tohma and J. D. Bierlein demonstrated an even more compact structure on the KTP waveguide forms the end of the extended laser cavity length (L). The laser output was focused into the KTP waveguide with a focusing lens (FL) and out of the waveguide with a collimating lens (CL). The dichroic beam splitter (BS) directs the 425-nm radiation to the sensor (S) in the cuvette. The fluorescence is detected by the photomultiplier tube (PMT) and the electrical phase is measured by the network analyzer (NA).

3 FIBER OPTIC OXYGEN SENSOR

We have previously demonstrated the detection of oxygen using phase fluorometry. The sensor material used was tris(4,7-diphenyl-1,10-phenanthroline) ruthenium(II) entrained in silicone.\(^6\) The fluorophore lifetime ranged from approximately 3.0 µs at 0% O\(_2\) to approximately 1.3 µs at 0% O\(_2\). Because of these extremely long fluorescent lifetimes, the modulation frequencies required to measure a large phase change upon the introduction of oxygen were less than 100 kHz. To adapt this sensor to a single-fiber optic-based system required a replacement for the blue LED optical source used in Ref. 6 because the LED does not couple well to single optical fibers. We used the frequency-doubled source shown schematically in Figure 1 as our optical source for the fiber optic-based sensor. The laser diode was commercially available (Spectra Diode Labs model SDL 5422 C), and rated at 100 mW of output power. The peak of emission was at 850 nm. As shown in Figure 1, the laser diode was coupled to the KTP waveguide with lenses and the wavelength was controlled by a grating forming the external cavity end reflector, with the first-order diffraction fed back to the laser diode. Thus the laser diode, lenses, KTP waveguide, and grating all form an external cavity. By adjusting the angle of the grating, the laser diode emission wavelength can be tuned. The laser output wavelength was adjusted to the peak of the second harmonic conversion for the quasi-phase-matched waveguide. The dichroic beam splitter inside the cavity reflected the light at 425 nm with 90% efficiency. Doubled output power after the beam splitter was 100 µW, although we typically operated with output powers from 10 to 20 µW. Using the 100-µW value, conversion efficiency was 0.1%. It should be noted that the sample waveguide we utilized was not the most efficient that has been produced.

The sensor was formed at the common end of a multimode fiber coupler with a 100-µm core diameter (Gould Fiber Optics). There is about 40 dB of isolation between the two arms of the fiber coupler. To form the sensor, the fiber was cleaved and the cleaved end dipped into silicone rubber and allowed to cure. Immersion in a solution of the Ru(II) complex in chloroform caused the silicone to expand while absorbing the dissolved Ru(II) complex. The fiber was then removed from the chloroform solution, trapping the Ru(II) complex within the silicone matrix as the chloroform evaporated and the silicone contracted to its original form. Finally, the sensor was washed with ethanol to remove any dye remaining on the matrix surface. The 425-nm light from the frequency-doubled laser was focused into one arm of the multimode fiber coupler. Accounting for coupling efficiencies and losses in the fiber, we estimate that the blue power delivered into the sensor matrix was about 4 µW. Since the diameter of the fiber is small, the intensity was sufficient to obtain a good signal-to-noise ratio for the data even with this small input power. The fluorescence was collected from the other arm of the fiber coupler. Any stray excitation was eliminated using a long-wavepass filter with a cutoff at 600 nm (Andover, Salem, MA). The fluorescence was detected by a Hamamatsu (Bridgewater, NJ) model HC120-07 compact PMT with an integrated high voltage supply and 200 kHz bandwidth amplifier. The signal output from the PMT was connected to a Princeton Applied Research (Princeton, NJ) model 5110 dual phase lock-in amplifier. The amplitude and phase data from the lock-in were collected by an Apple Macintosh computer using an A/D card to digitize the signals. The lock-in amplifier has a phase noise of 5 mdeg rms at 1 kHz.

The response of the oxygen sensor was calibrated in both air and water. The sensor was suspended in a 2-liter spinner flask and exposed to a mixture of O\(_2\) and N\(_2\), with the exact mix of the gases controlled by flowmeters. The total flow rate was maintained at 5 liters/min. Since the flowmeters were accurate to ±5% of full scale, most of the experimental error can be attributed to this source. The phase change in the output was recorded as a function of the percentage of oxygen. The total phase shift was about 23 degrees for a 0 to 100% oxygen change. Obviously, this includes the ranges for
bioreactors (0% to atmospheric concentrations) and oxygen sensing in clinical settings (0 to approximately 35%).\textsuperscript{5} The calibration curve is very similar to the one obtained in the gas phase.\textsuperscript{6} Figure 3 shows the response of the sensor to alternate purging by 100% O\textsubscript{2} and 100% N\textsubscript{2}, with phase and modulation values recorded every 30 ms. This single-fiber sensor exhibits the same robust features of the oxygen detector in Ref. 6, including sensitivity, stability, and repeatability.

4 CO\textsubscript{2} Sensor

We have also demonstrated a CO\textsubscript{2} sensor based on phase fluorometry.\textsuperscript{7} This sensor was in turn based upon a colorimetric sensor.\textsuperscript{20} Our CO\textsubscript{2} sensor was demonstrated using a commercial apparatus, an ISS K2 multifrequency phase fluorometer, with a helium cadmium laser emitting at 442 nm. The laser emission was externally modulated with a Pockels cell driven by a 25-W rf amplifier. Although these experiments demonstrated the conceptual possibilities for a sensor, the modulation and detection apparatus are too costly and bulky for a practical sensor.

Although the blue LED emits at useful wavelengths for this sensor, its modulation bandwidth was too limited to fully exploit our CO\textsubscript{2} sensor system, which is based on a lifetime change produced by fluorescence resonance energy transfer (FRET). The fluorophore lifetime range from FRET involves the nonradiative energy transfer of excited state energy between a donor and acceptor when the molecules are in close proximity to each other,\textsuperscript{7} and it can be a very efficient process. An acceptor molecule is chosen so that its absorbance depends on the concentration of gas to be sensed. Using FRET then allows the fluorescent donor molecule to be chosen for quantum efficiency, excitation and emission wavelengths, without constraining it to also be sensitive to the analyte gas. For efficient FRET, the emission of the donor must overlap the absorbance of the acceptor. In our CO\textsubscript{2} sensor, the donor molecule is sulforhodamine 101 (SR101) and the acceptor is pH-sensitive dye, m-cresol purple (MCP), both of which are mixed together in a phase transfer agent and then incorporated into an ethyl cellulose film.

In this sensor CO\textsubscript{2} diffuses through the cellulose film and forms carbonic acid with the water associated with the phase transfer agent, lowering the pH value. The absorbance spectrum of the acceptor is pH dependent, and thus the introduction of CO\textsubscript{2} leads to a decrease in the rate of the FRET, resulting in an increase of both the fluorescence intensity and lifetime. Again the phase fluorometry technique detects this change in lifetime by detecting the change in phase between the modulated excitation light and the modulated fluorescence. The phase of the modulated fluorescence is detected relative to the excitation light. Thus the introduction of CO\textsubscript{2} causes an increase in this relative phase. The phase is calibrated relative to the concentration of the gas. The average fluorophore lifetime is about 2 ns at 5% CO\textsubscript{2} and about 0.5 ns at 0% CO\textsubscript{2}.

The frequency-doubled diode laser source and experimental configuration is shown schematically in Figure 2. The source differs from that used in Figure 1 in one significant respect. As previously described, the frequency doubling section is followed by a DBR in the KTP waveguide. The DBR section diffracts the waveguide section into the backward direction, providing feedback for the external cavity laser. Again, the DBR section is fabricated so that the wavelength of the laser matches the quasi-phase-matched wavelength for efficient frequency doubling. By eliminating the bulk grating, we achieved a very compact structure on the order of 5 cm long. Further, based on simple theoretical estimates, the modulation bandwidth of this device should be about 1 GHz. We experimentally verified that it had a bandwidth exceeding 200 MHz.

The diode laser used was a 100-mW laser with an antireflection coating on the front facet (SDL AR 5419C). The front facet reflectivity was around 3% whereas the back facet had >95% reflectivity. The laser was biased at 89.89 mA, with an ac signal superimposed on the dc using a Bias-Tee (Picosecond Pulse Labs). The ac voltage was generated by the output channel of the network analyzer (HP 3577A/B), and was amplified using a 1-W, 40-dB amplifier (Mini circuits ZHL-4240). The output voltage from the amplifier was nearly 1.9 V. To obtain better frequency response, the transmission line driving the diode laser was terminated with a 47-ohm resistor. It is estimated that the ac current modulating the laser diode was about 40 mA. The laser output was collimated using a Fujinon (Wayne, NJ) lens and focused onto the segmented KTP channel waveguide. The laser was focused onto the crystal using a 12-mm focal length lens (Newport FL10B). The output beam was recollimated.
mated using a similar lens. The output power was optimized using an optical power meter. A dichroic beam splitter was positioned at 45° to the output beam. The beam splitter transmitted the infrared beam and reflected the blue, thus separating the fundamental and the second harmonic. The transmitted beam was absorbed in a beam dump, thus reducing the scattered light. The blue optical power was typically 40 μW, giving an overall conversion efficiency of 0.04%. As stated with the O₂ sensor, the sample waveguide we utilized was not the most efficient that has been produced, and the optical power was not optimized. The infrared residue in the second harmonic was further attenuated using short-wavepass filters (Schott Glass BG 39). The blue light was then allowed to impinge on the sensor held in a plastic cuvette. To minimize reflections from the cuvette surfaces, the surfaces were cut so that the optical signal was not reflected off the cuvette. Gas mixtures produced with flowmeters were introduced into the top of the cuvette. Since the flowmeters were accurate to ±5% of full scale, most of the experimental error can be attributed to this source.

The sensor film was coated onto a glass slide which was placed at a 45 degree angle to the cuvette face so that the reflections from the slide itself were directed away from the photomultiplier-tube (PMT) detector. The detector was placed at 90 degrees to the excitation beam, allowing efficient collection of the fluorescence while reducing interference from the much stronger blue excitation light. A PMT (Hamamatsu R928) biased at −1100 V was used as a detector. The output of the PMT was amplified by a 20-dB low noise amplifier and connected to the input of the network analyzer. The resolution bandwidth of the analyzer was set to 1 Hz to improve the signal-to-noise ratio. The network analyzer measured both the magnitude and phase of the fluorescence signal with respect to its internal reference signal. The manufacturer states that phase measurements are accurate to 1 degree. Typically 32 samples were taken and a weighted average was performed to reduce the scatter in measurements. We estimated that about 10 pW of fluorescence power was incident on the PMT.

Experiments were performed to characterize the CO₂ sensor and compare the results with those obtained from the ISS phase fluorometer. The fluorescence was quenched and unquenched using CO₂. The sensor is quenched (has least fluorescence) with no CO₂. Both the magnitude and phase responses were recorded for 0 and 5% of CO₂ in an atmosphere of N₂. This range is useful for measurements of bioreactor off-gases, but only measures to median levels in a clinical situation. Since the range of the sensor can be easily changed by replacing the acceptor dye in the sensor mixture, the range can be extended to include all clinical levels. Flowmeters were used for precise mixing of N₂ with CO₂ to obtain the exact percentage of CO₂. Since the phase change is dependent on frequency of modulation, the data were collected at six distinct frequencies spanning the frequency range from 15 to 95 MHz.

Figure 4 shows the measured phase difference between 5 and 0% CO₂ as a function of modulation frequency averaged over three different data sets taken on separate days. Measurements were taken at 95.42 MHz, the frequency at which the greatest phase change occurs. For a change of CO₂ from 5 to 0%, the phase change was 30 degrees. At this modulation frequency, the day-to-day variability of phase change measured was approximately 1 degree, which implies a detection sensitivity of approximately 0.5% CO₂ (variation around the average phase value at a CO₂ concentration of 5%) using the calibration curves of phase change versus CO₂ concentration. The robustness of the phase detection technique was demonstrated when we attenuated the blue excitation and hence the signal by 20 dB (at 95.42 MHz modulation) and observed the phase to be constant to within 1 degree. These results were similar to those obtained by the ISS instrument. Figure 4 demonstrates the need to have a high modulation frequency to achieve a high detection sensitivity. The blue LED used in Ref. 2 had a modulation bandwidth of approximately 6 MHz. The depth of modulation would be extremely small (less than 1%) at 95 MHz, leading to a signal that would be dominated by noise. In the case of the blue-doubled source in Figure 2, the depth of modulation of the blue light was 27% at 95 MHz. The depth of modulation as a function of frequency is shown in Figure 5. The two large variations are due to reflections caused by impedance mismatches. As shown in the figure, the source can be significantly modulated to at least 200 MHz.

**Fig. 4** Relative phase difference between a nitrogen atmosphere containing 5% and 0% carbon dioxide as a function of modulation frequency.
5 CONCLUSION

We have demonstrated a diode laser frequency doubled by a KTP waveguide as a practical source for fiber optic sensors of oxygen and sensors of carbon dioxide based on the technique of phase fluorometry. The source is compact, low power, focusable, and easily modulated up to frequencies as high as 200 MHz. These attributes recommend it as an excitation source for optical sensors in clinical and biological settings. Based on the experiments that we have described in this paper and recent results of butt coupling a laser diode to the KTP waveguide, we feel that phase fluorometric sensors for oxygen and carbon dioxide based on these optical sources have the potential for widespread commercial use. In addition, the source could be used with any lifetime-based sensor requiring blue excitation, high modulation frequencies, or coupling into a single-fiber optic.

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