Integration of an Emerging Highly Sensitive Optical CO_2 Sensor for Ocean Monitoring on an Existing Data Acquisition System SeaKeeper 1000™ Annual Report for FY10 (Oct 1, 2009 – Sep 30, 2010)

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LONG-TERM GOALS

Develop a high-performance pCO_2 sensor that is affordable enough to be deployed in geat numbers to autonomously monitor the overall patterns of CO_2 emissions and ocean acidification.

OBJECTIVES

- Meet the challenging requirements for ocean pCO_2 monitoring using an innovative sensor design based on high sensitivity fluorescence detection.
- Assemble the system with low-cost optics and electronics in order to make it affordable enough (target cost <$1,000) to be deployed in greater numbers.
- Make the entire system drift and calibration free by perfecting a patch renewal process using an innovative microfluidics-based approach.
- Integrate the system into an existing platform system, the Seakeeper 1000™, to leverage already proven deployment systems readily amenable to autonomous operation.
- Test the reliability and robustness of the prototype system in the lab and open waters with our partners NOAA and Seakeepers International.
- Commercialize sensors with our industrial partner Fluorometrix Corporation.

APPROACH AND WORK PLAN

- Scientific and/or technical approach

The novel optical sensor being developed for autonomous ocean monitoring works on equilibrium principles. When the pH-sensitive reagent solution residing behind a gas-permeable membrane is exposed to seawater, the CO_2 molecules present in the seawater diffuse across the membrane into the reagent and induce a pH change. After equilibrium is reached, the fluorescence is measured and the pCO_2 data are recovered from a calibration curve. A specific feature of our measurement technique is the use of excitation ratiometric approach. A violet LED (Light Emitting Diode) and a blue LED are used to excite the sensing reagent through an excitation filter and the pCO_2 dependent emission is measured through an emission filter by a photodiode. This approach is especially valuable from stability point of view – the chemical part that the sensor is based on is practically insensitive to the changes in temperature. Besides the ratiometric feature, the system also has a lot of other novel features including the ideal 90° separation between excitation and emission, the mirrored cavity, the beam combiner, and the renewal of the sensing reagent, etc. To greatly improve the sensitivity of the sensor, we ideally separated the excitation and the emission, leading to significantly reduced scattered light reaching the detector, thereby increasing the signal to noise ratio. The sensing reagent was placed in a mirrored cavity, allowing the excitation light to uniformly excite the dye. To address the photobleaching of the sensing reagent, we used a technique that automatically renews the sensing reagent after every measurement. This makes the measurements totally calibration-free.

- The key individuals participating in this work

Govind Rao, Ph.D., Professor, overall project management.
Yordan Kostov, Ph.D., Research Associate Professor, electronics and optics design.
Hung Lam, Ph.D., software and algorithm development.
Xudong Ge, Ph.D., Research Assistant Professor, reagent formulation and microfluidics design.
Robert Henderson, Graduate Student, system preparation and test etc.

- Work plans for the upcoming year

We will build the patch renewal system with gas-impermeable tubing, micro-pumps, and micro-valves, and program the whole process so that the measurements are done automatically. After the prototype system is built and programmed, its reliability and robustness will be thoroughly tested in lab. At the end of the upcoming year, a prototype system will be delivered to our partner for autonomous operation and validation.

WORK COMPLETED

- Designed and built the excitation and read out system with low-cost optics and electronics.
- Designed and built the microfluidic system including the equilibrium chamber and the measurement chamber.
- Optimized the sensing patch formulation, found the best dye or dye combinations to meet the requirements for measuring ranges, found the optimal dye and base concentrations to satisfy the required sensitivity, and found the best semipermeable material for pCO_2 sensing.
- Prototype systems were presented and examined at NOAA’s AOML location.

RESULTS

- Optoelectronics

The optical unit and the read out system have been designed and built. To meet the very high precision (1ppm) and stability (6 months) requirements, the optoelectronics system must have a signal-to-noise ratio (S/N) of ~2000:1 (as a comparison, the Varian Fluorescence Spectrophotometer only has an S/N of 400:1). Analysis of the behavior of our existing hardware made clear that analog synchronous rectification and averaging are sources of additional noise. As a result, we elected to remove the synchronous rectification switches and the low pass filtering and implement this functionality in the software. This approach is often termed “software lock-in”. The software lock-in relies on fast ADC (we have selected ADC 8613, a 500 ksps 16 bit SAR ADC), which is operated twice per period. Signal acquisition is performed at the maximum and the minimum of the signal, assuming sine wave signal. Subtracting the minimum from the maximum gives a value equal to twice the amplitude of the detected signal. Averaging these measurements 1024 times resulted in very low-noise measurement - the noise was less than 1 bit of the 16-bit value. This translates into an S/N of 16000:1 in signal measurement and approximately 8000:1 for the measurements of ratios, accounting that the signal was approximately 16000 counts.

As the modulation rate of the LED is 10 kHz, the total measurement takes 0.2 seconds (0.1 seconds for each LED). This strongly decreases the photo-bleaching of the sensing dye during the measurement and allows the use of LEDs with very high intensities, which in turn decreases the noise in the detection path.

For fluorescence excitation, LEDs with emission maxima of 400 nm and 460 nm are used. In order to provide uniform light path on both wavelengths and to ensure that the same spot is illuminated, a specialized optic device called a beam combiner was developed (Figure 1). Both LEDs (blue, B and Violet, V) are mounted inside a block which also accommodates a dichroic mirror (DM). The DM transmits the light of the violet LED and reflects the light of the blue LED. Both beams are shaped by an extended aperture, which also holds a short pass filter (SPF). This filter removes the long "red tails" of the LED's emission spectra. In this way, it is possible to achieve good spectral separation between excitation and emission. The beam combiner is also equipped with a 90/10% splitter (M), which allows 90% of the light to proceed toward the microfluidics but directs 10% toward the reference photodiode. In this way, all the optics is housed in a single block, which makes it shock proof and environmentally insensitive.

The use of proper optics and good detection techniques allowed us to evaluate the capabilities of our LED-based illumination unit. With this unit, significant drift was noticed. It is well known that LED intensity depends on two factors – current through the LED and the crystal temperature. As the current through the LED was fixed using a deep feedback controlled current source, our conclusion was that the LEDs' brightness drifts due to the increase of the crystal temperature as it heats up. In theory, it is possible to turn the LED on and wait until it thermally equilibrates; however, this still does not prevent the ambient thermal drift. For this reason, we have elected to attempt optical monitoring and compensation of LED brightness.

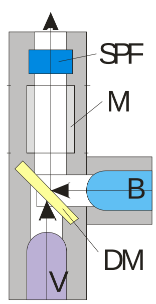

Figure 1. Beam combiner. B – Blue LED, DM - Dichroic Mirror, M- Splitter, SPF – Short Pass Filter, V – Violet LED.

Our first attempt was to introduce analog negative-feedback correction. While it stabilized the long-term drift, it introduced significant noise in the signal and led to substantial degradation of the S/N. It was concluded that for compensation purposes, the signal will be acquired and digitally processed. To this end, we implemented 2 photodetectors – one that monitors fluorescence and another that monitors the LEDs themselves. The amplification chain of the both detectors is adjusted in such way that the reference and the sensing signal are roughly the same magnitude, leading to a ratio that exhibits the same sensitivity in both channels. The version of the board (Figure 2) that performs these measurements has been assembled with the respective optics and coupled with preliminary version of the microfluidics. The system is currently being tested in realistic conditions in order to evaluate its accuracy, precision, temperature drifts, and other metrological parameters. We will use the system to study the efficiency of the excitation, decoupling between excitation and emission, as well as to implement novel noise and drift-suppression algorithms.

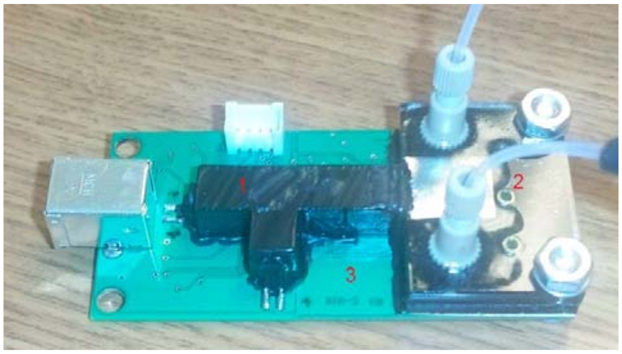

Figure 2. Picture of the assembled detection board with USB communication. All the electronic elements are on the bottom providing separation in case of spills. 1 - beam combiner, 2 - microfluidics, 3 - electronics board.

- Flowcell

The flow cell has been designed and built. The best semipermeable material for CO_2 sensing has been determined. Three different membranes including Teflon 2400, Teflon FEP, and PVDF were tested. Among them, Teflon 2400 has the best permeability for CO_2 and the required mechanical strength. In our initial design of the equilibrium chamber, a 50-μm thick flat Teflon 2400 membrane was stretched between two snake-shaped channels (Figure 3). For this design, the 90% response time is 9 minutes from air to nitrogen or from nitrogen to air. To further reduce the response time, Teflon AF 2400 tubing was procured for use in the equilibrium chamber (Figure 4), and the 90% response time was greatly reduced to only two minutes due to mass transfer in the radial direction instead of a single direction. Large inlet ports were added to prevent clogging by bio or marine fouling. UV LEDs were also included to sterilize the equilibrium chamber to reduce bio or marine fouling.

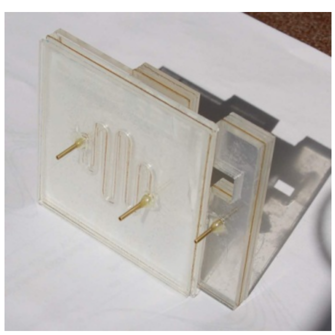

Figure 3. In the initial design of the flowcell, a 50-μm thick flat Teflon 2400 membrane was stretched between two snake-shaped channels in the equilibrium chamber.

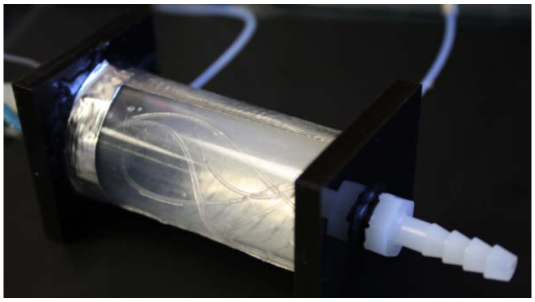

Figure 4. Teflon AF 2400 tubing was used in the new design of the equilibrium chamber. The 90% response time was greatly reduced to only two minutes due to mass transfer in the radial direction instead of a single direction.

To minimize dye retention, the elliptical well measurement chamber in our initial design was replaced by a snake-shaped channel (Figure 5). The dye volume necessary for individual measurements was reduced by 80% to 50 μL. Mirrored cavity was created using reflective aluminum sheets. The S/N for mirrored cavity was greatly increased by 60% compared with non-mirrored cavity. The new design is 50% smaller than the previous version.

- Dye formulation

The best dye to meet the requirements for measuring ranges has been identified. Three dyes including 5-(and-6)-carboxynaphthofluoresein, 6,8-dihydroxypyrene-1,3-disulfonic acid disodium salt, and 8-hydroxypyrene-1,3,6-trisulfonic acid trisodium salt (HPTS) were studied for sensitivity and stability for the specified measurement. HPTS is best for its stability although it has about the same sensitivity as the other two dyes.

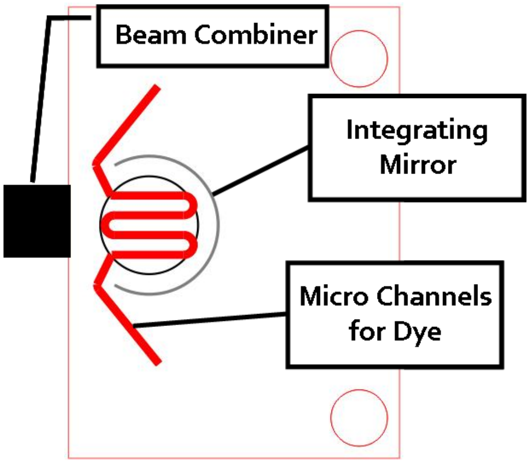

Figure 5. The elliptical well measurement chamber in our initial design was replaced by a snake-shaped channel, and the dye volume necessary for individual measurements was reduced by 80%.

The optimal dye and base concentrations to satisfy the required sensitivity have been determined. The fluorescence intensity is higher at higher dye concentration but will decrease due to inner filtering effect if the dye concentration is too high. The optimal dye concentration for HPTS is 50 μM. The optimal base concentration is that at which the dye has the best sensitivity to CO_2 in the range of 200-500ppm. Higher base concentration will move the most sensitive concentration range upward. The optimal base concentration was determined to be 20 μM.

- Fabrication technologies

Direct thermal bonding without the use of solvent or adhesive was tested and optimized. Thermal bonding is superior to adhesive or solvent binding because the optical and fluorescent properties of the microfluidic device are maintained during construction and the tiny channel geometries are created without any distortion or blocking. Dye entrapment at interface due to adhesive is also avoided. The microfluidic device made by thermal bonding is capable of >80 psi service without any leaking. The machined channels were solvent polished to increase their optical clarity. It was also found that annealing the material can reduce its internal stress and thus can reduce cracks occurring during fabrication or in use.

IMPACT AND APPLICATIONS

National Security

Global warming caused by increasing amount of CO_2 discharged into the environment by human activity is usually characterized as an environmental threat, but now it has been realized that it is also an issue of national security. Unchecked global warming could raise sea levels, change the amount and pattern of precipitation, and increase the intensity of extreme weather events and change the agricultural yields, leading to large-scale migrations, increased border tensions, the spread of disease and conflicts over food and water. All could lead to direct involvement by the United States military. The pCO_2 sensor being developed will have a great impact on understanding global warming and ocean acidification. As the sensor being developed will be highly sensitive, and highly stable, yet affordable enough to be deployed in great numbers, the overall pattern of greenhouse gas emissions in an area can be monitored autonomously. Accurately and precisely measuring is the first critical step to control global warning and to alleviate the severity of its effects.

Economic Development

The economic sector most affected by global warming will be the agricultural sector because global warming will seriously affect the number of rainfall. In some regions, the overall frequency of droughts will become longer and more intense. While in other areas, there will be too much rainfall, leading to flooding. Another globally important economic activity most affected by global warming will be fisheries. Due to their primitive nature, the output of fisheries is directly and strongly affected by variations in natural conditions. The pCO_2 sensor being developed will monitor the CO_2 levels and help alleviate the harmful effects.

Quality of Life

Fisheries are closely tied to human health and species health across the globe. Widespread changes in quality of life will occur if global warming continues at its current pace. More frequent heat waves and a significant increase in days with poor air quality could endanger the elderly and children.

TRANSITIONS (For the 4 NOPP evaluation factors below, please describe how the results (hardware, software, knowledge) are being utilized by others. Transition is taken to mean, "products which are being incorporated into more developmental (or operational) programs or have already been incorporated in other's plans.")

Economic Development (Delete this section if there are none)

Discussions are underway with a major manufacturer of environmental sensors to fast-track deployment of the pCO_2 sensors.

PATENTS

As a result of this project, an application for United States Letters Patent entitled "Fluorescence based sensors utilizing a mirrored cavity" was filed on January 25, 2010, and identified by United States Serial No. 12/692,752.