

# Advanced Fiber-Optic Monitoring System for Space-flight Applications

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## ABSTRACT

Researchers at Luna Innovations Inc. and the National Aeronautic and Space Administration's Marshall Space Flight Center (NASA MSFC) have developed an integrated fiber-optic sensor system for real-time monitoring of chemical contaminants and whole-cell bacterial pathogens in water. The system integrates interferometric and evanescent-wave optical fiber-based sensing methodologies with atomic force microscopy (AFM) and long-period grating (LPG) technology to provide versatile measurement capability for both micro- and nano-scale analytes. Sensors can be multiplexed in an array format and embedded in a totally self-contained laboratory card for use with an automated microfluidics platform.

## INTRODUCTION

Recycling of water resources is an essential component of Advanced Life Support Systems (ALSS) for extended missions in space. Aboard spacecraft such as the International Space Station (ISS), water is used for drinking, food preparation, personal hygiene, oxygen generation, and scientific experiments. In this closed-loop spacecraft environment, the majority of wastewater generated must be recovered, purified, and re-used due to the prohibitive costs of re-supplying the craft with water from earth. Consequently, continuous in-flight monitoring of microbial pathogens and chemical contaminants is critical to assure the quality of recycled water and to maintain crew health.

The crew itself contributes the vast majority of biological and chemical contamination present in space station wastewater and is particularly susceptible to waterborne illness due to the limited medical facilities available and the potential for prolonged flights to compromise the immune system [i]. Bacterial pathogens such as *E. coli*, which may have increased virulence in microgravity environments [ii], enter space station water supplies through shedding skin, sneezing and other bodily

functions. In addition to bacterial pathogens, chemical contaminants such as aqueous ammonia, ammonium cations, nitrate, nitrite and urea enter shipboard water supplies through the crews' metabolic degradation of nitrogenous biomolecules such as amino acids, purines, and pyrimidines [iii,iv].

Protection of crew health during prolonged space flight requires sensor technology that i) is suitable for monitoring an array of biological and chemical contaminants and ii) complies with unique operational limitations imposed by space flight including minimal power consumption, small system footprint, containment of reagents/samples, and ease of use. Presently an ideal water monitoring system that satisfies all of NASA's performance and operational specifications does not exist, and only a suite of labor intensive analytical techniques can determine accurately the suitability of water resources for crew re-consumption.

The goal of this research is to develop an advanced fiber-optic sensing platform for continuous water quality monitoring during prolonged space-flight. In recent years, fiber optic sensor technology has become increasingly attractive for development of robust, miniaturized water quality monitoring devices for a range of water contaminants including explosives [v], pesticides [vi], and various bacteria and toxins [vii]. The system we describe uses arrays of two complementary fiber-optic measurement schemes embedded in disposable laboratory cards for use with a totally self-contained, automated fluidics system. Using this single platform, we have demonstrated label-free detection of whole bacterial organisms and metal cations using immuno-based receptors and reactive polymers, respectively. Current research is focused on developing fiber optic sensor arrays for a full suite of biological and chemical contaminants present in recycled wastewater.

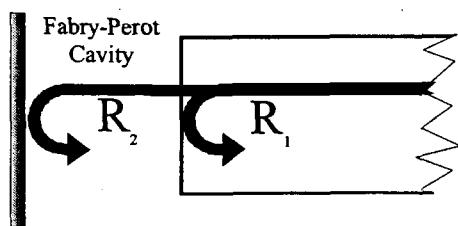
## THE ADVANCED MONITORING SYSTEM

The advanced fiber-optic monitoring system integrates novel atomic force microscopy (AFM)-based fiber-optic microcantilever (MC) sensors with long period grating (LPG) optical fiber sensors. The resultant dual fiber-optic sensor platform provides complementary detection capability for simultaneous screening of both micro- and nano-scale analytes (e.g., whole bacterial cells and metal cations, respectively) in a single device that meets many of NASA's critical operational specifications.

## SENSORS AND MEASUREMENT PRINCIPLES

### Microcantilever (MC) Sensor

Microcantilever interferometric sensors are based upon the principle that as organisms and other targets bind to an affinity ligand-coated microcantilever beam, the intermolecular adsorption and desorption, surface reconstruction and reorganization and conformational entropy changes induce a tensile or compressive surface stress on the beam [viii]. As a result, the biochemical ligand/target binding events cause the beam either to mechanically bend or to change resonant frequency in proportion to concentration of bound target. To ensure that beam deflections are due to binding events, null, non-affinity control sensors are interrogated simultaneously. Currently, promising results are being obtained with DNA-based formats which exploit the DNA hybridization "zipper-like effect" to induce sufficient chemomechanical forces to bend the cantilever [ix]. However, protein based-formats must often rely on surface chemistries that include secondary probes and hydrogels to cause the requisite surface stress. Initial results presented here demonstrate that bacteria can be detected using cell/ligand complexing.



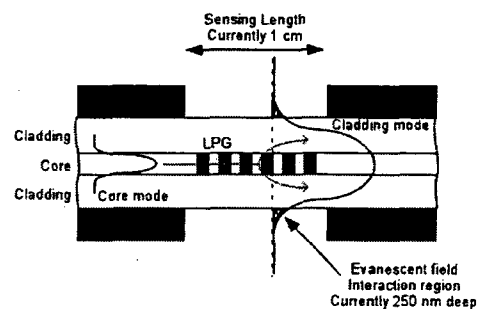
**Figure 1:** Interferometric sensor format used at Luna. Extrinsic Fabry-Perot interferometry (EFPI); R1 is the reference reflection and R2 is the sensing reflection.

Presently, microcantilever sensor technology relies on expensive and complex bulk optical techniques to monitor changes in beam deflection or resonant frequency. The transduction mechanism described here, however, uses low-cost and robust extrinsic Fabry-Perot interferometer (EFPI) technology. This is a distance measurement technique based on the formation of a cavity between the polished endface of an optical fiber and a reflective surface, shown schematically

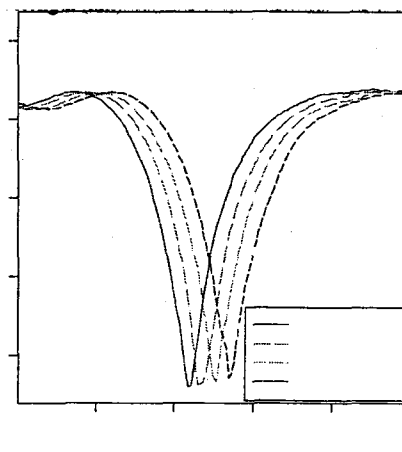
in Figure 1. Light is passed through the fiber, where a portion of the light is reflected off the fiber/air interface ( $R_1$ ). The remaining light propagates through the air gap between the fiber and the reflective surface and reflects back into the fiber ( $R_2$ ). These two light waves interfere constructively or destructively, based on operating wavelength and path differences, and travel back through the fiber to the demodulation and data acquisition system. In this manner, the actual gap is measured accurately to sub-nanometer resolution and thus deflection of a ligand-coated beam upon binding of specific targets can be determined easily.

### Long-Period Grating (LPG) Sensor

The long-period grating sensor filters light at a specific optical wavelength, determined in part by the environment immediately surrounding the sensing region of the fiber, thus creating a loss band whose location correlates to refractive index. Depending on the demodulation approach selected, the LPG will need to couple into either forward or reverse propagating cladding modes. A schematic of the LPG sensing platform is shown in Figure 2a with the spectral response to refractive index changes shown in Figure 2b.



**Figure 2a:** Schematic of a long-period grating sensor.



**Figure 2b:** Shift in wavelength of light coupled out of the LPG with refractive index change.

LPG sensors are formed by exposing a germanosilicate fiber to a spatially periodic intensity pattern generated from a high-power ultraviolet (UV) source [x]. Germanosilicate fiber is photosensitive such that optical fiber exposure to certain wavelengths of light, particularly 244 nm, will cause the refractive index of the glass to increase slightly. If the fiber is exposed to a periodic index variation, the refractive index of the optical fiber is modulated forming a structure analogous to the Bragg gratings formed by crystal lattices. Light that travels down an optical fiber can be related to a set of guided electromagnetic waves called modes. Different modes refer to the different paths that light can take while traveling along an optical fiber. Based on the grating periodicity, the phase matching condition is satisfied such that the forward propagating fundamental mode is coupled into propagating cladding modes and the evanescent wave extends out of the optical fiber. This field can extend up to one micron out of a typical 125-micron diameter optical fiber and is what creates the sensing region of the LPG. The spectral location of the loss band due to this modal coupling is a function of the difference in the effective indices of the guided mode and the corresponding cladding mode. The coupling wavelength,  $\lambda$ , for a specific loss band, is given by the expression,

$$\lambda = (n_g - n_{cl})\Lambda \quad (1)$$

where  $\Lambda$  is the grating period and  $n_g$  and  $n_{cl}$  are the effective indices of the guided and cladding modes, respectively. Variations in the values of  $\Lambda$ ,  $n_g$  and  $n_{cl}$  will shift the position of the coupled wavelength. However, as  $\Lambda$  is a constant parameter of the optical fiber and  $n_g$  is essentially independent of the external environment, measurable wavelength change is due entirely to  $n_{cl}$  (refractive index surrounding the optical fiber grating). To couple into reverse propagating cladding modes, Equation (1) becomes additive rather than subtractive. Therefore standard LPGs have a period of tens of microns while the modified reverse coupled LPGs have a period on the order of a micron.

## SENSOR COATINGS

MC sensors were developed for detection of *Escherichia coli* O157:H7 using antibody-based techniques. LPG sensors were developed for detection of divalent metals using a reactive carboxymethylcellulose (CMC) polymer.

### MC Sensor for detection of *E. coli*

Affinity anti-*E. coli* MC sensors were prepared using the direct coupling Lomant's method. Previous studies showed that ligands and polymers can be coupled to non-gold coated silica dioxide microcantilever beam substrates using conventional silane chemistry [xi]. For this research, however, we used more facile gold-coupling chemistry [xii] and thus obtained

microcantilevers with a single side coated with gold to facilitate directed target binding. After characterizing *E. coli* tester strains and anti-*E. coli* antibodies in conventional ELISA formats, we adapted gold-coupling chemistry to characterize the antibodies on the gold-coated micro-cantilever chips used with the fiber optic affinity MC sensor. The gold-coated microcantilever chips were activated with DTSP and the anti-*E. coli* antibodies were coupled to the microcantilevers using succinimidyl ester chemistry.

A direct coupling method employing Lomant's reagent, or dithio-dipropionate-N-succinimidyl ester [xiii] was used for the gold-coated microcantilever surfaces.

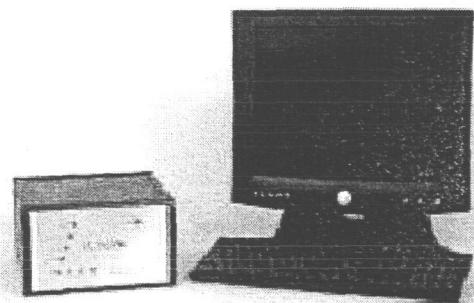
### LPG Sensor for detection of divalent metals

To model the performance of reactive polymers on LPG fiber optic sensors, a carboxymethylcellulose (CMC) polymer was applied to an LPG sensor to permit detection of divalent metal cations. Partitioning of divalent metals into the CMC polymer induces a change in refractive index that is subsequently measured by the LPG sensor. The response is reversible, depending on the partition coefficient and the concentration in solution. High sensitivity can be achieved for solutes that have a strong tendency to partition into the polymer and the response time is relatively rapid.

Prior to application of the CMC polymer, LPG sensors first were cleaned in acetone and boiling DI water and allowed to dry. Sensors then were silated in a 10% aminopropyltrimethoxysilane (APS) and dry methanol solution and incubated at room temperature. Sensors then were rinsed in methanol and allowed to dry overnight. Next, sensors were placed in a solution of 1% CMC and 20mM MES at pH 6.0 and incubated at room temperature. Sensors then were removed from the CMC solution and rinsed with MES buffer and deionized water and left to cure at 37°C.

## SENSOR INTERROGATION

MC and LPG sensors were interrogated simultaneously using a FiberPro 2.0 optical signal conditioning system (Figure 3) coupled with a MUX 8-Channel Expansion unit for multiple channel capability (Luna Innovations Inc., Blacksburg, VA, USA).



### Figure 3: Shift FiberPro USB with Display

#### FLUIDICS

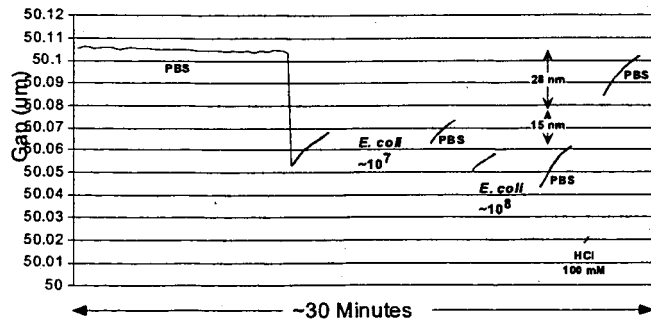
Automated and self-contained transfer of reagents was performed using a Micronics microFlow system integrated with a custom-designed, dual-sensor flow-cell (Seattle, WA, USA).

#### SENSOR DEMONSTRATION

##### Demonstration of MC sensor for *E. coli*

Tester strains of *E. coli* O157:H7 and 15224 (ATCC) were maintained on LB agar plates and grown on agar plates at 37°C for 24-48 hr prior to testing. Test suspensions were made from plates by removing a loopfull of cells and suspending them in PBS at 0.5 O.D.600 or  $\sim 10^8$  cells/ml. The sensors were equilibrated in PBS until a stable baseline was achieved with minimal drifting and then challenged with cells at room temperature. Figure 4 shows a binding response of the microcantilevers directly coupled to goat anti-*E. coli*. To determine the initial responses to the *E. coli*, sensors were challenged with  $10^7$  cell/ml followed by a challenge with  $10^8$  cell/ml to determine if saturation had been achieved. The sensors then were regenerated with and equilibrated with PBS for the next run. All reagents were brought to room temperature before testing to minimize temperature drifts.

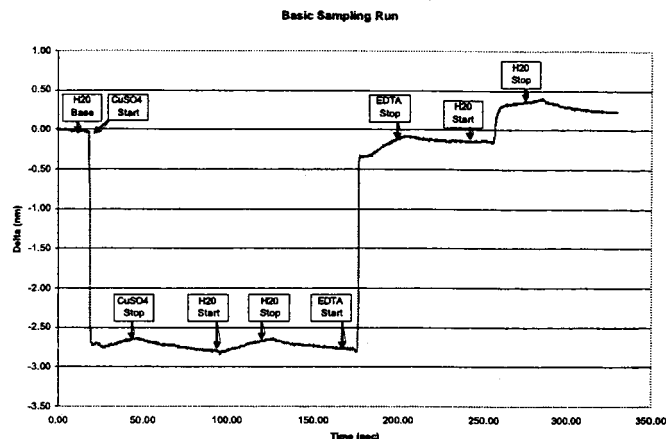
When challenged with  $10^7$  cells/ml the affinity *E. coli* MC sensor showed significant responses of  $\sim 40$  nm with a noise level less than 1 nm (Figure 4). Significant but smaller responses were seen at  $10^6$  CFU/ml, which is in the range of detection for other optical formats such as surface acoustic wave (SAW) devices [xiv].



**Figure 4:** Sensorgram of an anti-*E. coli* MC sensor challenged with *E. coli* cells. Following establishment of a stable PBS baseline, the sensor was challenged with  $10^7$  and  $10^8$  cells/ml.

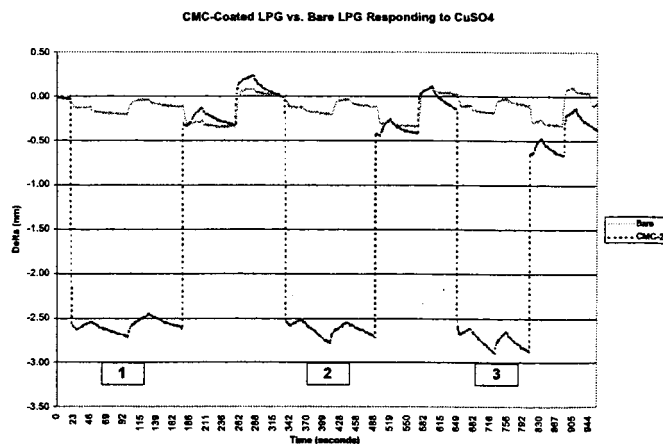
##### Demonstration of polymer-coated LPG sensor

Figure 5a shows data obtained using a CMC-coated LPG sensor base-lined in de-ionized water and then exposed to a 30 mM copper sulfate solution. CMC swells in the presence of the  $\text{Cu}^{2+}$  ion (as well as in the presence of other divalent cations), thus inducing a nearly 2.75 nm shift in refractive index that can be measured using the LPG optical fiber sensor. A 50 mM ethylenediaminetetraacetic acid (EDTA) solution, which has a greater affinity for the  $\text{Cu}^{2+}$  ion, is used to chelate the  $\text{Cu}^{2+}$  from the CMC polymer matrix and subsequently regenerate the sensor.



**Figure 5a:** Sensorgram of a CMC-coated LPG sensor challenged with  $\text{CuSO}_4$ . Following establishment of a stable baseline in deionized water, the sensor was challenged with 30 mM  $\text{CuSO}_4$ .

Figure 5b shows the response of a CMC-coated LPG sensor relative to a null (i.e., bare or un-coated) LPG sensor when challenged with a 30 mM copper sulfate solution. To demonstrate reproducibility, three cycles are shown.



**Figure 5b:** Sensorgram of a null (i.e., bare or non-coated) LPG sensor and CMC-coated LPG sensor challenged with  $\text{CuSO}_4$ . Following establishment of a stable baseline in de-ionized water both sensors were

challenged with 30 mM CuSO<sub>4</sub>. Three separate challenges are shown.

## CONCLUSION

The prohibitive costs of transporting fresh water re-supplies out of earth's orbit necessitates intensive recycling efforts during prolonged missions in space. Consequently, continuous monitoring of wastewater contaminants is critical to maintaining crew health. In spite of its important role in advanced life support systems, an ideal NASA-compliant water quality monitoring system has yet to be developed.

The advanced fiber-optic monitoring system described here has been demonstrated under controlled laboratory conditions for detection of a limited set of biological and chemical targets including *E. coli* and divalent metals, which may contaminate wastewater during prolonged missions in space. The combination of two different fiber optic sensing schemes provides complementary detection capability for simultaneous screening of both whole-cell biological pathogens (> 1 micron) and smaller chemical contaminants. The system is responsive to NASA operational specifications in that sensors are embedded in totally self-contained, disposable laboratory cards, the sample-handling process is largely automated, and most components have been miniaturized to provide a small overall system footprint.

Research is ongoing to further the maturity level of the system and to develop fiber optic sensor arrays for a full suite of biological and chemical contaminants that typically occur in recycled spacecraft wastewater. In addition to aqueous-phase monitoring, researchers at Luna Innovations and NASA MSFC are investigating the applicability of this technology for monitoring spacecraft gases and vapors as well.

## ACKNOWLEDGMENTS

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