Lab-on-a-Chip Biosensor for Interplanetary Life Detection Missions

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Abstract—Space research still consists of the major goal of the successful detection of life on another planet. The 2012 biological and life detection (BOLD) mission to Mars included the ARROW biosensor as one of its main life detection tools for the mission. Although the mission never went forward, the ARROW biosensor remains an obvious choice for interplanetary life detection missions. The sensor is cheap, small, lightweight, and very sensitive. All of these factors helped make it the candidate of choice for the BOLD mission to Mars and still make it a good candidate today. Since 2012, the ARROW biosensor has incorporated buried waveguides in order to improve its sensitivity and environmental stability and has improved dramatically in its detection capabilities.

I. INTRODUCTION

Human interest in reaching the planet Mars includes a wide variety reasons and goals, including the major goal of traveling to Mars with the idea of potentially inhabiting the planet in the future. This endeavor has several requirements in order to make it a possibility. One of those requirements is to determine whether or not life has ever existed on Mars and if it could potentially exist again.

The Biological and Life Detection (BOLD) mission to Mars was proposed in order to take on this major requirement – determining whether or not there was once any life on Mars [1]. One of the devices included in the proposal is a lab-on-a-chip optofluidic biosensor, called the ARROW biosensor, which has been developed for individual bioparticle detection [2]. The BOLD mission to Mars never came to fruition; however, this paper outlines that the ARROW biosensor remains a prime biosensor candidate for interplanetary biological testing and detection. There are three major advantages that this biosensor offers these types of missions.

First, the lab-on-a-chip technology, allows for the majority of the device to be packaged into a 1 cm x 1 cm chip that weighs virtually nothing [3]. In space travel an important consideration is the weight of the cargo [1]. All extra weight represents an extremely difficult feat in order to reach Mars. It is very favorable that the biosensing device is so small and adds little mass to the mission.

Second, the lab-on-a-chip technology also allows for the biosensor to probe an extremely low volume (~0.24 fl) of a sample [4]. This low volume excitation leads to the ability to detect, with a high sensitivity, and count individual biological particles that within a given sample.

The third major reason for including the ARROW biosensor in the BOLD mission to Mars proposal is the cost of the fabrication and design of the biosensor. Optofluidics piggybacks off the microelectronics industry, which uses parallel processes in order to make thousands of devices in parallel for a relatively low cost. This fact also allows for easy design changes in order to optimize the biosensor for this specific mission to Mars and its objectives.

This paper discusses ARROW biosensor that was included in the BOLD mission to Mars and expands on the most recent advances that make the biosensor an even more sensitive and robust platform than when it was included in the BOLD proposal. Specifically, we discuss the design of the platform, how it is made, how biosensing is achieved in the biosensor, and the most recent advances resulting in high quality biosensing results.

II. THE ARROW BIOSENSOR DESIGN

The ARROW biosensor uses a hollow waveguide that is capable of guiding light directly through a low refractive index solution. This allows excellent interaction between the sample and the light used to probe the sample. This special waveguide is called an anti-resonant reflecting optical waveguide (ARROW) [5-7].
The ARROW biosensor is a lab-on-a-chip, optofluidic device. The term lab-on-a-chip refers to the idea of shrinking an entire table-top size or bigger lab-like setup onto a single small die, or chip, using microfabrication techniques. It simply means that the biosensor is very small, which can be seen in Fig. 1(b) which shows and photograph of a completed ARROW biosensor. The term optofluidic refers to fact the biosensor uses a combination of optical components and microfluidics in order to accomplish its biosensing. Fig. 1(a) shows the basic structure of the ARROW biosensor and Fig. 1(b) is a photograph taken of a completed biosensor. The biosensor is resting on a standard penny in order to illustrate just how small these sensors really are.

As seen in Fig. 1(a), the biosensor is made up of two main types of waveguides. The solid grey waveguides represent solid rib waveguides that are composed of SiO$_2$ or in other words a glass. These waveguides allow for light to couple onto and off of the biosensor after passing through the hollow waveguide portion of the device. The blue waveguide is the hollow waveguide, mentioned above, that is called the ARROW waveguide. The sample will flow directly through this waveguide in order to achieve high light interaction with the sample being tested. The walls and the ceiling of the hollow ARROW waveguide are made of SiO$_2$, which is the same material that is used for the solid waveguides. The next section describes how we use this design in order to actually perform biosensing on the chip.

III. SENSING USING THE ARROW BIOSENSOR

The ARROW biosensor relies on a fluorescent signal in order to detect or sense a particle within a sample; unfortunately, most biological particles are not very fluorescent on their own. This means that the particles must first be pretreated in some way in order to be detected. The most common method to pretreat the sample and selectively make bioparticles fluorescent is the use of an intercalated dye.

There are many types of intercalating dye. Essentially, they are all a fluorescent particle that will intercalate or fit in between segments of a DNA strand. Some will fit better in single-stranded DNA and others are designed to intercalate better with double-stranded DNA. There are intercalating dyes that work in many different wavelengths. It is important to be using the correct excitation wavelength, based on the dye that is chosen for mixing.

Once a sample has been sufficiently mixed with the intercalating dye it generally enters the biosensor through a tiny reservoir that has been placed over the end of the hollow waveguide. A pressure is then applied to the sample in order cause it to flow through the hollow waveguide. At the same time light, from a laser, is coupled onto the biosensor through one of the solid core waveguides. This is depicted in Fig. 1(a) in green.

The laser light travels down the solid waveguides and intersects with the hollow core waveguide. Where these two waveguides intersect is called the excitation point. It is at this excitation point that the intercalating dye will fluoresce if it has attached itself to any DNA. If there is any fluorescent signal that will travel down the hollow waveguide, pass into the collection solid waveguide, and then off of the biosensor to an avalanche photodiode (APD) in order to be detected. If the intercalating dye found no DNA to attach to, then there will be no fluorescent signal given off. It is by this method that samples can be probed for any biological particle.

The idea behind detecting life on Mars in the BOLD proposal is to collect samples of Mars soil, mix them into a solution, add intercalating dye, and then run them through the ARROW biosensor. If any biological particles exists within the soil sample the ARROW biosensor will be capable of sensing it.

IV. ARROW BIOSENSOR FABRICATION

This next section focuses on the materials, processes, and methods used in order to make the ARROW biosensor. The entire fabrication process can be broken down into seven major fabrication steps, which are illustrated in Fig. 2

A. Deposit ARROW Layers

The first step in fabrication is to deposit a set of dielectric thin films, called ARROW layers. These layers are designed to possess extremely high reflection of visible light at glancing angles [2, 8]. The stack makes use of electromagnetic interference effects in order to achieve high reflectance. Essentially, the ARROW will act as a bottom optical buffer layer forcing light to guide in the desired waveguides that will reside on top of the layers. The thickness of these layers
The thickness of the $j$th layer, $t_j$, is determined by the index of the $j$th layer, $n_j$, and the index and thickness of the core, $n_c$ and $t_c$, respectively. The wavelength, $\lambda$, is also an important parameter that will affect the thickness required in the different layers [9].

$$t_j = \frac{\lambda}{4n_j} (2N - 1) \left[ 1 - \frac{n_c^2}{n_j^2} + \frac{\lambda^2}{4n_j^2t_j^2} \right]^{-1/2}, N = 1, 2, \ldots (1)$$

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### B. Deposit and Pattern Sacrificial Core

Once the bottom ARROW layers are deposited the next step is to define the hollow core waveguide geometry. We use a sacrificial material in order to achieve a hollow core on our lab-on-a-chip. Most often a negative photoresist, called SU8, is used as the sacrificial material. This is because SU8 can withstand relatively high temperatures (~300°C) without deforming, cracking, or reflowing. It is also relatively simple to remove the SU8 using a standard “piranha” acid mixture, which is made by mixing hydrogen peroxide (H$_2$O$_2$) and sulfuric acid (H$_2$SO$_4$).

In order to form the sacrificial cores the SU8 is spun-on over the ARROW layers. It is masked and exposed to UV radiation, and later developed. After development the SU8 requires some extra baking in order to prepare it for high temperature exposure without cracking.

### C. Pattern and Etch Pedestal

The next step was developed in order to improve the structural strength of the hollow cores. We found that the natural weak-point of the cores was near the bottom corners of the feature. This was due to the fact that the oxide overcoat deposited over the sacrificial cores generally included a crevice that extended toward this corner of the hollow core. The crevice formed because of the anisotropic nature of the plasma enhanced chemical vapor deposition of the SiO$_2$. The oxide grows vertically more rapidly than it grows horizontally, which causes a sort of bread loafing effect when the oxide is deposited over a step topology [10]. Fig. 3 shows an example SEM image of this bread loafing effect.

It was determined that if the hollow core waveguide could be raised up onto a pedestal, this would offset the natural crevice formed and the bottom corner of the...
hollow core. This hypothesis was proven correct and more results about the pedestal can found in the references included [3, 9, 11].

In order to eliminate alignment issues, the masking step developed for creating the pedestal was designed to be a self-aligned process. AZ4620, a thicker, standard positive photoresist, is first spun-on the wafer. Features are then exposed using standard photolithography in order to mask an area for the solid waveguides; however, then the entire wafer is flood exposed to UV radiation for a short amount of time. The photoresist is then slowly developed away in a low concentration developer until only the top of the sacrificial cores are exposed. Nickel is then deposited via E-beam and liftoff is used to remove nickel where it is undesired.

Once the nickel mask is defined, an anisotropic RIE dry etch is performed to first etch through the bottom ARROW layers and then further into the silicon substrate underneath. Once the etching is complete the wafer is submerged in nickel etch in order to remove the nickel mask.

D. Deposit Top Oxide Layer

After the pedestal is defined and etched the top oxide layer can be deposited. There are many methods to deposit SiO₂ or other transparent oxides onto a wafer. One very common method is standard chemical vapor deposition (CVD), which involves introducing two gas sources to the wafer surface, where they react and form a solid oxide over the wafer. In most cases the reaction requires quite a bit of energy in order to occur and so the wafer is heated to ~700°C. Oxides grown using this method are generally make dense, high quality, defect free films; however, the many ARROW layers already on our wafer and the sacrificial cores made out of SU8 cannot withstand such a high temperature process without sustaining significant damage.

For this reason, we deposit our oxides using plasma enhanced chemical vapor deposition (PECVD). PECVD also reacts source gases in order to form a solid precipitate on a substrate just like CVD, but in the case of PECVD instead of using high temperatures to force the reaction to occur, energy is added by forming a plasma in the reaction chamber using the reactant gases. This helps them react at relatively low temperatures (~250°C), which our devices can withstand without any damage. The gases used in the process recipe are silane (SiH₄) and nitrous oxide (N₂O).

There are some disadvantages to using PECVD SiO₂. Due to the low deposition temperature the oxide tends to be lower quality: less pure and less dense [12].

Another major concern is that the nonstoichiometric oxide possesses dangling bonds and forms nano-scop ic voids that allow it to absorb water from its surroundings [13-16]. Water absorption can alter the optical properties of the material post fabrication and cause undesired changes in device performance. In the next section, we discuss one of the undesired changes, which is the decrease in optical throughput in the waveguides on the device.

E. Pattern and Etch Solid Waveguides

Once the top oxide is deposited, photolithography is once again used to pattern the solid core waveguide on the wafer. Typically, a nickel mask is used as the etch mask. Once masked another anisotropic RIE etch is used in order to etch into the oxide and form the solid core waveguides. There are many important design considerations concerning the dimensions of the solid cores in order to ensure optimized device performance. These are discussed in more detail in the following references [17, 18]. The total thickness of the top oxide is also discussed.

Typically, the top oxide is 6 µm thick and the waveguides are etched 3 µm deep and are made to be either 4 µm wide or 12 µm wide in order to ensure optimized optical coupling throughout the biosensor.

F. Deposit Top Cladding Layer

This is a relatively new step that was added in order to bury the all of the core oxide under a protective, cladding layer. The cladding layer must have a lower index of refraction than the core oxide in order to ensure that successful waveguiding still occurs in the biosensors. If the index is not sufficiently lower than the core index then this layer fails to actually protect the waveguides from the adverse effects of water absorption discussed in the next section.

G. Expose and Etch Away Sacrificial Cores

The last step in the fabrication process is to expose the sacrificial cores and then etch them out in order to form hollow channels for biosensing. Once again, photolithography is used in order to pattern a mask. The mask exposes only the very edges of the sacrificial cores. HF is then used to etch through the top cladding layer and the core oxide layer and expose the ends of the sacrificial cores.

Once the cores are exposed they are etched away using a selective wet etch. Piranha, a mixture of hydrogen peroxide (H₂O₂) and sulfuric acid (H₂SO₄) will etch away the cores over time and leave the oxide layers unaffected. This mixture is usually a 6:4 ratio of
H$_2$O$_2$ to H$_2$SO$_4$ and is heated to 130°C to encourage a quick and yet gentle etch. These parameters are important because a more aggressive etch recipe will put the hollow cores under too much strain and cause them to pop and break. The broken hollow cores no longer hold a liquid solution and ruin the biosensor.

V. RECENT ADVANCES AND RESULTS

It was discovered quite a while back that PECVD SiO$_2$ absorbs water from its atmosphere, making our old waveguides quite unstable and requiring a damaging and time consuming 300°C bake out of the waveguides in order to ensure proper functioning [14, 16].

This problem was solved by adding a protective cladding layer over the core oxide that made up the waveguides. Fig. 4 shows the initial results achieved using our first cladding layer design. It is clear that our cladding layer provided some protection from the adverse effects of water absorption; however, it is also clear that the buried waveguides still decreased in optical throughput over time. After 20 days the buried waveguides were down near 50% of their original throughput.

Since then it has been discovered that increasing the index of refraction contrast between the core and cladding layers can greatly improve the protection the top cladding layer affords the waveguides. Fig. 5 is a graph that depicts our latest buried waveguide results using different index contrasting waveguides.

The first waveguides tested had a very small index contrast. The core oxide index was 1.457 and the cladding oxide was 1.452. Fig. 5 shows that these waveguides were very susceptible to adverse water absorption effects and after only a few days maintained only a small percentage of their original optical throughput.

The next waveguides depicted are the waveguides used in our first experiment. The core oxide index for these waveguides was 1.48 and the cladding index was 1.46. As mentioned above these waveguides obviously performed better than an unburied waveguide, yet still had a significant decrease in throughput loss after many days of high water exposure.

The third group of waveguides had a core index of 1.51 and the cladding index was 1.448. Fig. 5 clearly shows that these waveguides experienced relatively no decrease in optical throughput after 40 days of water exposure.

It is important to limit all forms of loss within the biosensors in order to ensure high detection sensitivities. Previous voyages to Mars have had ambiguous results when it comes to life detection [1]. Due to the high expense of the trip and the long turnaround time it is important that the device used for life detection be accurate and trustworthy. Any undesired optical losses in the biosensor represent a chance for the biosensors to miss the presence of a biological particle in a sample. The ability to make the waveguides completely environmentally stable is a
major step forward in making the sensors ready for actual use, such as a life detection mission to Mars. Now that waveguides have successfully been made water resilient, we have begun working on integrating these waveguides into actual biosensors. Once completed, these new biosensors will have no need for a 300°C bake out in order to perform correctly and will be much more stable than their predecessors.

VI. CONCLUSION

The ARROW biosensor is an optofluidic device that has previously been included in life detection mission proposals to Mars. While the BOLD mission to Mars did not take place, life detection on other planets remains a major goal of space and interplanetary research. The ARROW biosensor remains a viable option for including on a life detection mission because of its small size, weight, low power use, and simple, yet sensitive detection scheme. Since inclusion in the BOLD mission to Mars the biosensor has seen several major advancements that have made it even more stable, sensitive, and capable of performing well in an interplanetary life detection mission. One improvement is the addition of burying the waveguides under a high index contrast cladding layer in order to mitigate the adverse effects of water absorption in the oxide waveguides. These buried waveguides are in the process of being integrated into actual biosensor devices in order to demonstrate their improved biosensing capabilities.


