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Bio-Box: An Instrument for in-Situ Detection of Life Under Extreme Environments

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***Abstract*— The search for life in extreme environments on earth and places beyond earth has always been one of the biggest challenges for the human race. A lot of space exploration programs, each with new instruments for the detection of biosignatures are under progress. We have designed one such device named Bio-Box to detect some of the biosignatures of life at any potential site by carrying out in-situ biochemical assays of the soil present at the site. Bio-Box can be integrated with any surface mission sent for the search of extraterrestrial life. The device contains cuvettes which are assembled in a particular pattern and are loaded with chemicals that will be used to detect biosignatures in the soil sample. The collected soil sample is transferred into the cuvettes through a funnel. The Bio-Box has a spectrometer, which measures the absorbance of the sample in order to detect a colour change in the cuvettes which is due to the reaction taking place after addition of the soil into cuvettes containing the reagents. This is studied using a graphical output given out with the help of microcontrollers. The body of Bio-Box is made up of high-grade aluminium to give it a rugged design and is entirely wrapped with black insulation in order to make it impervious to light and thus ensuring proper functioning of the spectrometer. This paper explains the various structural features of the Bio-box and also describes its working and the methods to infer results from the output.**

Keywords—Life, Biosignature, Bio-box, Spectrometer, Biomolecules, Absorbance.

INTRODUCTION

This Composition of martian environment has always been a great mystery to find answers by international space programs for decades. NASA and other premier space organisation are constantly trying to unveil the mysteries about Martian surface and its biochemistry. The major objective of any mars mission is to search for life and for this we need to carry out soil sample analysis, which becomes a complicated task without human supervision. The bio-box developed aims at solving this problem as it does not require any human supervision. NASA spent more than $2.5 billion on its curiosity rover project out of which $16.9 million was spent on the science part [1]. Analysing soil helps us in identifying the possibility of survival of life and could help us in unraveling its life history. Bio-box is a device which can be coupled with any rover to perform in-situ biochemical tests to check the presence of life in the given soil sample and export the obtained results to the base station. It is fitted with an in-built spectrometer setup to perform in-situ quantitative analysis of biosignatures which in turn will prove life and can export the obtained values to the base station. It could function at extreme terrains and weather conditions, owing to its rugged and compact structure.

PRINCIPLE

The Bio-box is designed to perform the operations of an UV-Visible spectrometer in a way such that it is compatible with any rover or other surface missions to aid in the search for life at extreme environments.

A UV Visible spectrometer is a device capable of measuring the spectrum of light at wavelengths from 300 nm to 700 nm. To perform various biological assays autonomously, a spectrometer capable of measuring the emission and absorption spectra on-board a rover has been designed.

The spectrometer consists of 3 distinct parts:

1.An emission or reflection-based diffraction grating.

2.A light sensor

3.A actuation mechanism capable of changing the samples.Traditional spectrometers are large, bulky, expensive and power hungry. This cannot be incorporated into an on-board system.

The main parameters for an on-board system are:

- Low cost
- Portability and Low power consuming
- Ruggedness
- Capable of working and communicating in tandem with the rover processor.

STRUCTURE

Dimensions of the bio-box :
195 X 185 X 125(millimeters)

To ensure that all the aforementioned specifications are met , a spectrometer has been designed with the following parts :

COMPONENTS
Aluminum body
LED
Grating
Cuvette
Turntable
Light sensor/ CCD array
Servo motor

1) Aluminum Body:

The Bio-box is made of 6061-grade aluminum (also contains magnesium and silicon). It is anti-corrosive in nature and can withstand acidic nature of 2.8 pH and alkaline nature up to 8

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pH encountered during various chemical reactions. It is moulded into a cuboidal shape whose dimensions are 185mm x 125mm x 195mm. We used aluminum as it is anti-corrosive, cheap and could withstand extreme weather conditions. The bio-box is wrapped by a thick black insulating tape so as to prevent any entry of external light into it, done so because the spectrometer fixed inside needs to be void of light for its functioning [2][3].

Figure 1 : Aluminium body

2) Cuvette holder:

To arrange the cuvettes inside the aluminium body, a 3D-printed cuvette holder is used which was printed using ABS (Acrylonitrile butadiene styrene) as it is durable, slightly flexible and heat resistant. The cuvette holder is designed in such a way to hold the cuvettes in a circular arrangement with equal spacings between them so that the beam of light from all the cuvettes can be incident on the grating which is mounted at the centre.[4][5]

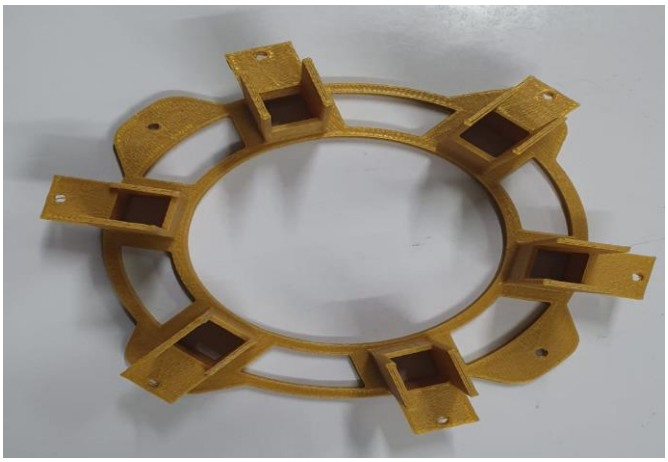

Figure 2 : 3D printed cuvette holder

3) Cuvettes

A series of quartz cuvettes are placed in the cuvette holder. The number of cuvettes are determined by the number of different assays performed which will be decided before the designing phase of the Bio-box in order to 3D-print the cuvette holder.[6]

Dimensions:
Cuvette- 45 X 125 X 12(millimeters)

4) LED Light

Each cuvette holder has a LED light attached to it. This LED serves as the light source for the measurement of absorbance by producing a coherent and high intensity light which should not diverge and pass through the cuvette efficiently.

5) Turntable

The Bio-box has a turntable which houses the spectrometer and grating. It is made up of 6061 aluminum and slightly elevated from the base. It is controlled by a servo motor and rotates the light sensor and the grating element which are mounted parallel to each other. The turntable adjustments are precise to avoid the deflection of beam when projected through the cuvette.

Dimensions:
Radius of the turntable - 45(millimeters)

Figure 3 : Aluminium body with the turntable

6) Diffraction Grating

A reflection grating made from a cd / dvd cutout has been used . A disk has been used in for the following reasons: a) Low cost – An actual lens / mirror based sensor would be prohibitively expensive. b) Weight / ruggedness : An optical disk is made of plastic which is more rugged and lighter than glass. c) Reflection grating – A reflection grating would reflect the spectra which reduces the space required. An emission type grating would require a longer chamber. d) By Bragg's law , the spacing between the grooves of an optical disk are of the appropriate dimension for the diffraction of UV-visible radiation.[7][8][9]

7) Spectrometer/ Light sensor/ CCD array

To sense the emission and absorption spectra throughout the bandwidth of UV-visible radiation , we need a densely packed array of sensitive light sensors. An ideal candidate is an 1-Dimensional Charge coupled device array (1D CCD array). We are using Sony ILX511B spectrometer sensor that can detect even slightest change in humidity and temp and give corresponding wavelength of the color emitted by the excited electron. A low cost Sony ILX554B array has been chosen for the following reasons: a) Low cost b) Compact

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size of 4cm c) 5V operation d) 2048 pixels giving an effective resolution of 0.19nm e) Pulsed array output.[10][11]

8) Servo Motor

The turntable can rotate 360° with the help of a servo motor on which it is placed. We use Servo motor-HS785H with a clock speed of 4.8 milliseconds for 360 clock turn shifts which is placed below the turntable to rotate the ccd array at prefixed angles to receive inputs from different cuvette samples.[12][13]

Dimensions:
Radius of the Axis-40.7 x 19.7 x 42.9 mm

9) Soil Transfer system

We use a funnel-pipe network system for soil transfer into the Bio-box (i.e.) the soil sample is dropped into the funnel which is connected to a series of tubes that in turn opens up into the cuvettes thus ensuring that the sample is equally distributed among the cuvettes. This system ensures equal distribution of soil into each cuvette by keeping the diameters of all the tubes equal.

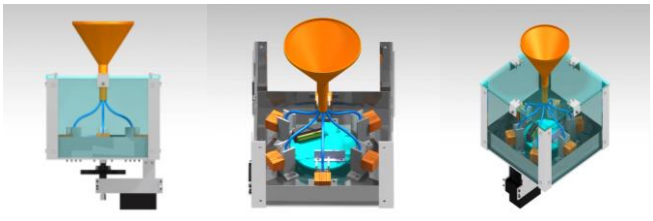

Figure 4 : Funnel mechanism used for soil transfer

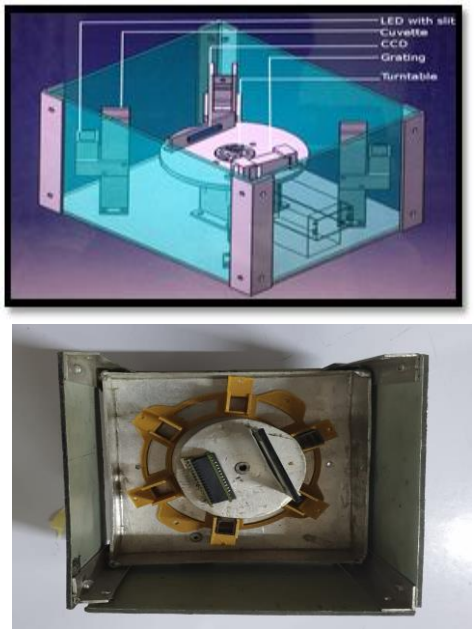

Figure 5 : a) 3D design of the biobox b) fabricated biobox with all components

WORKING

A. Different rovers have different arm mechanisms to collect soil samples and analyze them. The Bio-box requires the arm to drop the soil particles into the funnel after which the soil passes via the connecting tubes and drops into the cuvette. The diameters of all the connecting tubes are equal so that the soil is equally distributed among all the cuvettes. The soil sample reacts with the reagents present in the cuvette undergoing a colorimetric reaction. The LED lights are switched on one at a time and the turntable aligns itself with the corresponding cuvette whose absorbance is to be measured. The light passes through a narrow slit in the cuvette and provides a narrow slit source for diffraction. The incident slit light with the modified spectra incidents on the diffraction grating . The reflected beam is composed of the incident radiation split into corresponding constituents which in turn is detected by the spectrometer.[15]

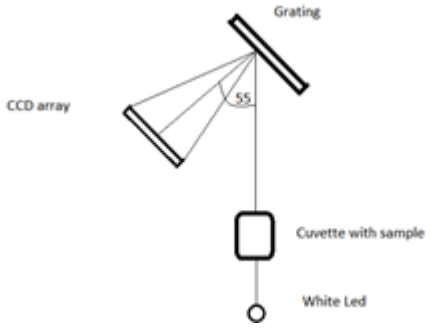

Figure 6 : Absorbance measurement of a sample using CCD array

RESULT DETERMINATION

Once light falls on the spectrometer we obtain the absorption / emission spectra for a particular incident radiation wavefront by mapping the pixel number to the wavelength (pixel number* resolution) and measuring the amplitude from a particular pixel. By calculations , it is pre-ensured that UV (300 nm) would fall on pixel 1 and Red (700 nm) would fall on pixel 2048.

An output graph containing pixel-amplitude curve is obtained using the My Spectral software whose code is incorporated within the main code.

According to Beer-Lambert's law, the absorbance value of a solution is directly proportional to the concentration of solute in them. The absorbance value is the amount of light absorbed by a solution when light passes through it. The light is absorbed by molecules present in the solution and hence the absorbance value increases if the concentration of these molecules increase.

The output graph shows the absorbance of the solution through the entire spectrum. The sudden deflections in the graph in a particular region shows that a solution with a color corresponding to that pixel number has been formed. If the required molecule is present in the soil sample it forms complex compounds with the reagent thus absorbing most of the light passing through them and thereby increasing the absorbance which is detected using the graph. Only colorimetric tests are carried out in the Bio-box so that the

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appearance of the anticipated colour indicates the presence of the biomolecule in search.
Using the absorbance values from control solutions, the concentration of biomolecules present in the sample can also be determined.

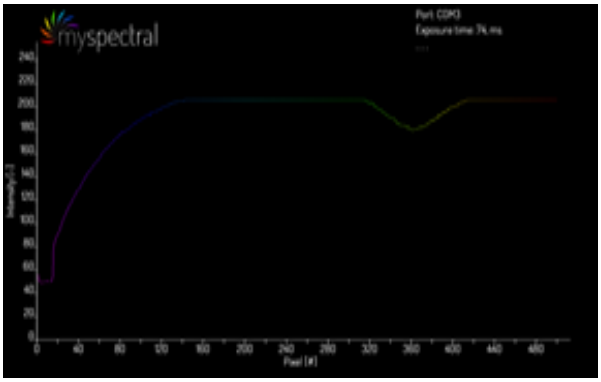

Figure 7 : Model output graph developed by MySpectral software

LIFE DETECTION

Detecting the presence of life form is the prime objective of our Bio-box. Biosignatures are the substances whose presence could prove the possibility of the existence of life form in a particular environment. Only colorimetric tests should be used since these tests produce a color change if the required biomolecule is present thus corresponding to higher absorbance values.
Life is a characteristic feature that differentiates entities that has the capability to undergo biological process from those that cannot.Those entities that doesn't come under this category are considered to be inanimate. The salient features of a living organism are listed below:
Movement: Every living being undergoes locomotion/movement in form or the other.
Respiration: Respiration is an exothermic reaction that happens within cells to generate energy by using our food as a substrate.
Sensitivity: The ability to detect changes in the surrounding environment.
Growth: A positive change in size of an organism over a given period of time.
Reproduction: The ability of an organism to give rise to offsprings. Excretion:
It's the process of removing waste from a living body.
Nutrition: The source of energy which is quintessential for performing our day-to-day activities.

A biosignature is any indication in the form an element, isotope, or molecule that helps us in determining extinct or extant life.[16][17][18]. Common parameters of life include its structure and composition and also its effective use of nutrients and the generation of energy. The most common bio-signatures are carbohydrates, proteins and lipids which the Bio-box is designed to test for their presence.

Carbohydrates
Carbohydrates are major components of microbes found in soil, they are present both as an integral part of cell structure and as a participant in several metabolic reactions [4]. Carbohydrates in living organism are prominently found in conjugated form(i.e) glycoproteins and glycolipids on the cell membrane. In the cytoplasm of eukaryotes, lysosomal degradation occurs which results in production of β-D-galactose, β-D- mannose, and β-D- glucose.Upon cell degradation they are discharged into the soil. These compounds are detected by performing Molisch Test. Molisch test answers all kinds of carbohydrates whether they are in free state or in conjugated form. The positive reaction produces a purple-coloured ring which is determined by the measurement of absorbance at 600 nm.[19]

Proteins
The major source of proteins in any living form is from its cell wall and cell membrane.
Proteins mostly occur in conjugate forms (i.e) in a combined form with another bio-molecule like protein or lipid. E.g: glycoprotein and lipoprotein. Apart from this, proteins are also found in its raw form in cytoplasm of certain microbes. Presence of protein is confirmed by biuret's test.
In the presence of peptides, the copper (II) ion (from biuret's reagent) forms an intense violet-coloured coordination complex in alkaline solution (NaOH). The protein concentration is determined by the measurement of absorbance at 540 nm.

Lipids
Lipids have their significant presence in the cell membrane in the form of glycolipids and lipoproteins. In a eukaryotic cell, lipids are synthesised in smooth endoplasmic reticulum.
At any given stage of a cell, there are significant presence of mono and triglycerides which are synthesized by the smooth endoplasmic reticulum(SER). They could easily be detected by performing Sudan (III) test to the soil sample. A positive sample has characteristic reddish-brown texture and a maximum absorption at 304 nm.[20]
In all these 3 tests , the concentration of the biomolecules are directly proportional to the absorbance at a wavelength characteristic of the particular reagent in accordance with Beer-Lambert's law. The absorbance at any particular wavelength is calculated by determining the variations in intensity value of the corresponding pixel number which in turn is calculated using the formula: pixel # = resolution * wavelength. A decrease in the intensity value shows an increase in the concentration of the required biomolecule.

FIELD STUDIES

The preferred spellin Team RUDRA - SRM Mars Rover is one of the teams participating in the University Rover Challenge which takes place at the Mars Desert Research Station annually. At URC 2017, the bio-box was incorporated with Team RUDRA's rover in order to select a potential site and to analyze the sample for life.
All lifeforms share certain common properties, one of which is the synthesis of proteins coded by the DNA which carry out the functioning of the cell. Team RUDRA planned to detect the presence of proteins bound by membranes by performing the Bradford's assay.
Bradford's Reagent protein assay is based on the colorimetric shift of the Coomassie G250 dye from green to blue due to

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change in the net charge of the resultant molecule. Attenuation in the 595 nm range is observed in proportion to the quantity of protein present. The quantity of protein is estimated by the absorption shift measured using our onboard spectrometer. In order to determine the quantity of extracellular and intracellular proteins, Bradford's test has been performed in 3 samples, 2 of which consist of Bradford's reagent in mixture with other agents like Triton X and SDS. Triton X is a cell lysing agent which releases membrane bound proteins by increasing the permeability of the cell membrane , as well as lysing the phospholipid bilayer using its polar group.

Sample 1 : Bradford's Reagent + soil sample

It is used to determine the presence of proteins in the soil. It indicates the level of extracellular proteins.

Sample 2: Bradford's Reagent +Triton X + soil sample.

Here Triton x is a Cell Lysing agent .

It enables the assay to determine the level of total proteins in the soil, that is the intracellular and extracellular proteins as well

Sample 3: Bradford's Reagent + SDS + soil sample :

SDS , a detergent, when added to samples containing protein , denatures them to their primary structure preventing the G250 dye from binding.

This gives the level of light that was not absorbed by the proteins.

From the output graphs obtained via MySpectral software, the presence of life was confirmed as the concentration of intracellular proteins was greater than the concentration of extracellular proteins which was indicated by the difference in the absorbance values. This shows the existence of membrane bound cells which are actively producing proteins by the processes of transcription and translation[21][22].

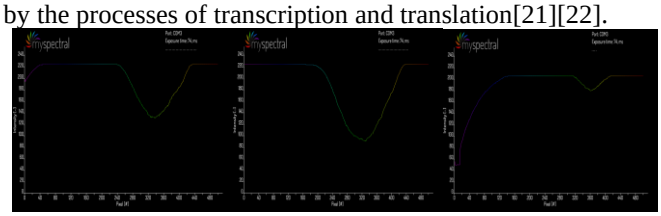

Figure 8 : Output graphs for protein detection
a) soil + Bradford reagent b) soil+Bradford reagent+Triton X
c) soil+Bradford reagent+SDS

APPLICATIONS

Bio-Box is a lightweight, easily mountable, versatile device and can perform a huge amount of chemical tests
, this makes it capable of detecting the presence of life. Bio-Box can be coupled with any rover and can be sent to the moon or mars mission. Alternatively, it can also be used for carrying out soil analysis for crops if modified slightly.

LIMITATIONS

Bio-Box can only be used once i.e the cuvettes have to washed and refilled with the reagents again in order to perform the testing at multiple sites. And this has to be done by the rover. In order to overcome this defect, we have planned to create sinks and reagent tanks to wash the cuvettes and refill the reagents.

Bio-Box only supports colorimetric chemical tests i.e any test which doesn't show colour change can't be used. In order to overcome this defect, we have planned to place a high definition camera inside the biobox along with the spectrophotometer. Bio-box doesn't support chemical tests that require heating as it can be risky for onboard electronics. In order to overcome this, we have planned to use infrared heating. The examination of rocks by Bio-box requires it to be powdered to test them within the cuvettes of the Bio-box which additionally requires the rover to have an arm with auger mechanism. We are expecting to implement these upgrades in our upcoming model.

CONCLUSION

Mars has been visited by a total of 10 spacecrafts, from Mars 2, a Soviet probe that crashed badly in the planet in 1971, to NASA's Opportunity, Spirit and Curiosity which might have carried Earth microbes that were contaminated during the launch. If one of those terrestrial rocks was contaminated with microbes, the organisms might have survived on Mars for a time but died eventually because of harsh climate but would have left signs in the geology there. Still, scientists and researchers are very confident that they can develop instruments to differentiate between microbes of Earth and Mars. Last but not the least, the effort taken to detect life on Mars will prove its worth at the right time for the life on earth.

Thus, the bio box is an appropriate device that can be used for future exploration of mars for the detection of life. We have tested the bio box at simulated Martian environments such as MDRS, Utah and were able to obtain proper results without any malfunction. The bio box can be used as a screening instrument which can examine the potential habitable sites on mars and suggest the best site which can be used for a future mars soil-return mission. Hence, we strongly believe that the Bio-box will join the search for life on mars and other planets in the upcoming years.

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