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N94-25357

NON-INVASIVE OPTICAL DETECTION OF GLUCOSE IN CELL CULTURE
NUTRIENT MEDIUM

Final Report
NASA/ASEE Summer Faculty Fellowship Program-1993
Johnson Space Center

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Date Submitted:	August 18, 1993
Contract Number:	NGT-44-001-800

ABSTRACT

The objective of the proposed research was to begin the development of a non-invasive optical sensor for measuring glucose concentration in the output medium of cell cultures grown in a unique NASA bioreactor referred to as an integrated rotating-wall vessel (IRWV). The input, a bovine serum based nutrient media, has a known glucose concentration. The cells within the bioreactor digest a portion of the glucose. Thus the non-invasive optical sensor is needed to monitor the decrease in glucose due to cellular consumption since the critical parameters for sustained cellular productivity are glucose and pH. Previous glucose sensing techniques have used chemical reactions to quantify the glucose concentration. Chemical reactions, however, cannot provide for continuous, real time, non-invasive measurement as is required in this application. Our effort while in the fellowship program was focused on the design, optical setup, and testing of one bench top prototype non-invasive optical sensor using a mid-infrared absorption spectroscopy technique.

Glucose has a fundamental vibrational absorption peak in the mid-infrared wavelength range at 9.6 μm . Preliminary absorption data using a CO_2 laser were collected at this wavelength for water based glucose solutions at different concentrations and one bovine serum based nutrient medium (GTSF) with added glucose. The results showed near linear absorption responses for the glucose-in-water data with resolutions as high as 108 mg/dl and as low as 10 mg/dl. The nutrient medium had a resolution of 291 mg/dl. The variability of the results was due mainly to thermal and polarization drifts of the laser while the decrease in sensitivity to glucose in the nutrient medium was expected due to the increase in the number of confounders present in the nutrient medium. A multispectral approach needs to be used to compensate for these confounders. The CO_2 laser used for these studies was wavelength tunable (9.2 to 10.8 micrometers), however, was to unstable across wavelengths to test the multispectral approach.

From this research further NASA support was obtained to continue the work throughout the year in which a more stable light source will be used at smaller, near-infrared, wavelengths. It is anticipated that a more compact, non-invasive, optical glucose sensor will be realized which can be used with a bioreactor on future space shuttle missions. It is also anticipated that a multispectral optical sensor may be used to determine the concentration of other molecules needed within the NASA bioreactor, such as fructose and galactose.

INTRODUCTION

The NASA Johnson Space Center - Biotechnology Group is currently involved in the development of a new class of cell culture vessel technology [1,2]. The rotating wall vessels are fluid filled cylinders with a silicone membrane oxygenator in which cells and microcarrier beads are horizontally rotated in free suspension resulting in a low shear, quiescent, environment for the culture of these cells. It has been shown that under these simulated microgravity conditions certain cells (i.e. Baby Hamster Kidney (BHK-21), human transitional epithelial bladder carcinoma cell line (T-24), etc.) provides for superior growth, less cellular damage, and less glucose utilization. Currently, the NASA/JSC Biotechnology Group is utilizing a set of *invasive* sensors (i.e. pH, CO₂, O₂, and glucose), off-line, to monitor these samples, to maintain the cells by manually increasing or decreasing the concentration of the various parameters, and to study the effects of feed back control systems on tissue development. As would be expected this approach has the drawbacks of potential contamination and subsequent death of the cells as well as the lack of real-time control. Our work focused on the development of a glucose detection system to overcome the above concerns by providing continuous, non-invasive, sensing of the glucose concentration within the cell culture medium.

Several non-invasive glucose monitoring methods have been proposed and investigated, particularly for blood glucose determination. One approach is polarimetry [3,4,5,6,7] in which a beam of linearly polarized light is directed through the aqueous humor of the eye and due to the chiral nature of the glucose molecule the light polarization rotates in an amount proportional to that of glucose. For this application, since the nutrient medium has a number of optically rotatory confounders, this approach was deemed inadequate. The second approach is a development based on the near infrared (NIR) (750 nm - 1300 nm) spectroscopic absorption of light due to glucose at certain wavelengths. This approach has been used extensively in agricultural food analysis but has been recently applied to blood glucose measurement in which the light is directed through the finger [8,9] and derivative or multivariate analysis techniques are used to quantify the glucose levels. Since NIR bands are due to weak overtones and combinations of fundamental molecular vibration absorption bands which can overlap significantly in a complex medium, such as the one used here, this technique was not initially considered. The last technique is also based on infrared absorption spectroscopy but in the mid-infrared region. Both transmission [10] and attenuated total reflection (ATR) [11,12] techniques have been used in this region. The glucose concentration was found to be directly proportional to the absorption peak at the fundamental vibrations for that molecule. In fact, Mendelson, et.al.[12] reported a resolution in the measurement of glucose concentration in whole blood of 30 mg/dl at the 9.6 μ m absorption peak using the CO₂ laser line at that peak. Therefore, using a transmissive optical approach and multiple CO₂ wavelengths it was felt that better than 30 mg/dl could be achieved.

EXPERIMENTAL SYSTEM

The optical path, as shown in Figure 1, began with a Laser Photonics continuous wave CO₂ laser with a maximum power output of 8 Watts. It is linearly polarized 100:1, and is tunable from 9.2 to 10.8 μ m using a grating at the output. Power stability of the CO₂ laser is controlled by air-cooling the laser cavity to a desired temperature set point. This also determines the power of a particular CO₂ wavelength at the output. A marginally

stable output of 400 mW at the 9.6 μm wavelength was used in these experiments. The laser and all optics were mounted on a 2 ft by 2 ft optical breadboard for this bench-top system.

The output beam of the laser was reflected off a gold-coated flat mirror and split with a zinc selenite (ZnSe) window into a reference and sample path. The split from the ZnSe window is polarization and thermally dependent but was set at an angle to give a 50/50 split.

Both the reference and sample paths were chopped at a frequency of 300 Hertz by an optical chopper. The blade was precisely positioned with a three dimensional positioner such that when one blade is transmitting the beam from one path, the opposite blade is blocking the beam of the other path. This caused a 180 degree phase difference between the two beams and allowed for the use of a single detector. With the single detector approach the need for detector matching was eliminated and an increase in the signal-to-noise ratio was achieved.

The two beams passed through their respective 20 μm path length ZnSe sealed cells. The reference cell contained double distilled water for on-line subtraction of water absorption in the sample solution. The sample cell contained the glucose solution to be analyzed. Both beams are focused with 4 inch focal length gold-coated spherical mirrors onto a single mercury-cadmium-telluride-arsenic (HgCdTeAs) detector. Since light was continuously incident on the detector from the two paths, a square wave difference signal, resulting from the phase difference, with an offset from zero was obtained. The difference signal represented the different absorptions of the two solutions and the offset was proportional to the power level incident on the detector.

From the detector, the signal was amplified and sent to a band pass filter with a center frequency of 300 Hz to obtain the difference signal and to a low pass filter with a cutoff frequency of .08 Hz to obtain the power signal. The difference signal was passed to a lock-in amplifier which locked into the fundamental frequency. The output of the lock-in amplifier was an offset signal proportional to the magnitude of the difference signal. The output of the lock-in amplifier and of the low pass filter were sampled at 100 Hz by an A/D board to obtain 100 samples for each signal.

The sampled signals were imported into Microsoft Excel for Windows for data analysis. The two signals were averaged individually and then the lock-in amplifier signal was divided by the power signal to remove small laser fluctuations. A linear regression of the signal ratio to glucose concentration was performed and the resolution of the system found.

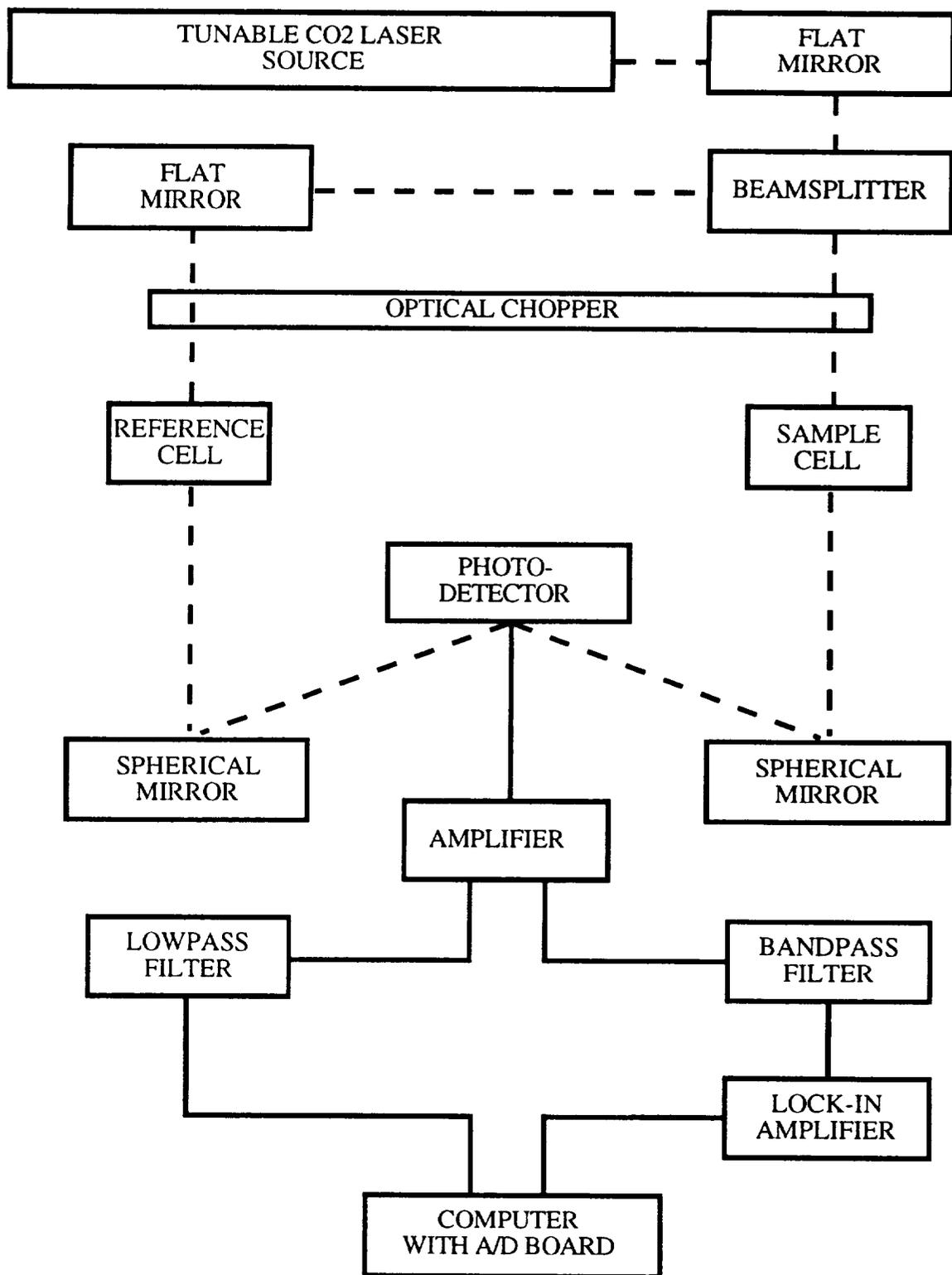


Figure 1.- Block Diagram of the Optical Glucose Sensor Developed for the NASA/JSC Biotechnology Group

RESULTS

The same set of solutions of glucose and water ranging from 100 to 500 mg/dl were analyzed over two consecutive days. The normalized scatter plots of these two runs are included in Figures 2 and 3. A linear regression was performed on the data of Figure 2 and the system resolution was 38 mg/dl. It was discovered, however, that the outlier at 500 mg/dl was due to saturation of the input to the A/D board by the lock-in amplifier. With this point removed the system resolution was found to be 10 mg/dl. A gain adjustment was made to the A/D board and the experiments were repeated the next day. The second run of glucose solutions, shown in Figure 3, showed little repeatability of the system. The obvious difference from the previous data was the decrease in magnitude of the slope. A linear regression was again performed and the system resolution found to be 108 mg/dl. It should be noted that the data had a negative slope which was merely a result of the change in the splitting ratio of the ZnSe window, however this was obviated with normalization. The outlier at 400 mg/dl was due to a momentary shift in intensity to one of the paths off the beam splitter because of laser polarization and power output variations due to thermal effects.

The GTSF solutions with varying concentrations of glucose from 100 to 1000 mg/dl were used in the final experiment. The data is shown in Figure 4 (Again modified to account for negative slope). The linear regression for the data gave a system resolution of 291 mg/dl. The decrease in system resolution was due to the multiple IR absorbing components in solution.

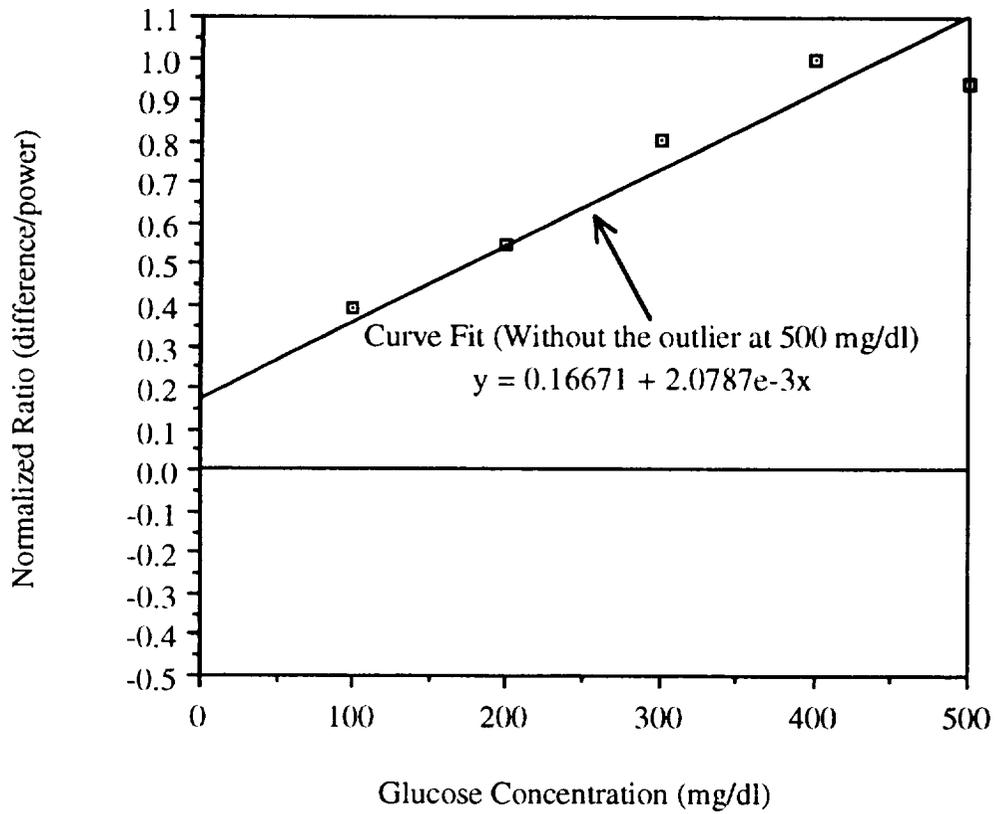


Figure 2.- Initial study of glucose in water showing the normalized ratio of the difference signal as a function of glucose concentration. The outlier at 500 mg/dl was due to saturation of the system.

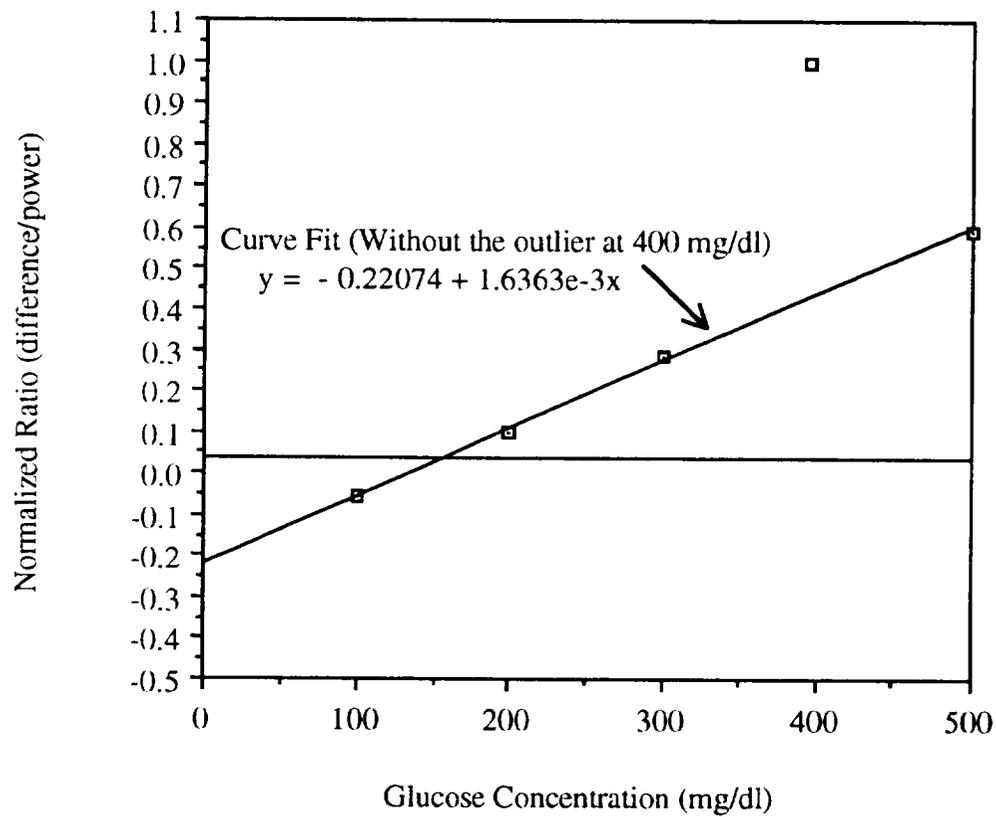


Figure 3.- Repeatability study of glucose in water showing the normalized ratio of the difference signal as a function of glucose concentration from the solutions used for Figure 2, one day later. The outlier at 400 mg/dl was due to shift in intensity of one light path.

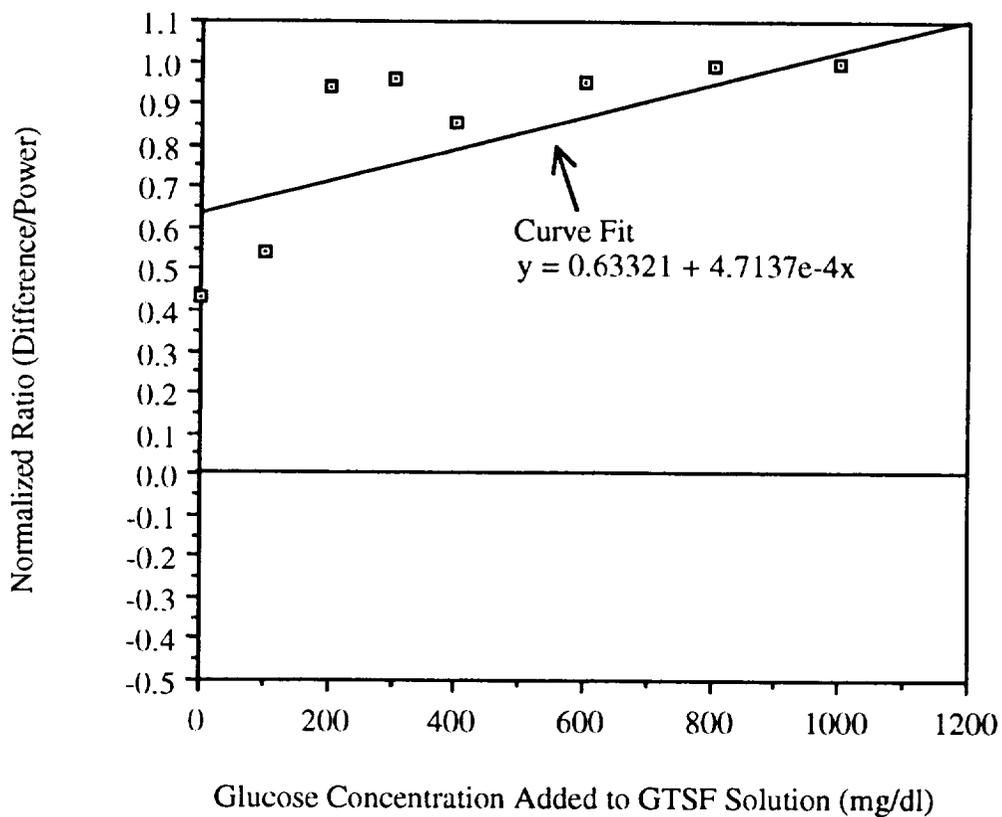


Figure 4.- Study of GTSF nutrient medium showing the normalized ratio of the difference signal as a function of glucose concentration. The result showed a decrease in sensitivity to glucose due to the large number of confounding molecules with overlapping absorption peaks.

CONCLUSIONS

In this report a non-invasive glucose measurement system using a mid-infrared absorption spectroscopy technique that was developed, built, and tested for the Biotechnology Group at NASA/JSC was described. The above results showed the potential for a very accurate glucose measurement system attaining, at times, 10 mg/dl resolution. However, the use of a CO₂ laser as the light source did not allow for repeatable measurements due to its thermal and polarization instability. As anticipated, using a single wavelength, the glucose could only be resolved with good accuracy in the single component (glucose in water) solution but not with the multi-component nutrient medium. It is felt that the use of multiple wavelengths and multivariate analysis would provide the needed 30 mg/dl accuracy in the multiple component cell culture nutrient medium, but the CO₂ laser used here did not show sufficient stability when tuning across wavelengths to perform such analysis.

As indicated, from this research further NASA support was obtained to continue the development of a non-invasive glucose sensor throughout the year in which a more stable light source will be used at smaller, near-infrared, wavelengths. It is anticipated that a more compact, non-invasive, optical glucose sensor will be realized which can be used with a bioreactor on future space shuttle missions. It is also anticipated that a multispectral optical sensor may be used to determine the concentration of other molecules needed within the NASA bioreactor and provide continuous closed loop delivery of various nutrients to the cells.

In addition, this technology, although currently applied to cell culture systems, could also be useful in the non-invasive monitoring of blood chemicals of the astronauts. This will allow for better health care monitoring with less risk to the NASA crew than with contemporary invasive methods such as venipuncture and subsequent analysis by various means following sample preparation. The current monitoring process is time-consuming, requires high to moderate levels of technical expertise, and delivers single time point rather than continuous, real-time data. The development of non-invasive sensors offers significant advantages over current modes. It would reduce the risk of complications due to invasive procedures, increase the availability of critical data with less crew time committed to producing the data, allow real time biomedical analysis and permit telemetry of the data in near real-time to earth stations for medical decision making.

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