

## **INSTRUMENTATION FOR PLANT HEALTH AND GROWTH**

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### **ABSTRACT**

**Comprehensive spectroscopic monitoring of plant health and growth in bioregenerative life support system environments is possible using a variety of spectrometric technologies. Absorption spectrometry and atomic emission spectrometry in combination allow for direct, on-line, reagentless monitoring of plant nutrients from nitrate and potassium to micronutrients such as copper and zinc. Fluorometric spectrometry is ideal for the on-line detection, identification and quantification of bacteria and fungi.**

**Liquid Atomic Emission Spectrometry (LAES) is a new form of spectrometry that allows for direct measurement of atomic emission spectra in liquids. An electric arc is generated by a pair of electrodes in the liquid to provide the energy necessary to break molecular bonds and reduce the substance to atomic form. With a fiber probe attached to the electrodes, spectral light can be transmitted to a photodiode array spectrometer for light dispersion and analysis.**

**Ultraviolet (UV) absorption spectrometry is a long-established technology, but applications typically have required specific reagents to produce an analyte-specific absorption. Nitrate and iron nutrients have native UV absorption spectra that have been used to accurately determine nutrient concentrations at the  $\pm$  5% level.**

**Fluorescence detection and characterization of microbes is based upon the native fluorescent signatures of most microbiological species. Spectral and time-resolved fluorometers operating with remote fiber-optic probes will be used for on-line microbial monitoring in plant nutrient streams.**

### **INTRODUCTION**

**Currently, there are two different approaches to the development of on-line, analytical, process monitoring in industrial, environmental and space applications: general purpose spectrometry and analyte-specific reagent-based sensors. The spectrometric approach that is emphasized here is characterized by the following features:**

- 1. The spectrometric approach is reagentless (no chemicals are immobilized or consumed);**
- 2. A wide range of analytes, chemical and microbial, can be measured;**
- 3. Chemometric algorithms are used to provide specificity; and**
- 4. Fiber-optic links are used as light transport vehicles, not as sensors.**

**For on-line process monitoring, a wide range of spectrometric techniques are candidates for selection, only a few of which will be discussed in this paper:**

- 1. Molecular absorption spectrometry**
  - a. ultraviolet/visible (UVAS)**
  - b. near infrared (NIR)**
  - c. infrared (IR)**

2. Atomic spectrometry
  - a. flame
  - b. absorption
  - c. emission
3. Fluorometry
4. Raman
5. Nuclear magnetic resonance (NMR)

In connection with space applications, Biotronics has experimented with the following techniques for measuring the specified analytes:

1. UV absorption
  - a. nitrates
  - b. iron
2. Atomic emission
  - a. potassium
  - b. magnesium
  - c. calcium
  - d. sodium
  - e. manganese
  - f. copper
  - g. zinc
  - h. molybdenum
  - i. pH
3. Fluorometry
  - a. bacteria
  - b. fungi

The primary goal of this paper is to review the progress and status of Biotronics instrumentation destined for use in bioregenerative life support systems. The main emphasis will be on a new form of atomic emission spectrometry under development, liquid atomic emission spectrometry (LAES).

#### ATOMIC EMISSION SPECTROMETRY

LAES, a new form of atomic emission spectrometry technology being developed at Biotronics, embodies the following characteristics:

1. Direct on-line measurement;
2. Measurement in liquid media; and
3. The use of electrical energy as a source to break atomic bonds that results in emission energy being released.

All atomic emission spectrometry systems require energy to break atomic bonds, which results in atomic spectral lines. In the past, the following energy sources were used to break these bonds to create atomic light emission:

1. Flame;
2. Electrothermal (graphite furnace);
3. Electric arc;
4. Electric spark; and
5. Inductively-coupled plasma (radio frequency field).

These energy sources can be used for atomic absorption, atomic emission and atomic fluorescence spectrometry.

#### LIQUID ATOMIC EMISSION SPECTROMETRY

The spectrometer used for this application is basically the same as that used for ultraviolet (UV) absorption spectrometry. A block diagram of a Hybrid Absorption/Emission Spectrometry (HAES) system is illustrated in Figure 1. This HAES system is designed to operate in either the absorption or the emission mode. In

the atomic emission spectrometry mode of HAES operation, the power supply generates a spark across two electrodes within an optrode containing a liquid medium, creating an arc for a short period of time. The arc energy generates a series of atomic emission lines. The emission light generated travels through fiber-optic cable to a spectrometer where the light is dispersed across the spectral wavelengths in a spectrograph. The dispersed light is detected by a silicon photodiode array in which electrical analog signals are generated and then converted to digital form. These signals are then processed by a microcomputer where estimates of analyte concentrations in the liquid medium are determined.

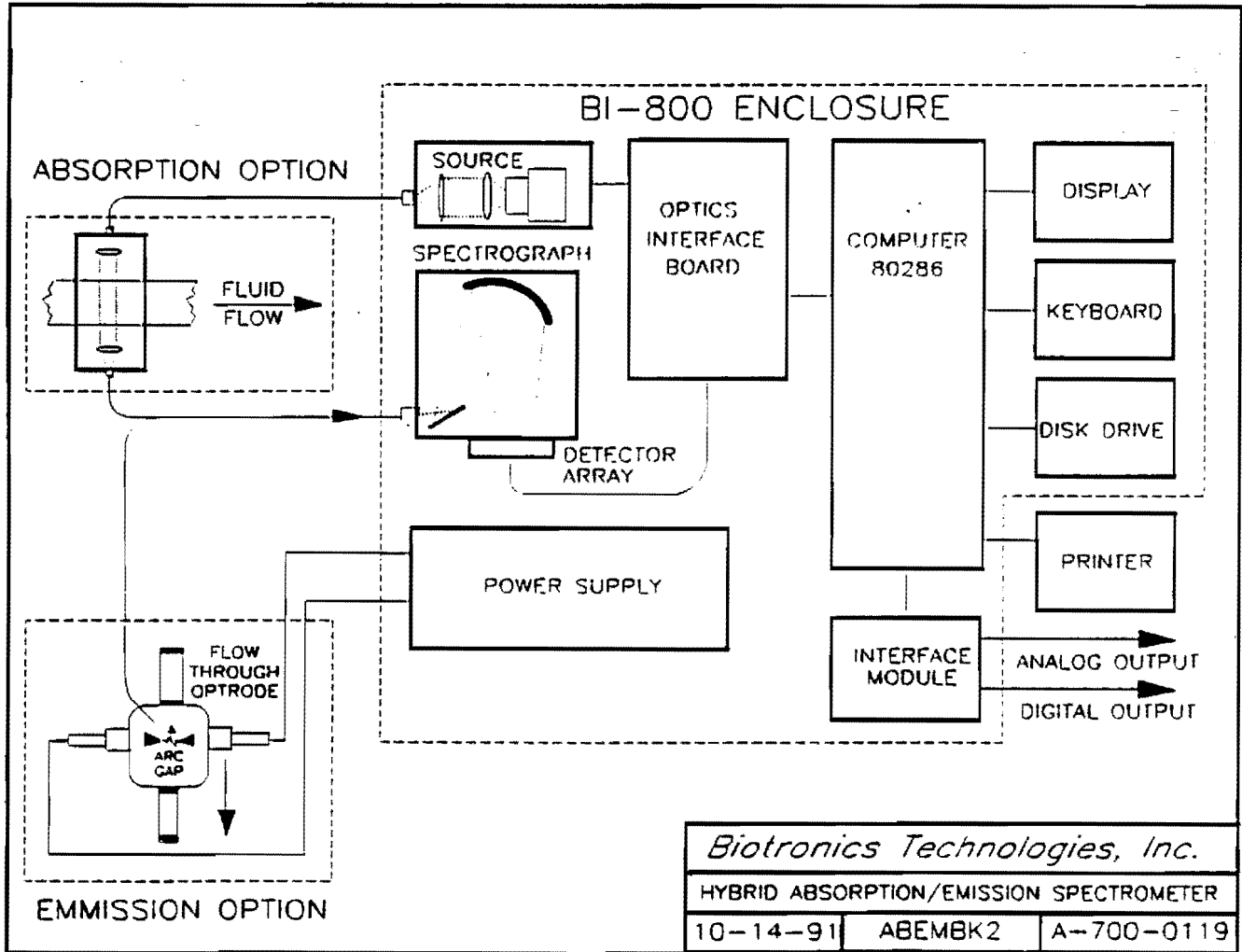


Figure 1

During on-line operation in a bioregenerative life support system, as a monitoring system of hydroponic plant nutrient solutions, a sidestream of the plant nutrient solution would continuously flow through the system optrode. Periodic measurement of atomic spectra produced would lead to the detection, identification and quantification of ionic nutrients in the solution. The basic principles of this system were previously explained in a paper, On-Line Monitoring of Water Quality and Plant Nutrients in Space Applications Based on Photodiode Array Spectrometry /1/. This paper will describe some of the problems encountered during the development of the LAES system and the approaches used for the solution of these problems.

### LAES Development Problems

An initial design challenge in LAES development involved the extension of the spectral range of the basic array spectrometer used for UV absorption spectrometry to include the visible and NIR spectral ranges as

well as the UV range (200–400 nm) for which the spectrometer was originally designed. It was necessary to extend the spectral range out to 1100 nm because atomic emission emphasized the use of the visible and the very near infrared (VNIR) spectral regions. While in the UV range, atomic absorption spectra change only gradually in intensity; atomic spectra register sharp changes in intensity in the visible and NIR regions. These fast changing spectra necessitated a higher degree of resolution in the spectrograph component of the LAES instrument.

The development of a high-voltage power supply to provide the arc for the electrodes was another major stage in LAES instrumentation development. Initial experimental results were severely degraded from a lack of current control. The variations in intensity caused by current fluctuations were greater than those caused by changes in chemical concentrations. This problem was solved by the design of a new, fast-response current control loop for the power supply.

Inadequate spark generation was another initial system design problem. In LAES, the spark is used to initiate the arc. The spark produced during the initial stages of development was not capable of delivering a reliable and rapid series of discharges. Extremely high voltages could have been used to initiate the arc eliminating the need for a spark; however, this would have greatly increased electrode wear. Since then a very effective spark circuit with a higher peak voltage has been developed.

Determining the correct material to be used for the electrodes within the optrode also required extensive experimentation. The first material selected was tungsten because this material has been extensively used for electric arc generation. This tungsten material eroded quickly; however, leading to rapid changes in the gap between the electrodes, producing erroneous concentration estimations. Copper was the next electrode material employed. These copper electrodes performed better, but electrode wear was still too rapid to be practical. Also, because of the electrolytic action of copper in the arc, plating occurred during system operation. A third material, gold, was then selected. An 18k gold alloy was experimented with first, but again the alloy plated silver onto the anode. The use of pure 24k gold eliminated this problem. Gold electrodes have now been incorporated in the LAES prototype design.

The ideal solution to the electrode material problem would be the development of a system that produces an arc that has no contact with the electrodes. The arc would float in liquid space and thereby eliminate electrode wear completely. Such a system is currently being evaluated at Biotronics for possible introduction in 1993. This system, designated the plasma current concentrator, has shown great promise in early testing.

Each atomic element can be identified through the atomic lines that it produces upon excitation. The atomic emission line wavelengths are well known and described extensively in the literature. A partial list of wavelengths applicable to the elements of interest here is shown in Table 1. The strength and high resolution of atomic emission lines, as compared to those generated through molecular absorption, simplifies the nature of the analytical algorithm requirements. Initially, one wavelength was used to estimate the analyte concentration based on the intensity of a single line. This method proved adequate, but instrument sensitivity and accuracy were limited by the interaction of elements in more complex media. Normalization based on energy was attempted, but errors greater than those consistent with the performance goals were still being experienced. Multiple-wavelength statistical regression analysis was then implemented. This method was able to account for the interaction between the elements in a complex medium. A multiple-wavelength stepwise regression software package to select wavelengths for the development of ionic nutrient algorithms has been employed at Biotronics for the prototype instrument.

**TABLE 1 Atomic Spectral Lines for Nutrient Analysis**

<u>Element</u>	<u>Wavelengths (nm)</u>	<u>intensity</u>
Sodium	589.0, 589.5	2000, 1000
Potassium	766.5, 769.9	1800, 900
Calcium	393.3, 396.8	4200, 2200
Magnesium	285.2, 383.8	6000, 500
Hydrogen	656.3	3000
Iron	373.5	700
Manganese	403.1, 403.3	2060, 1400
Copper	324.7, 521.8	5000, 100
Zinc	636.2	500
Molybdenum	379.8, 386.4	3200, 2800
Oxygen	777.1	1000
Boron	249.8	500
Sulfur	469.5	500
Phosphorous	253.5	80
Nitrogen*	411.0	1000
Chlorine	479.4, 481.0	200

\* Singly ionized form

#### Review of Test Results

Considering both absorption and atomic emission spectrometry measurements, seven of the fourteen plant nutrients present in hydroponic plant nutrient solutions have now been measured at Biotronics with the required levels of accuracy. These elements include the following:

1. Nitrogen (nitrates)-UV absorption
2. Iron-UV absorption
3. Potassium-LAES
4. Calcium-LAES
5. Magnesium-LAES
6. Sodium-LAES
7. Phosphorous-LAES

It's encouraging to note that all three of the macronutrients (nitrate, phosphorous and potassium) and four other nutrients of major importance can now be measured. Chlorine, sulfur and the micronutrients manganese, zinc, molybdenum, copper and boron are also under experimental investigation. Improved system sensitivity will be required to successfully measure these elements. Some encouraging experimental results are beginning to emerge regarding these low-level analytes. The delivered instrument will be able to monitor at least seven of the fourteen plant nutrients, with the possibility of four more being added. Some recent liquid atomic emission spectra for potassium, calcium, magnesium and sodium are displayed in Figures 2, 3, 4 and 5.

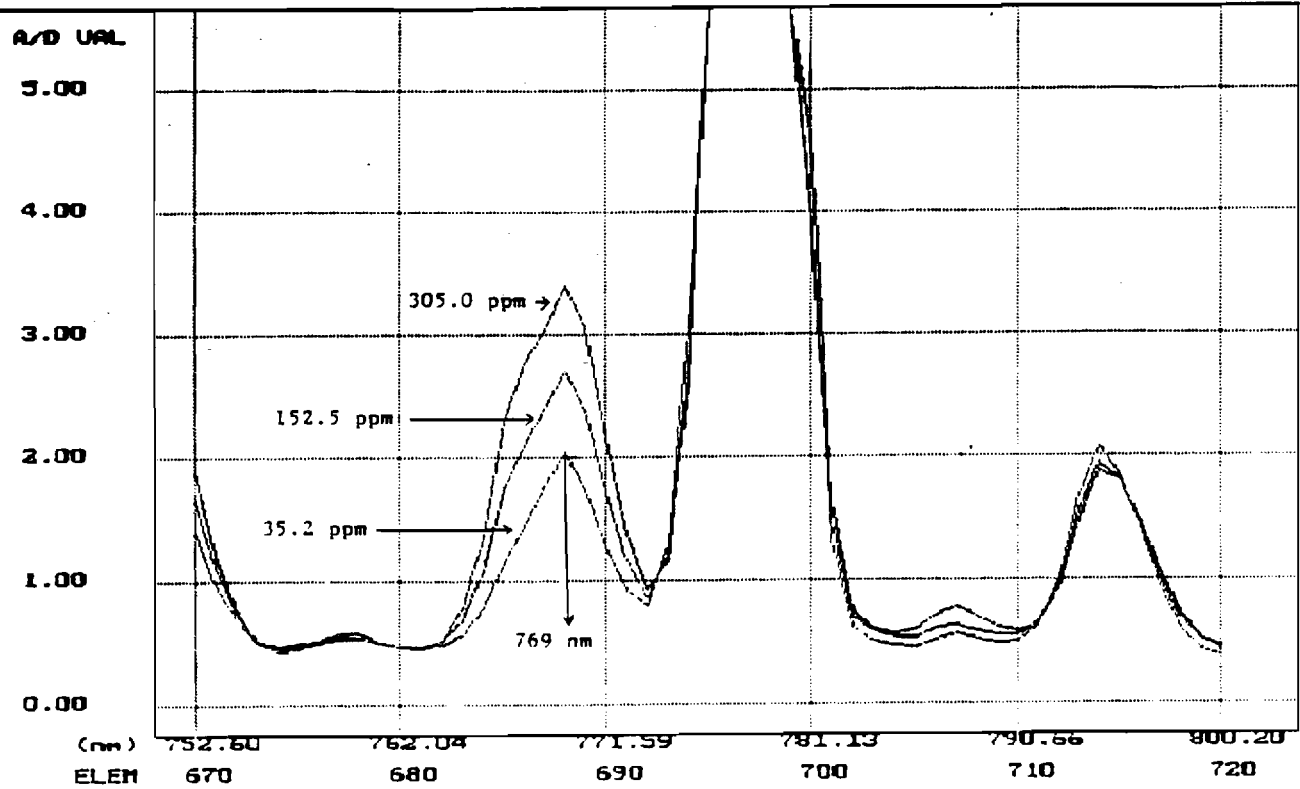


Figure 2. Potassium Emission Spectra

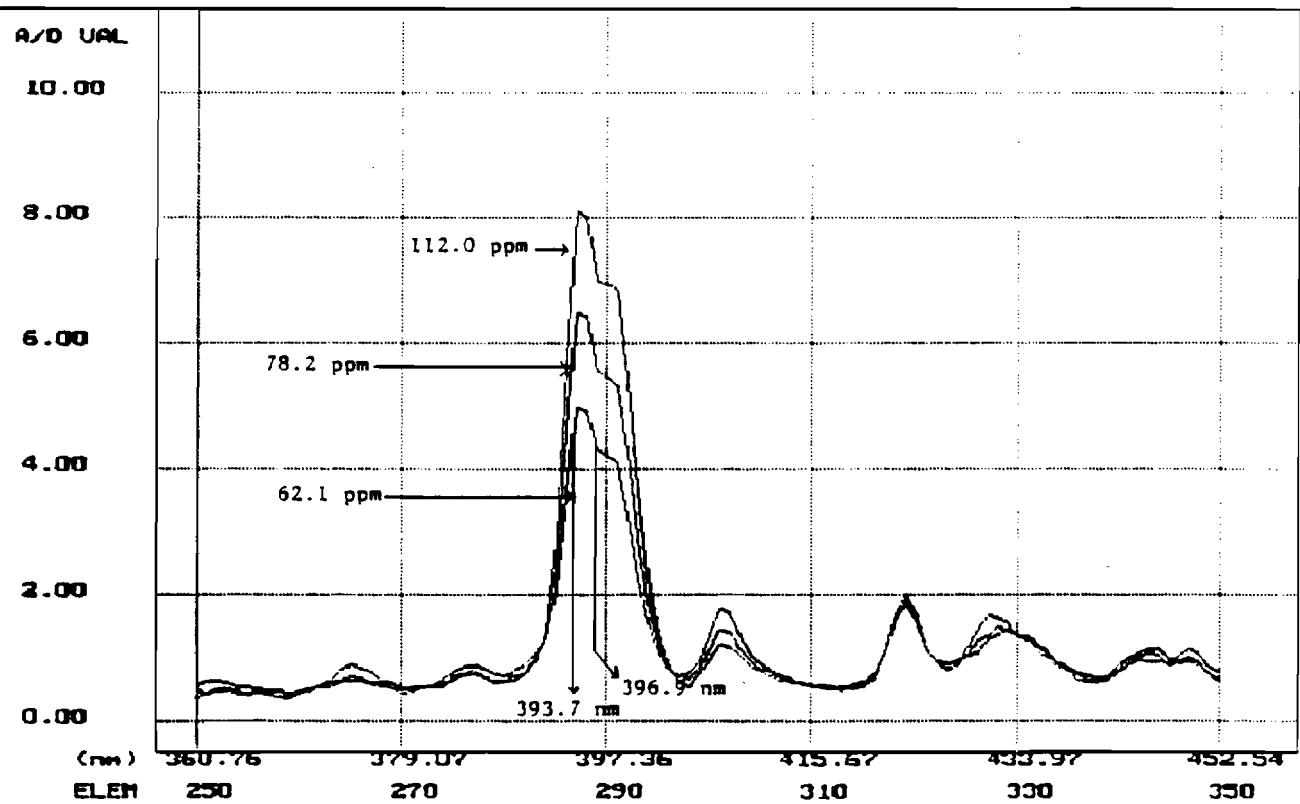


Figure 3. Calcium Emission Spectra

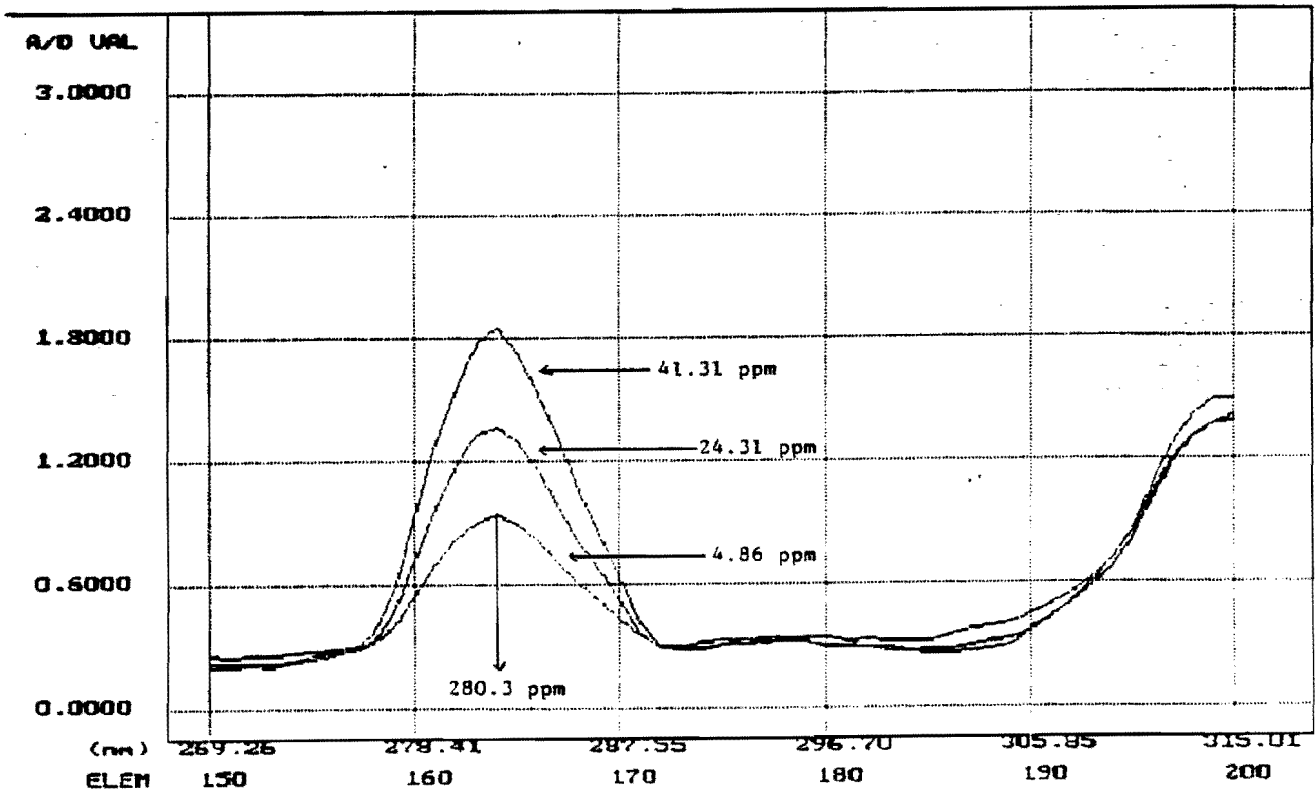


Figure 4. Magnesium Emission Spectra

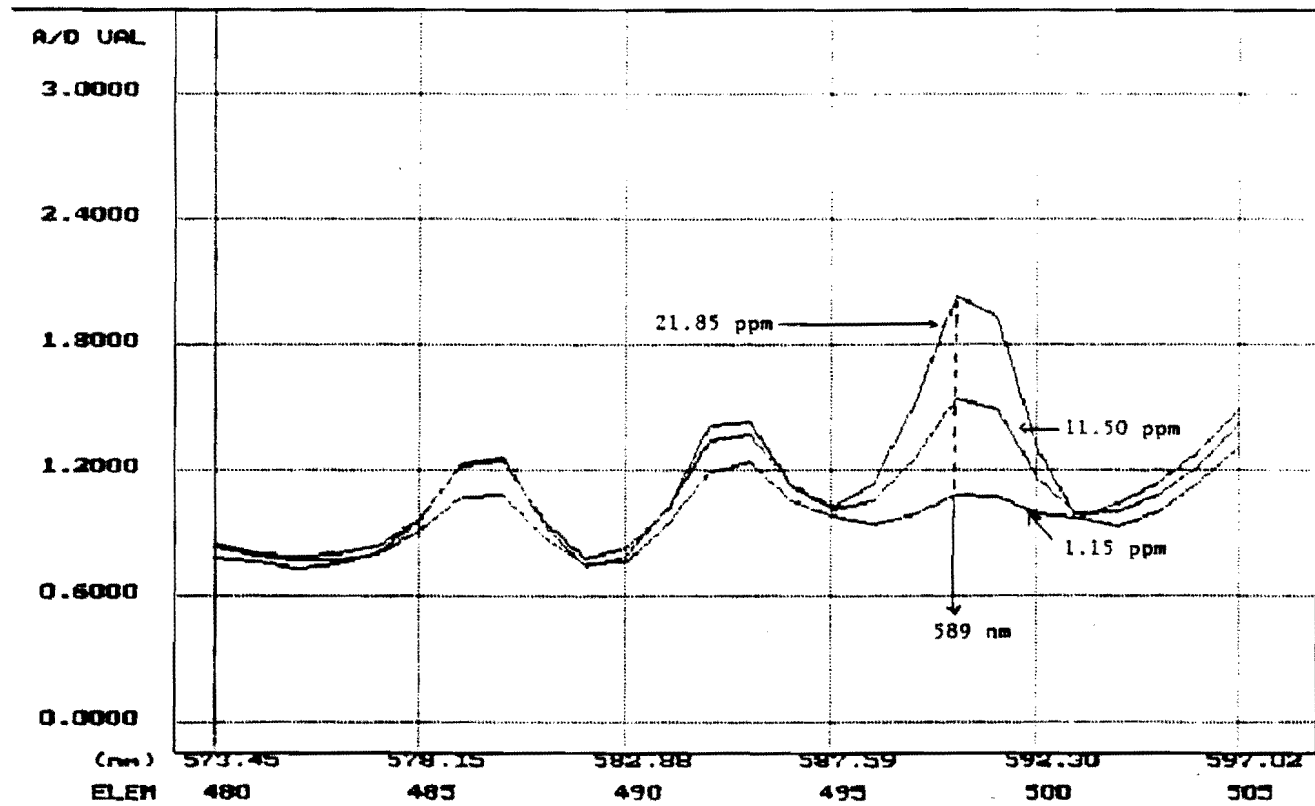


Figure 5. Sodium Emission Spectra

## MICROBIAL FLUORESCENCE

Certain forms of bacteria and fungi can limit the effectiveness of biomass production in bioregenerative life support systems. Nutrient solutions used in these systems need to be monitored to measure total microbiological populations and to detect the presence of individual pathogenic species of bacteria or fungi at concentrations that are harmful to plant growth. Microbiological monitoring needs also include drinking water that must be monitored to assure that it is safe for human consumption. Several Controlled Ecological Life Support System (CELSS) bioreactors using biological processes will also require a means of rapid or continuous analysis of total microbiological populations and individual microbial species for control purposes.

Conventional analysis techniques such as culturing and microscopic assays cannot provide the real-time information needed for close control of pathogenic microbes, biological processes and pure water supplies. Conventional optical monitoring techniques, such as optical density analysis or cell mass analysis, are not able to distinguish between living and dead cells, a distinct disadvantage in a "closed-loop" system where process solutions are continuously recirculated. None of these conventional analysis techniques are able to provide on-line microbial identification.

Because of the need to provide for both automation of microbial monitoring tasks and close control of microbiological parameters in bioreactors, NASA/Kennedy sponsored an SBIR instrumentation research project to evaluate fluorescence as a feasible technology for on-line microbiological analysis.

### Principles of Microbial Fluorescence

Luminescence. Fluorescence is a special case of the more general phenomenon of luminescence, the emission of light by matter following absorption of some form of energy. The types of luminescence are classified according to the mode of energy input causing the luminescence: fluorescence and phosphorescence (light), chemiluminescence (chemical), electroluminescence (electrical energy) and sonoluminescence (sound). In all categories, absorption of some form of energy by the luminescent matter precedes the emission of light. In the case of microbiological analysis, light is the energy source. Fluorescence decay time. Absorption of light excites electrons in the molecular structure of matter. In most materials, this light energy is converted to heat with no emission of light. If the matter is fluorescent, light is emitted but decays rapidly (nanoseconds) after the excitation source is removed. If the emitted light persists for longer periods (micro or milliseconds) after the excitation source is removed, the phenomenon is known as phosphorescence.

Absorption and emission spectra. Fluorescence and phosphorescence are characterized by two spectra: an absorption spectrum (as the excitation light is absorbed) and an emission spectrum. Matter that is phosphorescent will usually exhibit both a fluorescent and a phosphorescent spectrum, but typically only one of the spectra will be sufficiently strong enough for effective measurement. Each compound that exhibits these characteristics can be identified by a spectral signature that is unique for the compound.

Use of fluorescence for microbial analysis. Fluorescence is well suited to microbial analysis because all living cells contain both nicotinamide adiene dinucleotides (NADPH) and adenosine triphosphate (ATP), metabolic cofactors that are natural fluorophores. Because the relative amount of these and other fluorescent substances (such as tryptophan or tyrosine) present in the cell will be different for each species, it should be possible to identify a unique fluorescent signature for each species. Even if there are a number of different species present in a solution, the combination of their individual signatures will predictably contribute to the overall fluorescent signature for the solution. Differences in decay time for each signature may also aid in detection of individual species. Pattern recognition (chemometric) techniques should be able to process fluorescent signature information such that the individual species and their respective concentrations in the solution can be identified.

### Instrumentation for Microbiological Analysis

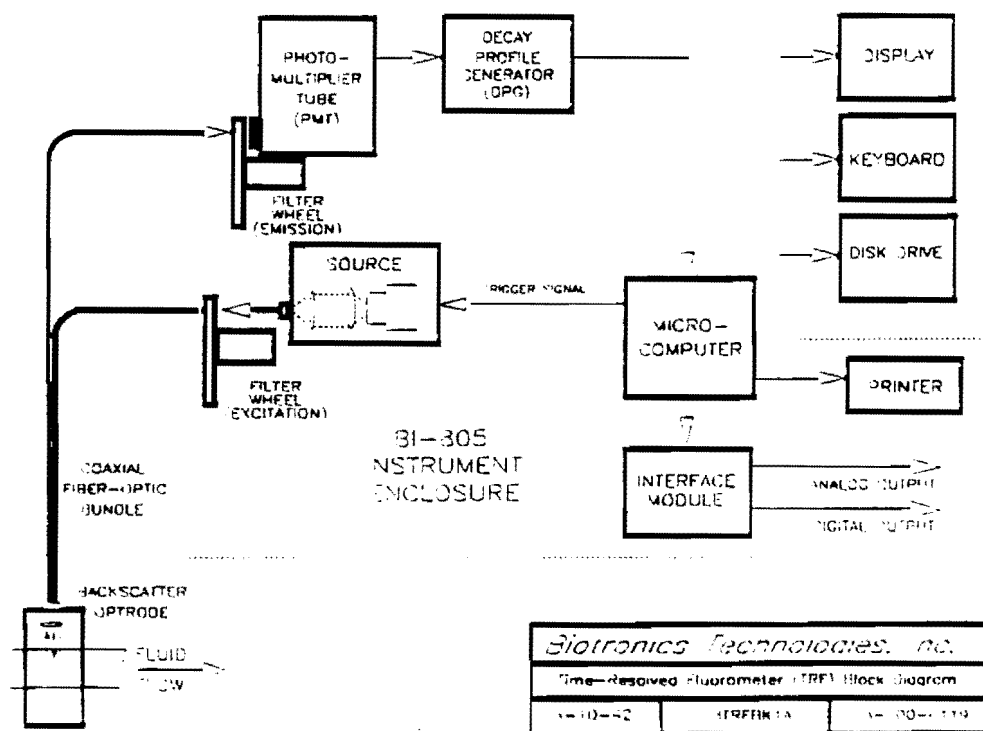
Two different instrument configurations are being evaluated in initial experimentation: a spectral fluorometer and a time-resolved fluorometer. A conventional laboratory luminescence spectrometer is being used to characterize samples for excitation and emission spectra throughout the project. This spectrometer measures fluorescence in static samples contained in a cuvette. Samples can be analyzed

using either transmissive or reflective (back-scatter) techniques. The instrument uses a pulsed xenon discharge lamp for excitation and a photomultiplier tube (PMT) for detection. A pair of monochromators (excitation and emission) scan the full range of UV, visible and NIR fluorescence. Such a monochromator-based instrument, however, is not suitable for on-line process monitoring.

In an on-line version of a spectral fluorometer, the excitation monochromator is replaced by a multiple waveband filter wheel. This filter wheel provides a set of 8 spectral wavebands using 8 bandpass optical filters. The emission monochromator scanning function is performed by a CCD photodetector array that is capable of rapid scanning of the emission spectrum consistent with the needs for on-line process monitoring. This CCD array fluorometer will allow for precise measurements of the emission spectra but only partial use of excitation spectra because the excitation filters have only limited resolution. This loss of excitation spectral information limits the potential specificity performance of this instrument and argues for serious consideration of time-resolved fluorometry.

Another instrument, a time-resolved fluorometer (TRF), is also undergoing experimental evaluation. This instrument uses a pulsed light source (laser or lamp) to excite a sample and measure the decay time period. The instrument employs eight excitation bands and eight emission bands. Unlike the spectral fluorometer, however, which is restricted to using spectral data from excitation and emission fluorescence, time-resolved fluorometry is also capable of measuring either single-component decay times or complex multiple-component decay functions. Complex decay functions are separated into their component parts using mathematical deconvolution techniques. The time-decay signatures should be unique for each microbe. The feature vector for each process measurement will be determined by the number of wavebands (64) times the number of points in each time profile. Typically, there will be about 100 data points in each time-decay profile, producing 6,400 elements in each feature vector.

A block diagram of a typical time-resolved fluorometer (TRF) configuration for microbial analysis is shown in Figure 6. A pulsed light source (nitrogen laser or nitrogen or deuterium lamp) transmits light through a fiber-optic link to a back-scatter optrode that reflects fluorescence back to a PMT detector. The electrical output of the PMT is processed through a decay-profile generator (DPG) that generates a complete lifetime function for input to a deconvolution program that determines the decay times and concentrations of the individual components making up the complex lifetime function. The potential of time-resolved fluorometry in microbial analysis appears very promising, but extensive experimental data are still lacking. Additional experimentation must be carried out to verify the sensitivity and specificity of this technology. Because of the variability of lifetime functions between microbes, even within a species class, time-resolved fluorometry seems the best choice for maximal specificity in microbial analysis.



## Fluorescent Microbiological Analysis Development

The primary requirements of fluorescent microbiological analysis are instrument sensitivity and specificity. Sensitivity is defined as the smallest number of colony forming units (cfu/ml) detectable by the system. Typical ranges encountered in the plant growth chambers of the CELSS Breadboard Project are  $10^3$  -  $10^7$  cfu/ml. Therefore, a sensitivity of at least  $10^3$  cfu/ml (1,000 bacteria/ml) is a reasonable requirement. This level of sensitivity should be easily achieved using a time-resolved fluorometric instrument.

Specificity, which is defined as the level of microbial species identification possible with fluorescence, is a much more difficult issue. Identification of bacteria can occur at three levels of classification:

1. Genus level - e.g., Pseudomonas;
2. Species level - e.g., Pseudomonas pickettii; and
3. Strain level - e.g., various strains of Pseudomonas pickettii.

Experimental experience to date indicates specificity limitations in the spectral fluorescence identification of microbes. Excitation spectra appear to contain more information than emission spectra for microbial investigation. Many fluorescent emission spectra appear very similar at the species level of bacteria. Because on-line fluorescence monitoring doesn't allow for the use of monochromators for reasons of reliability and speed of response, an on-line spectral fluorescent instrument probably cannot be developed with sufficient resolution to make use of the detailed information present in fluorescent excitation spectra. Consequently, project development at Biotronics currently emphasizes spectral band information collected with excitation and emission filters together with decay-time analysis of samples.

## Status and Prospects

Fluorometric identification and quantification of microbial populations offers exciting possibilities for advancing the state of the art in microbiological analysis. Significant new market potentials exist particularly in environmental monitoring and medical diagnosis. Primary uncertainty in this developing technology relates to the degree of specificity achievable in real world situation. Is identification possible at the species level of bacteria? At the strain level? How large a data bank is required before the technology becomes useful in CELSS and other real world applications? Hopes are high at Biotronics to answer these questions to some degree in the coming year. LAES also has a tremendous range of potential applications in laboratory, industrial process and environmental markets. Direct measurement of atomic elements is of particular importance in environmental monitoring of toxic metals in air, water and soil. LAES represents a shining example of the spinoff value of NASA-sponsored space technology.

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