STATE OF UTAH

CONTRACT

DP-2 6/88

CONTRACT # **961741**

1. **CONTRACTING PARTIES:** This agreement is between the State of Utah, Department of Community and Economic Development, referred to as STATE, and
   University of Utah
   Office of Sponsored Projects
   309 Park Building
   Salt Lake City, UT 84112

   Referred to as CONTRACTOR
   Fed. Tax ID No.

   Legal Status of Contractor
   [ ] Sole Proprietor
   [ ] Non-Profit Corporation
   [ ] For-Profit Corporation
   [ ] Partnership
   (XX) Governmental Agency

2. **GENERAL PURPOSE OF CONTRACT:** To provide for a Planning Grant to perform market research necessary to preparation of a proposal to create a Center of Excellence, namely the **CENTER FOR NOVEL APPLICATIONS OF FIBERS.**

3. **PROCUREMENT:** This contract is entered into as the result of the procurement process on requisition # N/A.

4. **CONTRACT PERIOD:** This contract is effective 1 November 1995 and will terminate on 30 June 1996, unless otherwise extended or terminated in accordance with the terms and conditions of this contract.

5. **CONTRACT COSTS:** CONTRACTOR will be paid a maximum of $10,000.00 for costs authorized by this contract.

6. **ATTACHMENTS INCLUDED AS PART OF THIS CONTRACT:**
   Attachment A - Standard Terms & Conditions
   Attachment B - Special Provisions
   Attachment C - Budget

7. **DOCUMENTS INCORPORATED INTO THIS CONTRACT BY REFERENCE BUT NOT ATTACHED HERETO:**
   All other governmental laws, regulations, or actions applicable to services provided herein.

8. **COMPLETE ON COST REIMBURSEMENT CONTRACTS ONLY:**
   a. AUDIT INFORMATION: Provide the name address and telephone number of the STATE staff person responsible for the contract audit and review function: Earl S. Maeser, 324 South State Street, 5th Floor, Salt Lake City, Utah 84111 (801) 538-8829.
   1. What audits and reviews are required of this contract?
   Financial? Yes X No __ | Program Compliance Yes X No __
   How Often? Quarterly | How Often? Quarterly
   By Whom? Vaughn S. Walsh | By Whom? Rod Linton
   b. RELATED PARTY TRANSACTIONS: Are any declared by CONTRACTOR? Yes __ No X
   See RELATED PARTIES - Attachment A, Paragraph 10.

IN WITNESS WHEREOF, the parties sign and cause this contract to be executed.

Contractor Name

Contractor Title

CONTRACT RECEIVED & PROCESSED BY DIVISION OF FINANCE

Director, Division of Finance

DCED Financial Manager

Roderick J. Linton, Director, COEP

Richard J. Mayfield, Director, DBED

Director, Division of Purchasing

Utah Centers of Excellence Program (COEP)
A New Center Proposal

Date: February 21, 1996

University: University of Utah
College of Engineering
2480 M.E.B.
Salt Lake City, Utah 84112

Project Title: Center for the Applications of Fibers as Sensors (CAFS)

Principle Investigator: Joseph D. Andrade
Dept. of Bioengineering
2480 M.E.B., University of Utah
Salt Lake City, Utah 84112
(801) 581-4379 phone; (801) 585-5361
joe.andrade@m.cc.utah.edu

Amount: For Year 1 $116,810
For Years 1-2: $228,955

Duration: Year 1: 7/1/96 - 6/30/97
Years 1-2: 7/1/96 - 6/30/98

Signatures:

J.D. Andrade
Principal Investigator

K.L. DeVries
Assoc. Dean for Research
College of Engineering

R. Glass, Director
Office of Sponsored Projects

T. Major, Director
Ofc of Technology Transfer

R. Koehn, V.P. for Research

EXECUTIVE SUMMARY:

All of life on Earth requires energy and the transformation of energy. All of that life, ranging from bacteria to man, uses, and is completely dependent on, two small and similar molecules which are involved in, and indeed critical to, all biological energy transformation processes, and are therefore involved in practically every biochemical reaction. Those two molecules, adenosine triphosphate (ATP), and nicotinamide adenine dinucleotide (NADH), can now be directly analyzed using disposable, dipstick, quantitative, direct-reading, fiber-based sensors. The readout involves a special bioluminescent reaction, using an enzyme originally derived from fireflies in the case of ATP, and an enzyme originally derived from bacteria in the case of NADH.

It is indeed very fortunate that nature has developed bioluminescent reactions for each of the two energy molecules in all of bioenergetics. It has only been relatively recently that the scientific community has had access to quantities of firefly luciferase and bacterial luciferase, the two enzymes involved in producing light from ATP-dependent and NADH-dependent reactions, respectively. Our group has been working with the firefly luciferase reaction for five years, and more recently has been working with a local company, Protein Solutions, Inc., to make this the basis of a generic sensing technology for ATP. In addition, we have developed means to use the human eye as the quantitative detector for the light output of these reactions.

Literally hundreds of biochemical reactions involve ATP, and, in particular, a large number of carbohydrate reactions. Since there are many disorders of carbohydrate metabolism of interest to the medical and health care communities, we now have the means to develop and apply cheap, inexpensive sensors for the analysis of those carbohydrates. Such analyses are not now routinely performed, except for glucose, because of the expense involved in utilizing normal, analytical procedures for carbohydrates.

We propose the development of a 6 channel carbohydrate sensor -- in year one for urine analysis and in year 2 for sweat analysis. Such sensors are to be applied as a component of an intelligent diaper and an intelligent sweatband. Diaper applications are of interest for wellness analysis and monitoring, both in the hospital and the home environment, as well as for the partially incontinent geriatric population. The sweat application is of interest to the serious athlete, the aerobic exercise enthusiast, and in industrial environments where substantial physical effort is required.

Such sensors also have application to other non-invasively derived biological samples. We propose to explore the feasibility of tear and contact lens applications in Year 3; of human and dairy milk analysis applications in Year 4; and of plant, horticultural, and botanical applications in Year 5. We have sufficient experience and expertise with firefly luciferase, ATP-dependent specific reactions to move forward with carbohydrate sensors immediately. Our experience with the bacterial luciferase system is much more limited, and we propose to expand our limited work on this system in Years 1 and 2 to bring it to the point where we can develop a complementary set of sensors based on the specificities and capabilities of the bacterial bioluminescent detection system.

With both ATP and NADH-specific sensors in hand, we can move on to consider analysis which involve both chemicals, which would then make it possible to develop disposable multi-channel sensors for amino acids, vitamins, and a wide range of other classes of bio and organic chemicals.

Only about 15% of the Center's resources in the first two years would be committed to the development of the bacterial detection system. The remaining 85% would

be committed to the development of the prototype carbohydrate sensors using ATP-detection, which is already well developed. We expect that by the end of the first year of funding the carbohydrate/urine sensor technology will be transferred to our corporate partners in the medical, self-diagnosis/consumer products (diapers)/education product areas.

At the conclusion of Year 2 of Center funding, the prototype sweat carbohydrate sensors will be completed, and the technology transferred to our corporate partners in those areas.

The U.S. disposable diaper market is approximately $4 billion annually, dominated by two major companies. The market is $12 billion world wide. An intelligent diaper, with chemical analysis capabilities, would be expected to achieve a 10% market share, which should exceed $1 billion world wide.

The sweat sensor market is more difficult to estimate, although there are over 5 million people in the U.S. who exercise seriously, and that number is rising steadily. Each of those folks spends roughly $100 to $1,000 per year for exercise and related equipment. We anticipate an initial annual market for a sweat sensor for non-medical applications in the range of $10 million annually, with possibly an additional $10 to $25 million for medical and related applications, particularly in subsequent years.

The longer range developments in the tear, milk, and plant area are not as well defined, and it is premature to provide estimates at this time.

The technology is applicable to woven and non-woven fibers, such as are already widely used in absorbent products, including diapers and sanitary napkins, and in textiles and clothing, such as in sweat bands and sweat clothing. Fibers are very light, very strong, very versatile, and have very high surface areas and surface to volume ratios. This gives them an unusual set of surface properties appropriate to our novel sensor applications. Fibers are existent micro-fabricated, micro-processed material which are not yet being developed for micro-devices. We do not at this time propose any development for wearable textiles or clothing, although that could be a long-range possibility. It is important to note that fibers and textiles are a $200 Billion/year industry in the United States and account for somewhere around 15% of the total GNP. The potentials are clearly enormous.

The center's technology transfer and commercialization plans have both short, moderate, and long-term components. Our local corporate collaborators include:

- Protein Solutions, Inc., a Salt Lake City based educational products company;
- HP Diagnostics, a Salt Lake City based immunosensor company for clinical diagnostics;
- ARUP, Inc., a major clinical chemistry and clinical diagnostics laboratory in the region;
- A semi-local corporate collaborator is Kimberly-Clark, which has Utah manufacturing facilities but R & D activities in Roswell, Georgia.

We request a two year budget of $228,955.

Table of Contents

Cover Page 1
Executive Summary 2
Table of Contents 4

1. Background 5
   1.1 Technology Definition 5
   1.2 Rights 6
   1.3 Status 6

2. Rationale 7
   2.1 Objectives 7
   2.2 Methods 8
   2.3 Schedule 12
   2.4 Expected Results 12
   2.5 COEP Support 12

3. Commercialization 13
   3.1 Definition of Products 13
   3.2 Maturity of Technology 13
   3.3 Uniqueness of Technology 13
   3.4 Technology Transfer 14
   3.5 Market Analysis 14
   3.6 Market Projections 14
   3.7 Economic Impact 15

4. Management and Personnel 15
   4.1 Background of Key Personnel 15
   4.2 Responsibilities 16
   4.3 Organization 16
   4.4 Oversight 17
   4.5 Coordination 17
   4.6 Personnel Needed 17

5. Budget 17
   5.1 Funds to Date 17
   5.2 Current & Pending Support 18
   5.3 Total Budget 18
   5.4 COEP Budget 18
   5.5 University Financial Support 18
   5.6 Budget Justification 18
   5.7 Financial Plan for Self Sustenance 20

Appendices:
1. References/Bibliography
2. Reprint/Abstract
3. Biographical Sketches: Andrade, Stewart
4. Planning Grant Update:
   1. Report by Ideas to Products, Inc: *Markets for Novel Diagnostic Methods Using Fibers*
   2. *The Disposable Intelligent Diaper Market*, by P. Triolo, business consultant
5. Letters of Support: Kimberly-Clark, ARUP, Inc., Protein Solutions, Inc., HCP Diagnostics

SECTION 1: BACKGROUND*

1.1 Technology Definition:

Several technologies and technology concepts are under development which form the basis of CAFS.

Technology One: Eyeball Detection

Patterns and images are much easier to detect, to remember, and to process than numbers. Man is exquisitely constructed to deal with visual patterns and pattern recognition. The design, development, and application of analytical devices which are self luminescing and generate a specific pattern which can be directly correlated or related to multiple analyte concentrations, has enormous potential benefits. In addition to greatly simplifying routine measurements in a range of analytical, environmental, biotechnological, and medical/diagnostic laboratories, such technology has the potential for being applied widely in schools, education, and in the home for a variety of monitoring, diagnostic, and other measurement purposes.

Working with Protein Solutions, Inc., our laboratory has developed a unique bioluminescence sensing technology which allows the spatial position of light to be directly correlated with chemical concentrations [1,2]. Thus, rather than developing a sensor which responds to chemical composition by changes in intensity or wavelength/color, these sensors change the spatial position of their emitted light as a function of chemical composition, thus allowing the human eye to be used as an efficient, effective, and unambiguous detector, eliminating the need for instruments.

Technology Two: Dry Enzymes & Gels

Convenient, easy to use, disposable biosensors [6-8] require the complete dehydration of the active biochemicals, including the enzymes and photoproteins involved in bioluminescence, and then allow these to be rehydrated at a later date with nearly full retention of activity and function. This process is based on the unique carbohydrates found in many desert organisms which must tolerate dehydration/rehydration cycles. We have developed means of producing films and coatings of luminescent materials on a variety of fibers using such carbohydrates and the successful drying and reconstitution of such films with high activities [3].

Technology Three: Recombinant Enzymes

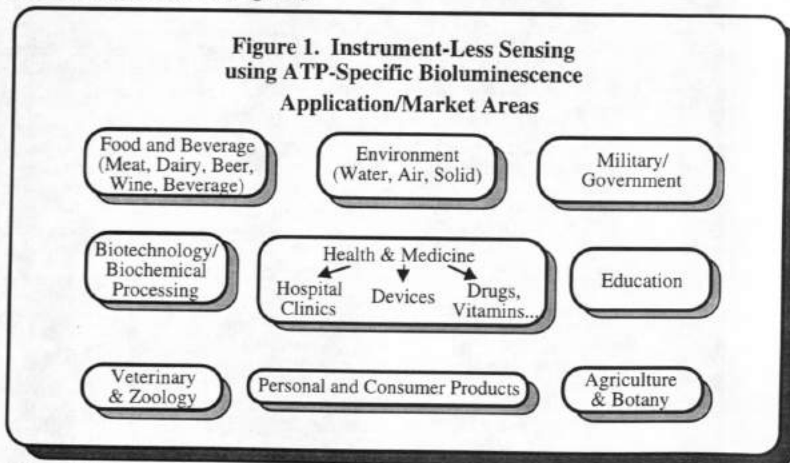
A major expense of bioluminescence-based detection systems using firefly luciferase has been the expense of that enzyme. The firefly luciferase gene has been expressed in *E. coli* and the recombinant enzyme is available, although it is still very expensive. Dr. Russell Stewart, a new member of the Department of Bioengineering faculty, has in the last year produced a newly engineered firefly luciferase, expressed in *E. coli*, in high yield and high activity, which is easily and inexpensively purified and immobilized and is now readily available in inexpensive form [3,4].

These three key technologies have been combined as a prototype, quantitative, ATP-specific biosensor, which is the enabling technology for this Center. In addition, technology two and the techniques and processes used in technology three, will be applied to the other enzymes required to expand the ATP sensor to a carbohydrate-specific sensor. These three technologies have been developed over the past four years and form the basis

* References are in Appendix 1.

of Mr. C-Y Wang's Ph.D. dissertation in the Department of Bioengineering [3]. Mr. Wang has agreed to stay on at the conclusion of his Ph.D. defense in May 1996 as a post-doctoral fellow and key researcher for this Center, thereby enabling the most efficient application of the work to the development and testing of practical sensors and prototype products.

These three technologies: Detection of concentration as a function of linear position; ability to dehydrate and rehydrate with nearly full restoration of activity; the ability to produce enzymes by recombinant means, allow the development and commercialization of a wide range of personal, dipstick based, chemical sensors for health care, environmental, and home monitoring applications, as well as for extensive application in the education products area (Figure 1).



1.2 *Rights*

Technology 1 is already the basis of a provisional patent application [1].

Technology 2 is in the public domain -- although we expect to produce application-specific patents [3].

Technology 3 has been disclosed and a patentability opinion is now being obtained. Its application to fiber-based geometries for personal sensors will be the subject of patent applications [3,4].

1.3 *Status*

Protein Solutions, Inc. (PSI) has a right of first refusal Technology Transfer Agreement with the University of Utah, and has exercised its option to technology 1 and 3 above. PSI has a major corporate interest in general applications of glowing luminescent fibers and material, especially in the development of educational materials and kits. PSI is an educational products company and plans to market an educational kit around novel fiber sensors within the next 18 months.

SECTION 2: RATIONALE.

2.1 *Objectives*

The overall research objective is to develop direct reading sensors for the measurement of glucose, galactose, and other carbohydrates in urine [5,12,13], employing spatial luminescent ATP detectors. The sensors have been shown capable of yielding a visible light output whose position is indicative of ATP concentration. When simple sugars, such as glucose and galactose, are phosphorylated by their respective kinases, they consume ATP. These changes in ATP concentration can be sensed by our luminescent detector, and the decrease in ATP concentrations are proportional to the concentrations of the sugars present.

We propose to apply the three unique and novel technologies discussed in 1.1 above to the application areas indicated in the Executive Summary and in Figure 2. The sensors will have the characteristics noted in Figure 3.

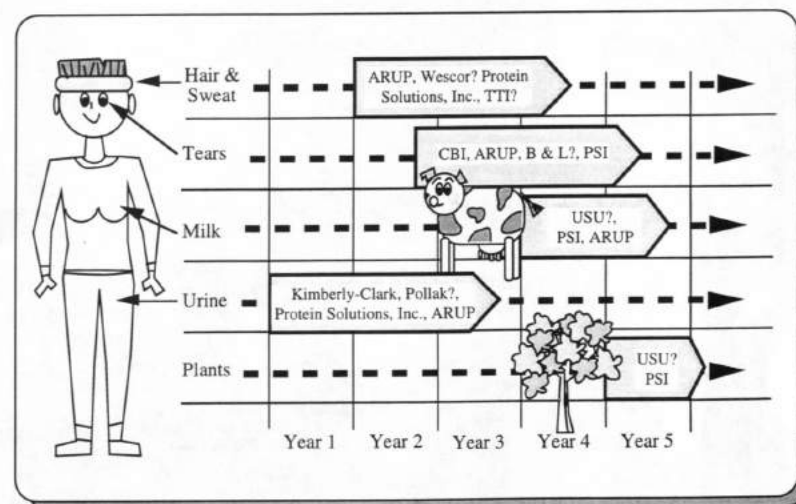


Figure 2. Center for Applications of Fibers as Sensors (CAFS) Topic Areas, Time Plan, and Industrial Collaborators

USU = Utah State University
PSI - Protein Solutions, Inc.
ARUP = Associated Regional University Pathologists, Inc.
? = These firms have not been formerly contacted, yet are likely to be involved.

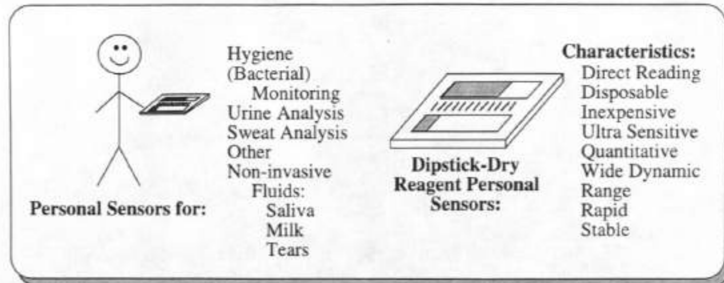


Figure 3. Application areas (left), schematic and characteristics of multi-channel direct reading sensors (right).

Project 1) Development of a urine dipstick sensor which will read out the presence of six carbohydrates of diagnostic importance [5,22-23] via position sensitive luminescence. The spatial position is directly related to chemical concentration [1].

Project 2) Development of a multi-channel sweat sensor which will read out the presence of six chemicals in sweat of interest [17-19,24] to the exercise/physiology community. This development will build on the experience in Project 1 and will be of primary focus in year 2 of the Center's operation.

Project 3) Principally in Year 3, we will focus on the analysis of low molecular weight solutes in tears which are of potential diagnostic significance and of interest in assessing the compatibility and wearability of contact lenses. We expect that this will also be a 6 channel device.

Project 4) Both human and bovine milk contain a wide range of low molecular weight carbohydrates, amenable to analysis by our unique technologies. In consultation with a mammalian nutrition experts, and with the milk and dairy folks at Utah State University, we will select a set of six analytes appropriate to our technologies, which are of major interest in milk and develop a prototype sensor specific to milk application by the end of Year 4.

Project 5) ATP and NADH, two major bioenergetic molecules of interest here, are of course, the key players in the bioenergetics of plants (actually NADPH). Although our experience in that area is more limited, by careful consultation with botanical and horticultural experts over the next several years, we anticipate developing one or more prototype sensors, each with an array of analytical channels of direct interest to plant physiology and pathology.

2.2 *Methods*

Task 1: Prepare and Characterize Kinases

Glucokinase (enzyme 2.7.1.2) and galactokinase (enzyme 2.7.1.6) will be obtained from Sigma Chemical Company, characterized, purified, and their respective stabilities and

activities determined, as will the kinases for lactose, fructose, xylose, and arbinose [5]. This work will be performed by Dr. C-Y Wang in the laboratory of Dr. Russell Stewart. Dr. Stewart will also assess the feasibility of producing these enzymes by recombinant means. Before proceeding with studies incorporating the enzymes in gels, we will further assess the ability of our sensor to detect changes in concentrations of sugars in solution. These experiments will be performed to obtain estimates of the concentrations of analytes and reactants that are required, and to identify any unanticipated interferences or incompatibilities of the system which can be addressed before proceeding to development of the gels described below.

Task 2. Enhance Stability of Enzymes and ATP

Our present ATP sensing technology incorporates firefly luciferase, an ATP "consumase" (apyrase), and trehalose as a preservative and stabilizer, into agarose gels. The gels are dried and stored. Results of previous investigations have demonstrated that luciferase and apyrase maintain their activity when incorporated into the agarose gels [3]. When rehydrated with an aqueous solution of an analyte sample containing ATP and luciferin, the preserved luciferase and apyrase are activated, yielding a light signal proportional to the concentration of ATP present in the sample [1,3].

We will preserve the kinases by incorporating them into gels containing trehalose or sucrose as a preservative. Agarose, trehalose or sucrose, and kinase concentrations in the gel will be optimized along with gel preparation conditions to obtain a dehydrated gel which can be rehydrated with maximal kinase activity.

In most of our previous studies, ATP has been the analyte. Here the ATP concentration will be modulated by controlling its reaction rate with monosaccharides. Appropriate kinases (galactokinase, glucokinase) will be used to modify the reaction rates ATP is provided in one of the device layers. ATP must also be included in the test system, and it's stability will be evaluated by assessing light output of sensors that incorporate different ATP gels as a function of storage time. We will also experiment with dual and even triple enzyme gels. Gels will be dried at different rates, subjected to storage under different temperature conditions, rehydrated at different rates, and evaluated for enzyme activity.

Task 3. Device Design

The prototype, multi-channel sensors design is not yet fixed. We will work closely with our key corporate collaborators in the first year, Protein Solutions, Inc. and Kimberly-Clark, as well as with ARUP, to determine the most practical design.

In the case of the Intelligent Diaper [see Appendix 4-2, Ref. 25,11], this would likely be a small patch or pad containing a capillarity wick which would draw the urine from the major urine storing region of the diaper up to the sensor, maintaining the sensor in a relatively dry and isolated part of the diaper. In this manner a controlled volume of urine can be delivered to the sensor, thereby allowing it to function as a conventional dipstick-type device [13-16; See also Appendix 4-1].

To get an idea of what such devices might look like, the reviewer would be encouraged to go to a corner drugstore and look for a typical urine dipstick for glucose, preferably a multi-channel dipstick [also App. 4-1]. Common sticks have anywhere from one to ten channels of analyte information. It is important to point out that, with the exception of glucose, the analytes we detect are different than those in the conventional urine dipstick, and basically would represent a second tier of analysis, generally for

inherited metabolic [22,23,5] diseases which don't show up immediately. We feel that there is no point at this time in trying to duplicate the technology that is already widely and inexpensively available with the conventional urine dipstick. That technology could, of course, be incorporated in the final products, together with our technology, at a later date.

For non diaper applications, the device would be configured as a dipstick, with a sample region of volume drawing and delivery region, and a six channel analytical region -- very roughly indicated in Figure 3 [6-8].

The aqueous solution of the sample is introduced to the dehydrated gel which is immediately hydrated, allowing reactants in that layer to act on the sample solutes. The reacted solution then migrates, by capillarity wicking to the next area for processing. The last area is the light-producing luciferase layer, which receives the ATP concentration, modulated by the front-end enzymes, and converts it to a light signal. The light signal is produced in a distinct spatial pattern detected by the naked human eye

The sample containing the analyte will be deposited on the fiber device and spread by a capillarity and rehydration process. This technology is already applied in existing dry reagent chemistry diagnostic kits [6-8].

For laboratory work leading to device optimization (Figure 4), the luciferase and other enzymes are deposited as circular spots along one dimension of the device, each dot of increasing enzyme concentration. The physical support for this lab device is a strip of filter paper sandwiched between two polyester films with an agarose coating. One end of the upper film has an opening to allow for delivery of the analyte.

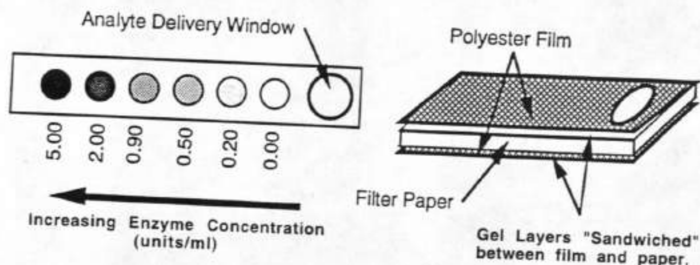


Figure 4. Diagram indicating incorporation of gel spots of increasing apyrase concentration for laboratory characterization. This is not a prototype sensor design.

The analyte is delivered to the sample window. A detectable, visual readout is made three minutes after application of the analyte solution. The design exploits the wicking properties of the filter paper and the capillary action of the gel film sandwich. The test strip geometry allows sample delivery to each gel "dot" within one second of analyte delivery. The time limiting factor for the reaction is the time required to rehydrate the enzyme gel.

The sensor concept is based on the fact that, for a given luciferase/luciferin concentration, minimum concentration of ATP is required to produce a "measurable" light output. Above that concentration, light is visible; below that ATP concentration, no light is detected. If a uniform concentration of ATP is delivered to a series of sensors (dots with controlled concentrations of enzyme), a detectable light signal will only be produced when enzyme levels are relatively low in relation to ATP concentrations.

The intent is to determine ATP concentration as an indicator of the concentration of carbohydrate. This is accomplished by reacting the ATP with the carbohydrate in the presence of varying concentrations of the appropriate kinase. The phosphorylation of the sugar and the dephosphorylation of the ATP occur before the ATP reaches the luciferase gel layer where light emission is initiated. Thus, the kinases serve as a mediator of ATP concentration by modulating its reaction with the carbohydrate.

The sample, consisting of unknown concentrations of carbohydrates in urine will be introduced to the test system. The sample solution will wick into the device and begin to rehydrate the uppermost gel containing the ATP. The ATP will be solubilized by the water, and diffuse, together with the luciferin and carbohydrates to the kinase gel layers. There the concentration of ATP will be depleted in direct proportion to the concentration of carbohydrate present. The ATP solution diffuses further and enters the luciferase layer, reacting there with the solution of luciferase and luciferin. The depleted ATP gives a light signal inversely proportional to the concentration of carbohydrate in the test sample.

The light is focused or waveguided for detection by the unaided dye. Comparison with an empirically derived table correlating carbohydrate concentration with obtained light pattern allows for a rapid estimation of the concentration of carbohydrate in the sample (Figure 5). The gradient in kinase concentration allows for assay of a wide range of carbohydrate concentrations.

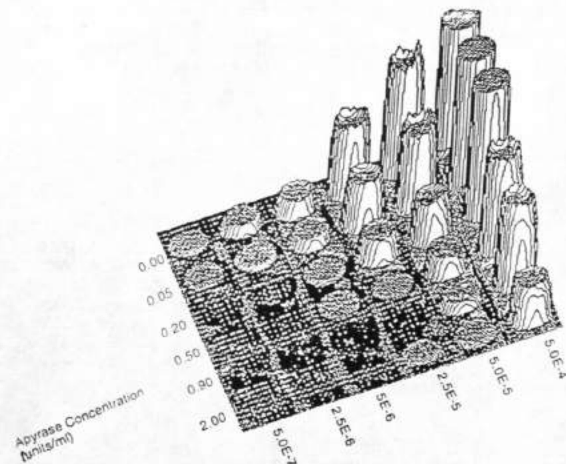


Figure 5. Typical CCD 3-D Profile of Light Intensity for a Six by Six Detector Array.

Task 4. Testing

Known concentrations of carbohydrate solutions will be introduced to the devices, and the light output and position determined. Studies will assess the sensitivity, reproducibility, and specificity of the system in the presence of urines of different dilution. The sensors will yield a light output whose position is indicative of carbohydrate concentration.

A number of disinterested volunteers will also participate in a series of experiments to determine the detection limits of the light directly emitted from test strips, using their naked eyes in various lighting conditions. CCD camera patterns (Figure 5) and visually determined patterns will be compared to see if any particular training or experience is required for disinterested, unaided observers to accurately interpret the results.

In addition, short term storage, dehydration, stability, and experiments to assess the reproducibility of the assay will be performed.

Task 5: Analysis of the Bacterial Luciferase System and a Comparison of its Advantages and Disadvantages with the Firefly Luciferase System.

As indicated briefly in the Executive Summary, about 15% of the effort for the first several years will be devoted to analysis and development of the bacterial luciferase system as a component detection system and a generic detection technology for NADH. This is the work of Mr. Dong Min, and he has already made considerable progress in this regard. The bacterial luciferase system is slightly more complicated than the firefly luciferase in the sense that a second enzyme is involved. The availability of a sensing technology for NADH and NADPH opens up an even wider range and set of applications for these position-sensitive, direct reading, bioluminescent sensors. We therefore think it is very important to direct a small percentage of the overall effort to this technology.

2.3 *Schedule*

Refer to Figure 2 and Project Listing on page 8.

2.4 *Expected Results*

Refer to Section 3.1.

2.5 *Impact of COEP Support*

The COEP funds and commitment will make it possible to develop the project areas proposed, permitting the University and the State to have a claim to these technologies. It will also permit our local Utah partners to be involved in these technologies, and to begin the development of products, and strategies, with which to take advantage of these new opportunities. The Center designation and Center funds will provide the data, experience, and environment which will enable the Utah firms to more effectively compete for SBIR and STTR grants, and enable the Center to seek major government support for projects. We have a major proposal in front of the National Science Foundation on a part of this application, which will require a significant matching component. Designation of Center status, and Center funds, will greatly increase the possibility of this major NSF award.

The goal is to utilize COEP funds in a catalytic and seed money manner with which to obtain more extensive government grant and contract funds.

SECTION 3: COMMERCIALIZATION.

3.1 *Products and Services*

As indicated in the Executive Summary and in the Time Plan figure, during the course of the first two years of COEP funding, we propose to develop two multi-channel sensors of carbohydrates -- that is, monosaccharides. One designed specifically for application to urine and primarily in an intelligent diaper format [11,25,2], which we expect to be transferred to the Kimberly-Clark Corporation for intelligent diapers, a very major potential market [Appendix 4-2].

A second sensor, which would be the primary focus of Year 2 of the Center's activity, is similar in concept, but designed for sweat analysis -- primarily for the exercise physiology and wellness community. Here, our corporate collaborator is not yet firmly established. It may be Wescor in Logan, although only very preliminary discussions have been entered into at this time. It may be a major athletic exercise equipment manufacturer. A possibility is Vetta Sports in Park City. But again, only very preliminary discussions are in place.

Both sensors, urine and sweat, will be utilized by Protein Solutions, Inc. in their rapidly evolving Labless Lab line of kits for educational [26] and self-monitoring/wellness purposes. PSI expects to have such a kit on the market by late 1996, and those kits would incorporate our prototype sensors by mid-1997.

We also anticipate developing a close collaboration with one or more companies who sell family or home doctor software. One of the leaders if Applied Medical Informatics in Salt Lake City. They sell Medical House Call, a CD ROM, personal computer-based system, based in large part on the highly successful Iliad software system used in medical schools throughout the world to teach medical students how to diagnose. At present these home-based systems have no chemical information. The eventual availability of simple, easy-to-use, direct-reading, multi-channel sensors, which could provide 10, 20, or even more channels of analytical chemical information, would allow these home-based software packages to be far more effective and useful. These areas will be considered and developed during the first two years of Center support, with the assistance of our marketing and business development consultants.

3.2 *Maturation of Technology*

This technology is already quite mature in the sense that simple prototype sensors for ATP and for glucose have already been demonstrated [5,13-16,23]. The ATP technology is the basis of a hygiene monitoring, i.e., bacterial contamination, sensor presently under development by Protein Solutions, Inc. The glucose sensor was developed for feasibility demonstration. It is relatively straightforward to extend that experience to other carbohydrates using other kinase enzymes. The concentrations in urine are such that there are no particular sensitivity problems and the enzyme specificities are such that there are no major interference problems.

3.3 *Uniqueness*

Because of the expense and reputation of luciferase enzymes, and for other reasons, others have not adopted this mode of multi-channel analysis and detection. A major reason is that whenever bioluminescence is used, it has been normally assumed and expected that one must measure intensity, and measuring intensity of small numbers of photons is an expensive and difficult proposition. Hence, there has been almost no interest in bioluminescent or chemiluminescent systems for direct reading dipstick application [6-10]. We, too, struggled with this limitation for many years and it was only several years ago that we invented a means [1] to essentially transform an intensity signal to a spatial signal. The best metaphor is that of a thermometer, whereas one measures the position of the liquid level or the length of the color band, rather than the color itself, or its intensity.

This is, indeed, already done with dipstick-type devices, such as tests for cholesterol, pregnancy, hormone, and related tests which you can buy in the corner pharmacy, but it has never been accomplished for luminescence systems. The provisional patent application on this invention has been submitted [1], and the formal patent will be submitted before July 1.

We are also investigating even other ways of presenting the information, particularly in multi-channel form, so the reader can deal with those six and, much later, even more channels of information in a very easy and effective manner [20,21].

The technology is indeed unique. The ability to detect *the* major molecule of bioenergetics -- ATP -- which is intrinsically coupled to literally hundreds of important biochemical analytes, together with the other technologies which we have described, provides a uniqueness and a set of opportunities which we feel must be developed and exploited.

3.4 *Technology Transfer*

The University's Office of Technology Transfer (T. Major, Director), CAFS' PI (J. Andrade), and CAFS' participating faculty (Andrade, Stewart) all have experience in industrial/business collaboration and interaction, in patent development and acquisition, and in technology transfer. Our experience with the Center for Biopolymers at Interfaces and the Center for Controlled Chemical Delivery provide a strong basis for the development of technology transfer processes for CAFS.

The key campus participants and representatives of interested local firms will meet in July to develop an objective, effective, technology transfer mechanism. The steps will likely include:

- Indication of general interest and involvement in CAFS;
- Specific interest in a CAFS technology and/or product concept;
- Direct support and/or active participation and involvement in the specific technology and/or product;
- Direct support of patent and related technology protection costs;
- Commitment to utilizing the technology and in product development, marketing, and sales, with appropriate investment and sales targets;
- An appropriate royalty payment mechanism to the University of Utah and commitment to contract and related support of CAFS;
- Means for University of Utah royalties to support ongoing CAFS activities.

3.5 *Market Analysis* [See Appendix 4-1, 4-2]

This will be performed with our corporate partners shortly after the Center is launched. Our Business consultant will work closely with our Corporate Partners to develop market analyses and additional product concepts.

3.6 *Market Projections* [See Appendix 4-2, 4-2]

These were indicated very briefly in the Executive Summary. Given the magnitude of the $12 billion/year international diaper market, which is a very technological and highly competitive [11] field, we anticipate that an intelligent diaper might attract up to 10% of that market, over a billion dollars/year. The diaper would also be popular in geriatric environments and in newborn clinics and hospital areas.

As we indicated earlier, the sweat market is much more difficult to estimate at this time, although we are working with a number of firms and a number of consultants [App. 4-1] to try to refine the possibilities there. It is important to note that there is a growing interest and activity in the use of sweat monitoring of drug metabolites and drugs of abuse, particularly in the forensics community [17-18]. As these applications develop, they will help lead the way to more extensive use of sweat for other analytical purposes.

At this time our technologies do not lend themselves to the analysis of drugs of abuse, although there is the distinct possibility that that would be possible in the future.

3.7 *Economic Impact*

We expect our activities to significantly enhance our corporate partners' ability to secure SBIR/STTR Phase I and Phase II funding as well as to help launch totally new product areas which will provide new jobs and increased sales.

Our hope and expectation, of course, is that Kimberly Clark indeed will elect to move with an intelligent diaper, based on Center technologies and, if so, given the location of the Center, and the fact that Kimberly has a manufacturing facility in Utah, that those devices would indeed be manufactured locally.

Given the interest of Wescor in Logan in sweat analysis, and Protein Solutions, Inc. in Salt Lake City in sweat and urine analysis, we certainly expect that these devices would indeed be manufactured locally.

Bausch & Lomb, Inc., has interests in moving ahead on tear analysis devices. Dr. Paul Valint will represent them on our Industrial Advisory Board.

SECTION 4: MANAGEMENT AND PERSONNEL

4.1 *Background of Key Personnel*

The Principal Investigator is J.D. Andrade, Professor of Bioengineering and Director of the Center for Integrated Science Education at the University of Utah. Joe has been involved in bioengineering, biomaterials, and medical device activities for the past 25 years. He is a fellow of the American Institute of Medical and Biological Engineering, former Chairman of the Department of Bioengineering at the University of Utah, has had extensive experience in analytical and diagnostic chemistry, and has strong interests in cost-effective health care technologies and education of the general public. His biosketch is attached.

Joe edited the recent book, *Medical and Biological Engineering in the Future of Health Care*, University of Utah Press, 1994. Chapters 2 (K.H. Keller), 18 (J. Wennberg), and 24 (J.D. Andrade) are particularly relevant to this proposal.

Dr. Russell Stewart, Assistant Professor of Bioengineering, is an expert on motor proteins and on the bioengineering applications of proteins and enzymes. He has been working closely with J. Andrade and C-Y Wang for the past 2 years, and is the inventor of the biotinylated luciferase, expressed and prepared *in vivo* by recombinant means [4], which is the basis of the present generation of ATP-specific sensors. As indicated earlier, Dr. Stewart's laboratory would be responsible for the preparation, purification, and characterization of all proteins used in these sensors. Dr. C-Y Wang will conduct much of his work in Dr. Stewart's laboratory.

Dr.s Karin Caldwell and K. Owen Ash are other faculty involved in CAFS. Their biosketches will be provided upon request.

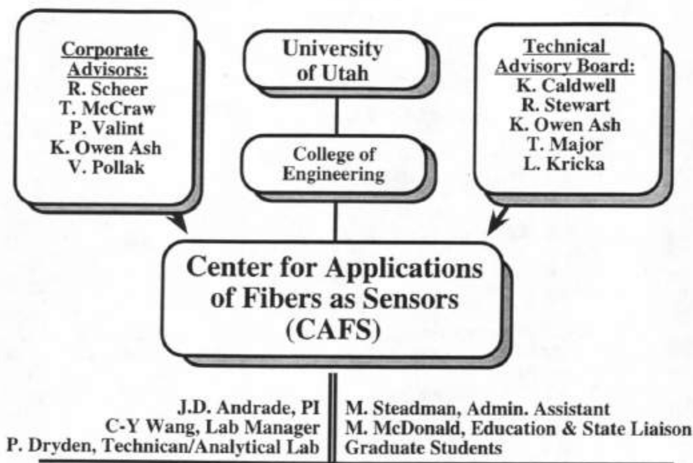
4.2 Responsibilities

J. Andrade will be responsible for the overall direction and management of the Center. He will be assisted by Mary McDonald and Mindy Steadman in administrative and office matters. Dr. Russell Stewart will be responsible for the protein components. Dr. C-Y Wang will be responsible for the key tasks and day to day work and operation of the activities. He, together with Dr.s Andrade and Stewart, will supervise the several graduate students involved, as well as Mr. Paul Dryden, a technician who will assist in many aspects of the project, and with the maintenance and operation of the Trace Element Analysis Facility (see also Section 5.7).

4.3 Organization

Overall organization is indicated in Figure 6 below. Our Advisory Boards will consist of two groups, those representing our corporate participants or potential participants: R. Scheer (Protein Solutions, Inc.), Tim McCraw (Kimberly-Clark), Paul Valint (Bausch & Lomb, Corp., which as a particular interest in application of these sensors to tear analysis), K. Owen Ash (ARUP), and Victor Pollak (HCP Diagnostics). The academic side of the Advisory Board will include, in addition to Andrade, K. Caldwell, Director of the Center for Biopolymers at Interfaces and Acting Chairman of the Department of Bioengineering 1996-97; Russell Stewart, described above; again K. Owen Ash (he holds both industrial and academic hats); Tom Major, representing the University's Office of Technology Transfer; Dr. Larry Kricka, head of the Clinical Laboratories and Professor of Pathology at the University of Pennsylvania. Dr. Kricka is internationally known for his work in the application of bio- and chemiluminescence to chemical diagnostics in clinical chemistry [9].

Other advisors will be added as appropriate, in particular one or two from the Exercise and Sport Science community, and possibly from the Food and Nutrition community. And, as we develop our collaboration and interaction with the Utah State University folks in the milk, dairy, and plant horticulture areas, we will of course invite those appropriate individuals to be full-fledged participants.



J.D. Andrade, PI
C-Y Wang, Lab Manager
P. Dryden, Technican/Analytical Lab

M. Steadman, Admin. Assistant
M. McDonald, Education & State Liaison
Graduate Students

Figure 6. Organization

4.4 Oversight

The project will be managed by J. D. Andrade, PI. Oversight will be provided by an on campus advisory panel which will consist of Mr. Tom Major, Director, Office of Technology Transfer and Dr. Karin Caldwell, Director of the Center for Biopolymers at Interfaces.

4.5 Coordination

The PI, the faculty participants, and the advisory panel, working closely with the industrial collaborators, will attempt to coordinate with and involve all others in the state who have interest or activities in the area of novel applications of fibers as sensors.

4.6 Personnel Needed

This is covered in Section 5.6 on budget justification.

SECTION 5: BUDGET

5.1 Funds to Date

J. Andrade has previously received grants from the Army Research Office and from the Office of Naval Research for his earlier immunosensor studies. These funds, together with the several million dollars in sponsored research over the past 15 years in his laboratory, have provided the technical basis for much of the work proposed in this center. An existing National Science Foundation STTR grant to Protein Solutions, Inc. has provided the resources for the development of the generic bioluminescence-based sensor for ATP, based on the position sensitive quantitation principle [1,2].

The COEP has provided a planning grant which is now being used for prototype development and marketing studies, as well as contact and interaction with corporate partners and participants. Protein Solutions, Inc. provides a small, ongoing contract on the development of firefly luciferase for biosensor applications.

5.2 *Current and Pending Support*

Protein Solutions, Inc. has several SBIR Phase I and an STTR Phase II application pending at the National Science Foundation and the Department of Agriculture. All of these small business grant applications include sub-contracts to the Center. We have submitted a large preliminary proposal to the National Science Foundation for a major effort on bio-photon-based devices for sensing applications.

Protein Solutions, Inc. provides ongoing support for the development of firefly luciferase biosensors.

The ARUP, Inc. recently donated a Perkin-Elmer ELAN 500 ICP mass spectrometer to the Center. This instrument is now being set up, with the aid of University funds, as a trace element analysis facility, which will be administered by and used in the Center. This will provide a key analysis and characterization for the fibers and reagents used in the luminescent sensors.

5.3 *Total Budget*

We plan for the Center and its activities to grow, as indicated in Figure 7 to an annual funding level of 1-2 million dollars/year. This will enable activity on a range of projects with specific product development and commercialization focus for our corporate collaborators. Such a funding level will provide the resources for ongoing research and development of new concepts and technologies which will permit a reasonable stream of new and novel products for our Utah corporate participants.

5.4 *COEP Budget* (next page, Table 1)

5.5 *University Financial Support*

The Institution is providing a significant percentage of J. Andrade's time, an indirect costs waiver, and the space and facilities in which this work will be carried out, including providing space and resources for the Center for Biopolymers at Interfaces. The University is providing space, equipment, and staff resources for the Center for Integrated Science Education. All three centers are integral participants in this project.

In addition, the University has provided $20,000 for establishing a trace element analysis facility which will be a key part of, and administered by, this Center. Trace element analysis is necessary as a way to screen fibers and reagents because a number of the enzymes involved are sensitive to trace element concentrations. Indeed, another project, which is not part of the Center at this time, would be to use that trace element sensitivity as a means to develop sensors for those trace elements. But that is another story for another time.

5.6 *Budget Justification*

No salary is requested for J. Andrade. His salary can be considered part of the University match. Most of the day-to-day lab work will be supervised by C-Y Wang and conducted by "Dr." Wang (Ph.D. expected 5/96) and the two graduate students. Dryden will provide technical and laboratory support.

5.7 *Financial Plan for Self-Sustenance* (See Figure 7.)

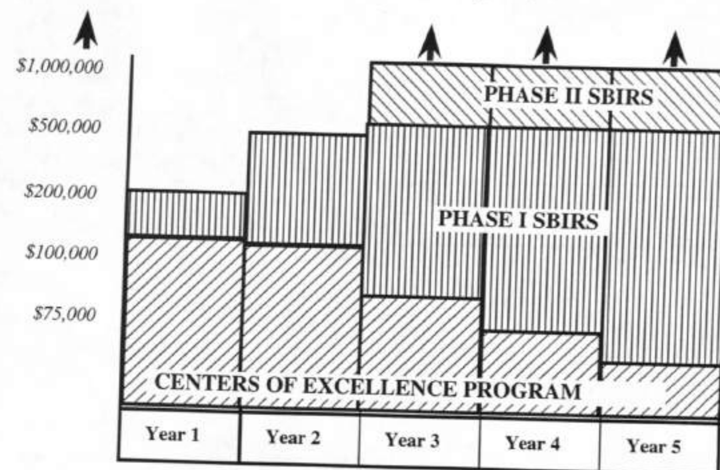
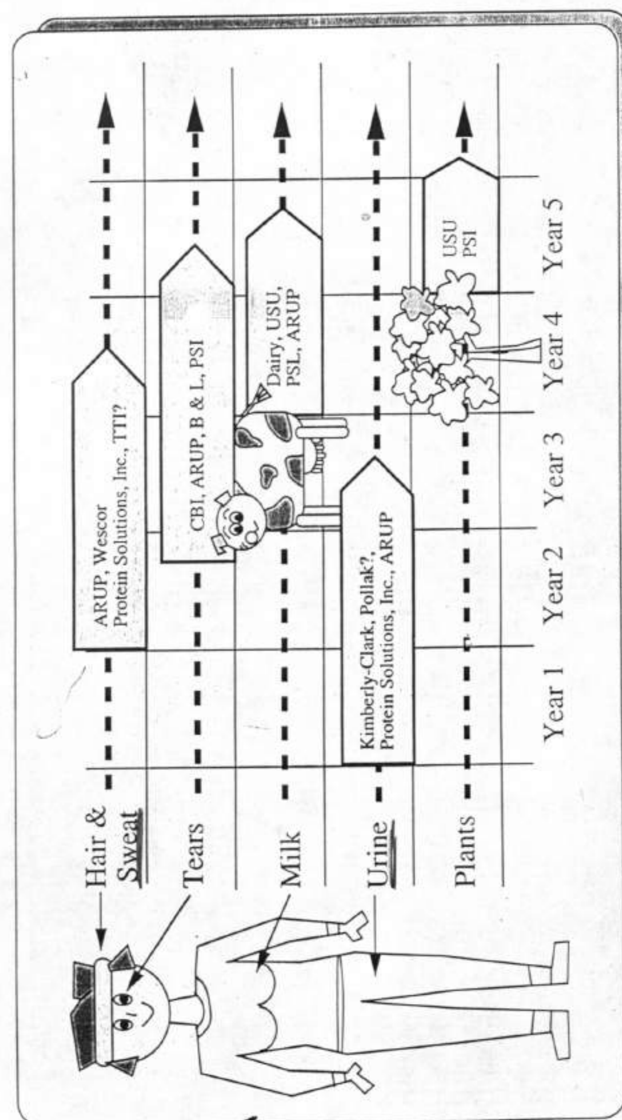


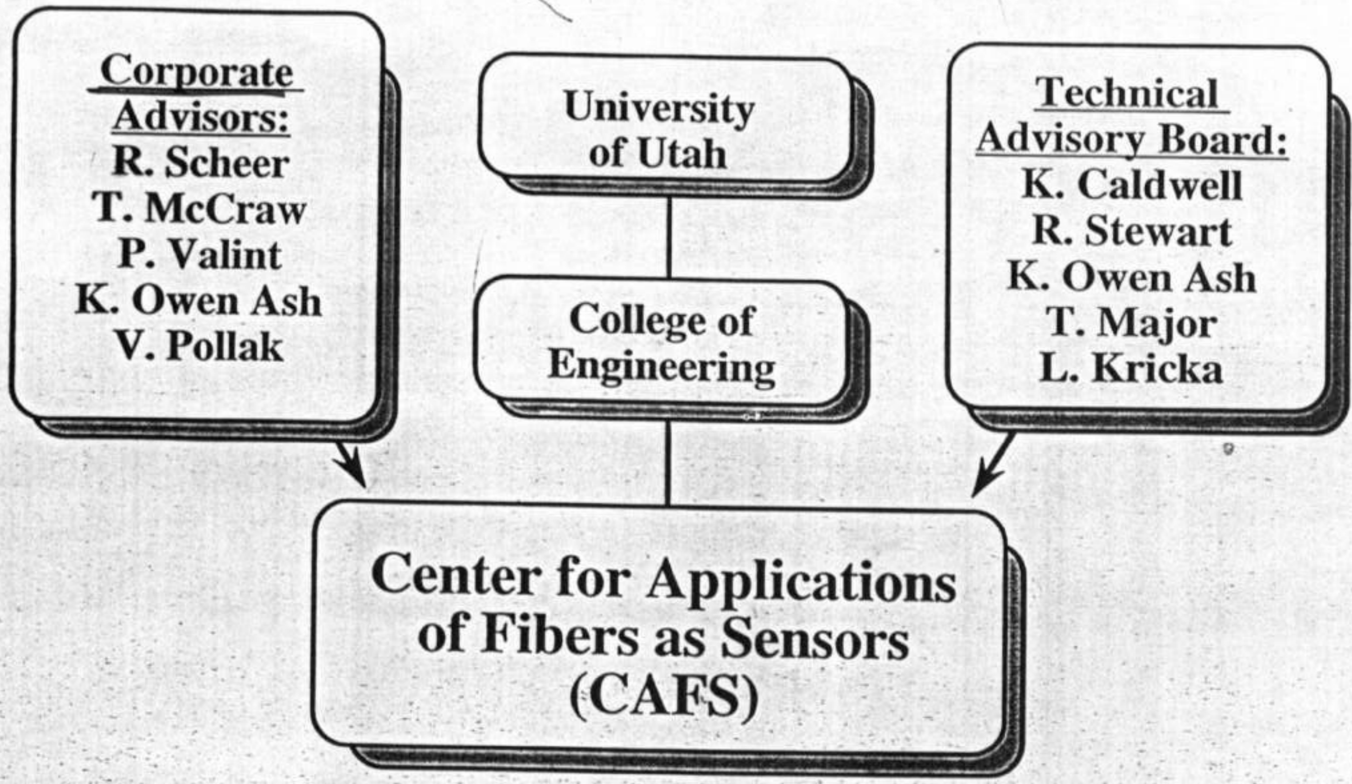
Figure 7. Estimated Economic Impact

REFERENCES/BIBLIOGRAPHY

1. J.D. Andrade, et al., "Method of Measuring Chemical Concentration and/or Light Intensity Based on Spatial Separation and Resolution," U.S. Provisional Patent Application filed 8/2/95.
2. J.D. Andrade, et al., "Direct Reading Biosensors," Proc. Int. Symp. Controlled Release **23** (1996). Included as Appendix 2.
3. C-Y Wang, Ph.D. Thesis, University of Utah, May, 1996.
4. C-Y Wang, et al., "Biotinylation of Firefly Luciferase," to be submitted to *Biochemistry*, 1996.
5. D.L. Ross and A.E. Neely, *Textbook of Urinalysis*, 1983, pp. 151ff.
6. Allen, M.P., and Singh, P., "Instrument-Free Quantitative Test Systems," in *Diagnostics In The Year 2000*, P. Singh, et al., ed.s, 1994.
7. Coolen, R.B., "Anticipating the clinical laboratory of the future from a dry chemistry perspective," *American Clinical Laboratory*, June 1994, 17.
8. Diebold, E., et al, "Chemistry on a stick," parts 1 & 2 in *Chemtech*, Aug. 1991, 462 and Sept. 1991, pp. 547.
9. Kricka, L.J., et al., *Anal. Applic. of Bioluminescence and Chemiluminescence*, Acad. Press., 1989.
10. B.J. Sedlak and G. Kyle, "Bioluminescent Technology for Reagents, Diagnostics...," *Genetic Engrg. News* 9/15/96, p. 8.
11. P. Berman, "...Diapers are High Tech," *Forbes*, Oct. 10, 1994, pp. 78.
12. S. Fox and A. Fields, "...Carbohydrate Technology...," *Gen. Engrg. News*, Nov. 15, 1995, pp. 8.
13. M. Tuchman, et al., "Screening Newborns...Urine...," *Ped. Res.* **30** (1991) pp. 315.
14. D. Kaye, "Dipsticks for Diagnosis of Urine...," *J. Amer. Med. Assoc.*, **274** 91995) pp. 868.
16. G.P. Zaloga, "Urine Testing at the Bedside," *Consultant* (June, 1993) pp. 90.
17. E.J. Cone, et al., "Sweat Testing...," *J. Anal. Toxic.* **18** (1994) pp. 298.
18. "Sweat Testing," Symposium, Amer. Academy Forensic Sciences, Feb., 1995.
19. W. Barlow, "Sweat Test," *Amer. Lab.*, June, 1989, pp. 6.
20. W. Vogt, et al., "...Pattern Cognition and Recognition...," *Trends Anal. Chem.* **3** (1984) pp. 166.
21. N. Honda and S. Aida, "...Medical Data by Face Method," *Pattern Recog.* **15** (1982) pp. 231.
22. J. Fernandes, et al., ed.s, *Inborn Metabolic Diseases*, Springer-Verlag, 2nd ed., 1995.
23. N.W. Tietz, ed., *Clin. Guide to Lab Tests*, 3rd ed., Saunders, 1995.
24. M.E. Houston, *Biochem. Primer for Exercise Science*, Human Kinetics, Inc., 1995.
25. D. Everhart, et al., "Disposable Absorbent Product...," U.S. Patent 5.468.236, Nov. 21, 1995.
26. J.D. Andrade and R. Scheer, "The Labless Lab™," *Proc. 2nd Int. Conf. on Intelligent Materials*, C.A. Rogers, ed., 1994, Technomic Publ. Co., pp. 35.



Center for Applications of Fibers as Sensors (CAFS)



J.D. Andrade, PI
C-Y Wang, Lab Manager
P. Dryden, Technican/Analytical Lab

M. Steadman, Admin. Assistant
M. McDonald, Education & State Liaison
Graduate Students

Organization