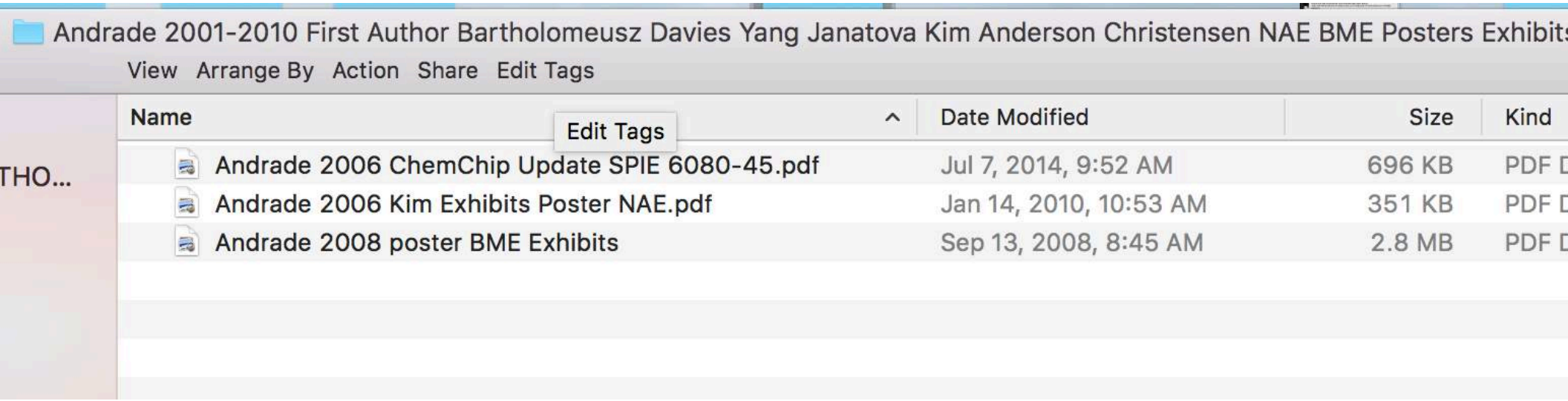
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Multi-analyte bioluminescence-based disposable ChemChips for home-based application: Update

Joseph Andrade, Daniel Bartholomeusz, Rupert Davies, Xiaoyun Yang, Jarmila Janatova
University of Utah Dept. of Bioengineering 50 So Central Campus Dr.
Salt Lake City, UT 84112-9202

ABSTRACT

Bio- and chemi-luminescent based biochemical sensors are being developed in a multi-well single use format for multi-analyte applications employing a single step, disposable, easy to use and interpret ChemChip. We briefly review and summarize earlier and ongoing work. We also agrue for far more, rather than less or limited, chemical data in all areas, and particularly in education, health, and medicine.

Keywords: Biosensors, lab on a chip, point of care, bioluminescence, luciferase, metabolites, immunoassay, science education, health care costs.

1. CHEMICAL ANALYSIS FOR ALL

We live in a world where conclusions are drawn and decisions are made about chemical effects, pathologies, and therapies with little or no measurement of the chemicals involved. We are generally ignorant of the presence and concentration of chemicals in our bodies, environments, foods, etc. unless particular events bring them to our attention. A recent example is the 'discovery' that the Great Salt Lake has extremely high mercury levels and that fish in the western states have high mercury levels. Our society is largely ignorant of the Periodic Table unless a particular measurement or situation brings one element to our attention—then we become 'concerned' and possibly react.

Our professional communities tend to feel that too much chemical information is inappropriate, costly, and counter-productive, in part because our society is generally uneducated and uninformed about chemistry and allied fields. There is also concern that too much chemical information may lead to liabilities and related legal and regulatory concerns.

The health care community is generally advised to minimize the ordering and acquisition of biochemical and related information unless such data will aid in confirming a diagnosis or is needed to help regulate a chemical therapy—for example, therapeutic drug monitoring. The practice of acquiring chemical data from patients for research or curiosity purposes is actively discouraged—usually based on cost containment and liability arguments.

This situation is in place for several reasons:

--the chemical awareness, literacy, and education of our general population is terrible. Most of our elected and appointed officials have little or no understanding of chemicals, concentrations, methods of measurement, etc.;

--the scientific – and especially the chemical – communities have been very ineffective in educating and involving the general public in such topics and issues;

--the media and communications fields are largely disinterested unless they can build on a scandal, tragedy, liability, failure, etc. -- something that makes 'news' – then they become very interested, albeit superficially;

--the availability of means and methods for chemical analysis are restricted, specialized, expensive, and generally unavailable to the wider community.

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This situation is likely to change significantly within the next decade or so, due to modern chemical analysis technologies, and especially lab-on-a-chip and related approaches. Most of the work to date has been focused on specialty, competitive, potential income generating opportunities—the standard 'business model' approach.

Just as the production and distribution of video and music has moved from specialists to literally anyone with some interest and motivation, we feel that the 'control' of acquisition of chemical information by specialized instruments, labs, and experts will be dramatically impacted by the ready and inexpensive availability of ChemChips which could be acquired and used by anyone. Responsible interpretation of the results is another matter.

The easy availability of chemical identification and concentration information will empower and induce the asking of questions, which will in turn fuel a need for more chemical information and education. Modern science museums or centers are likely to respond to such a need with far more interesting, personal, and extensive exhibits and activities related to chemistry (see www.utahsciencecenter.org, for example).

We expect that ChemChips will eventually become available for elemental analysis, for biochemical analysis, for drug analysis, etc. The very limited availability of simple kits and devices for consumers, such as glucose measurement via glucometers, urine test strips, lead test kits, etc. represent the tip of the chemical analysis iceberg.

Our small contribution to this impending revolution in acquisition and availability of chemical data is summarized in this paper.

2. METABOLITE CHEMCHIPS

The background to our work in this area is fully presented and illustrated in Ref. 1. The text below serves to update that earlier review.

Our development of a disposable, dipstick-type, quantitative analytical device for the measurement of multiple analytes in small sample volumes utilizes analyte-specific enzyme reactions coupled to bio- and chemi-luminescent detection platforms[1]. As nearly all analytes of interest can be coupled to these luminescence reactions, a wide range of analytes can be measured with the same device. The luminescence-based assays are inexpensively packaged in a stable form within an array of analytical wells (see Figures in Ref. 1). A photodetector array incorporated into a handheld device could measure the various analyte concentrations by the proportional light intensities from the array of luminescent assays. The highly sensitive nature of luminescence-based analyses means that more analytes can be measured from smaller sample volumes.

Many diseases and clinical conditions have biochemical origins, consequences, or markers that can be used to predict, diagnose, or manage these pathologies. Effective management of some pathologies require regular, routine clinical chemistry measurements of various analytes. A well-known example is glucose and glycosylated hemoglobin measurements necessary for the management of diabetes[2]. Other diseases such as the inborn metabolic diseases phenylketonuria (PKU)[3] and galactosemia[4] also need regular monitoring of relevant analytes.

Although clinical chemistry and analytical laboratories can routinely measure most biochemicals important to metabolism, in practice most of the major metabolites are rarely determined. Indeed, the direction in clinical medicine has been toward ordering less chemical tests rather than more, because of the interest in minimizing healthcare costs and thus in reducing “unnecessary tests.” The expense of most tests lies in the cost of central or commercial reference laboratories and in our health care system's very inefficient and high cost reimbursement mechanisms. Given the current expense of laboratory testing, cost-benefit analyses argue against more comprehensive biochemical analyses, except in high-risk or related situations. However, if the cost of clinical chemistry tests were significantly decreased, the cost-benefit analyses would be different, likely leading to recommendations for more rather than fewer tests.

Luminescence-based analysis has the advantage that it is generally 100 to 1,000 times more sensitive than common spectroscopic/colorimetric methods[5]. Chemiluminescence is light produced by compounds undergoing specific oxidation reactions, while bioluminescence is when these reactions are catalyzed by enzymes. The most well-known example of bioluminescence is the firefly; however, other organisms employ similar reactions to produce light; e.g.,

bacteria, fish, and fungi. All bioluminescent reactions employ an enzyme called "luciferase," which facilitates the oxidation of an energetic substrate, called "luciferin," into an excited state, where it emits a photon. There are many different luciferases and luciferins with at least 30 different known bioluminescent reactions in nature.

The most common chemiluminescence reaction involves the oxidation of luminol, which produces blue (450 nm) luminescence. This reaction can be used to measure hydrogen peroxide (H2O2) and can be catalyzed by transition metals or horseradish peroxidase. The yellow-green (580 nm) bioluminescence of fireflies is based on the enzyme-catalyzed oxidation of firefly luciferin utilizing adenosine triphosphate (ATP) as a highly specific co-reactant. The blue (490 nm) bioluminescence of marine bacteria is closely coupled to a reduced nicotinamide adenine dinucleotide (phosphate) [NAD(P)H]-dependent enzyme reaction.

Thus, Mother Nature has literally given us two unique, ultrasensitive, and highly specific reactions for the measurement and monitoring of ATP and of NAD(P)H, while luminol chemiluminescence can be used to measure H2O2. When analytes are coupled to ATP, NAD(P)H, or H2O2 via metabolic reactions, the intensity of luminescence is proportional to the concentration of the specific biochemical of interest in the sample. The reactions are sensitive to ATP or NAD(P)H over five orders of magnitude in concentration[5] without dilution or modification of the sample fluid. Since most of biochemistry depends on ATP and/or NAD(P)H or can be linked to H2O2, practically all metabolic reactions or metabolites can be monitored or measured by luminescence via one or more enzyme-linked reactions (see a biochemical or metabolic pathways chart[6] or www.expasy.ch/cgi-bin/search-biochem-index). There is a large body of literature on the development of sensors for ATP, NAD(P)H, and H2O2-dependent processes, using the firefly luciferase, bacterial luciferase, and luminol chemiluminscence reactions, respectively[5].

The increased sensitivity due to the high signal-to-noise ratio intrinsic to luminescence measurements means only small sample volumes are required, thus making it possible to simultaneously measure many different metabolites. Measuring 10 to 20 different analytes may be possible within the 10 microliter sample volume now used to measure only glucose with commercial glucometers. With increasing demand for minimally invasive sampling to reduce patient discomfort and inconvenience, sample volumes will probably decrease. Modern glucometer test strips now require only sub-microliter samples.

Inexpensive and reliable analyses of many biochemicals at the point of care (POC) would enable clinicians to diagnose and treat even complex diseases and pathologies. Biochemical reactions do not exist or operate in isolation, and every reaction is obviously dependent on many other reactions through the principles of biochemical networks, reaction kinetics, and equilibria[6]. We must have the tools to move beyond mono-parameter chemical paradigms. Most of the papers and studies we read about in the press and even in journals deal with only one chemical parameter—with mono-parameter based hypotheses or correlations. Biochemistry, medicine, and biology are not that simple. We need devices that can easily and inexpensively measure the many relevant biochemical parameters to provide the information base to more fully understand and effectively treat biochemical diseases in shorter time.

Generally, many of the analytes needed for one disease are also important and relevant to other diseases, due to the interrelationship and highly interconnected nature of metabolism[6]. Thus, the economic problems associated with developing a home assay for rare conditions such as PKU or galactosemia are minimized by producing one sensor that is useful for many applications and will be invaluable to clinical research. Simultaneous measurements on multiple metabolites could be performed on multi-analyte chips within a few minutes, at relatively low cost, empowering research laboratories in the study of metabolic networks, and possibly uncovering significant diagnostic correlations among interconnected and/or previously unlinked metabolites.

Part of our interest in this project is patient empowerment—getting patients to assume responsibility and control of their own diseases. If, for example, PKU patients are given their biochemical information, particularly their phenylalanine concentration, on a very regular basis, they will, in turn, utilize that information to regulate their own diet[3], much the way diabetics do by monitoring their glucose levels. By providing the information patients want, we give them the feedback which empowers them to improve their outcomes by allowing appropriate biochemical control of their disease (drugs, diet, etc.). This information empowerment means that patients, through direction from their physician, can monitor and manage their conditions away from the hospital, thus reducing patient visits and overall health care costs.

The background to the selection and development of analyte-specific assays is given in Ref. 1. We focus on homogeneous (single stage) rather than heterogeneous (multi-stage) assays, and on enzyme availability and/or cost. The advantage of a homogeneous assay is that all the reactions occur in the same place at the same time, meaning the ChemChip can be quite simple. A heterogeneous assay would require one set of reactions to be completed before the sample is moved to the next stage. This is easily accomplished in more sophisticated ChemChips, such as the ChemCD developed by Bartholomeusz, et al. and discussed in the next paper[7].

As enzymatic reactions may have very different pH optimums or other conflicting reaction requirements, the challenge is to optimize the reaction conditions to provide a useful analytical output. Potential assays are assessed by using enzyme kinetic models solved by numerical methods. The best-performing assays from the kinetic simulations are then evaluated in aqueous assay format and empirically optimized.

We have studied and implemented assays for:

Galactose , Glucose, Creatine, Creatinine, Urea , and ATP, using the firefly luciferase platform;
Phenylalanine, Lactate, Galactose, Glucose, Glutathione, and NADH using the bacterial luciferase platform; and
Glucose, Creatine, Creatinine, and H2O2 using the chemiluminescence platform[1].

Recent additions to the list include Phosphate, Glycerol, Pyruvate, and Arginine.

The optimized aqueous assay serves as a starting point for the lyophilized or dry-reagent assay. Enzyme stability is key to the development of biosensors. Selecting enzymes that are inherently robust is important so that they retain activity after deposition and lyophilization (freezing and drying). Lyophilization stabilizes enzymes for long-term storage by reducing both mechanical and chemical degradation. Although lyophilization generally increases the long-storage stability, the process itself can also degrade the enzyme. However, with appropriate stabilizing additives and preservatives, the degradation during lyophilization and long-term storage can be minimized. The enzyme solutions and chemiluminescence cocktails must be stable for long periods, ideally 12 months and longer. We have assessed and adopted various additive mixtures for maintaining activity after lyophilization[1,8]. Bovine serum albumin (BSA) is used for surface passivation and as a stabilizing agent.

Each well of the ChemChip contains a “cocktail” of reagents specific to the particular analyte and/or specific analyte concentration range. The reagent cocktails are prepared in a lyophilized form for stability. The assay arrays are tested with reference blood or urine and validated against standard and available methods.

Our ChemChip prototypes have been made and described by Bartholomeusz, et al.[7]. Work is also underway on an inexpensive CCD camera applied to the measurement of integrated luminescence from a multiwell ChemChip.

3. Drug ChemChips: ImmunoChips

We have recently extended our ChemChip interests and activities to include luminescent immunoassays in order to access analytes not readily measurable by enzyme-based reactions. Homogeneous immunoassays require no separation or washing steps (for antibody-bound and unbound components), whereas heterogeneous assays generally require one or more separation steps. Some commonly used homogeneous immunoassay techniques include Enzyme-Multiplied ImmunoTechnique (EMIT) and Cloned Enzyme-Donor Immunoassay (CEDIA). We have chosen CEDIA, based on the reagents and kits available from Microgenics and Promega[9].

The CEDIA method uses an enzyme acceptor (EA) and an analyte-labeled enzyme donor (ED). The presence of free analyte ties up the drug-specific antibody, allowing ED and EA to assemble, producing active enzyme. Low or no drug in the sample results in the binding of antibody to the ED-drug conjugate, preventing the assembly of the intact enzyme. The marker enzyme activity increases with increasing analyte concentration. The marker enzymes in the CEDIA assay usually involve H2O2 and can thus be coupled to the luminol chemiluminescent reaction. Yang, et al recently enhanced the assay performance dramatically by using a bioluminescence-based readout[9].

Our first ImmunoChip application is therapeutic drug monitoring relevant to epilepsy and related conditions (Xiaoyun Yang's PhD work, University of Utah). Epilepsy is a relatively common and serious neurological disorder which often requires long-term management. Medication with anti-epileptic drugs (AEDs) is the mainstay of therapy. Using the blood level as a guide, therapeutic drug monitoring (TDM) aids in determining the most effective dosage of a drug, identifying toxicity, and detection or confirmation of poor compliance. By using a one-step homogeneous immunoassay – CEDIA (Cloned Enzyme Donor Immunoassay) -- platform, we are developing a disposable, quantitative analytical device for the measurement of multiple drugs in small sample volumes. Such a device could be used for the diagnosis and management of clinical conditions at the point-of-care (POC) and eventually in the home environment.

CEDIA immunoassays were modified and enhanced for high sensitivity and for ImmunoChip application for the three AEDs in the study: Carbamazepine (CBZ), Phenytoin (PHT) and Valproic acid (VPA)[9]. The luminescence-based one-step CEDIA platform provides a sensitive, simple and relatively fast technique for detection and quantitation of CBZ, PHT and VPA. This one-step CEDIA platform has shown to be suitable for immunochemical detection and quantitation of these three AEDs in a homogeneous manner.

ImmunoChip fabrication and methods for reagent dispensing are being investigated in our laboratory[7].

This work is being extended by Jensen to develop a steroid hormone chip using CEDIA-based methods (Jelena Jensen, PhD work, University of Utah). This is a much more challenging problem due to the low concentrations and thus very high sensitivity required for steroid measurements. Her goal is to to develop a less invasive, disposable point of care luminescence-based multi-analyte assay platform to measure cortisol and other major steroid hormones, allowing more frequent analysis and faster results of multiple steroid assays, thereby improving diagnosis and maintenance of steroid-dependent endocrine disorders.

Steroid hormone levels regulate growth, metabolism, homeostasis and reproduction as part of the hypothalamic-pituitary-adrenal axis (HPA Axis). Hormone levels fluctuate with monthly cycles, circadian rhythms and induced stress. It is therefore desirable to measure hormones at various times throughout the day, week and month. Steroid hormone levels are adjusted biologically by inhibition and negative feedback systems and the levels of various hormones are influenced by each other. The most desirable indicator of hormonal status is given by the relative changes in various hormone levels with respect to each other, rather than by one isolated hormone measurement. Steroid hormones are present in low levels in the body, are small molecules and are lipophilic. These factors do complicate measurements of steroid hormones, but do not preclude a fast reliable method of measurement.

An existing homogeneous monoclonal antibody immunoassay that is rapid, of high specificity, and able to detect low levels of analyte from low sample volumes is being evaluated and modified.

The steroid ImmunoChip output will be delivered in a user-friendly visual signature that shows a picture of the patient's hormone levels compared to the normal standard reference values. Cortisol will be used as the model steroid with which the immunoassay platform is developed to be followed by similar protocols for testosterone and estradiol. Ideally, all the major steroid hormone assays will be present on one multi-analyte "hormone chip".

4. Data and Information Perspectives

Reliable information-processing methods (InfoWare) are being incorporated to extract useful information and simplify interpretation, enabling the optimal use of ChemChip and related devices in both research and POC settings. InfoWare refers to the means and methods for simulating analytical assays, calibration, estimating the device's total analytical error, and the processing of multiparametric data with the goal of developing visual patterns, easily interpretable by patients and care givers.

InfoWare involves (PhD work of Y Al-Sheikh, University of Utah):

--Simulating the chemical assays;

--Applying mathematical and statistical tools in experimental design to chemically optimize the assays and acquisition of experimental data in reference and appropriate biological fluids;
--Establishing mathematical and statistical models to generate the most reliable off-board calibration methods;
--Establishing mathematical and statistical models to perform on-board calibration features;
--Applying mathematical and statistical tools to estimate total device analytical error, extract useful diagnostic information, and develop visual diagnostic patterns that are easily interpretable by patients and care givers.

How can we deal with 10-20 different channels of chemical information? How can physicians, patients, and family members effectively deal with the interactions among many different metabolites, nutrients, and drugs? Fortunately, advances in data analysis, parameter presentation, and visualization—coupled with appropriate modeling, simulation, and sensitivity analyses—allow this challenge to be effectively addressed.

A simple but highly useful approach to multidimensional "visualization" is the use of radar, spider, or star plots (all synonomous) utilizing radial, polar, or even spherical 3-D coordinate systems to present multidimensional data[10]. We are now using this approach to present multiparameter clinical chemistry data so that the visual pattern generated by the locus of points on the spider plot is designed to reflect particular disease states and metabolic conditions. Although such plots are incorporated in some plotting and graphical analysis packages and software, and widely used in certain specific fields such as sensory assessment, they have not been widely applied in most other areas of science. There has been limited use in clinical medicine, which has demonstrated that such approaches have enormous potential. Perhaps the clearest example of an n-dimensional radar plot visualization method applied to clinical medicine and clinical biochemistry is the work of Cerra et al.[11] dealing with the nutritional management of metabolic stress. Their study of the role of branched chain amino acids in the stress response presented the data in a unique radial or star plot, similar to those that we are developing and using.

5. ACKNOWLEDGMENTS

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SPIE is an international technical society dedicated to advancing engineering and scientific applications of optical, photonic, imaging, electronic, and optoelectronic technologies.

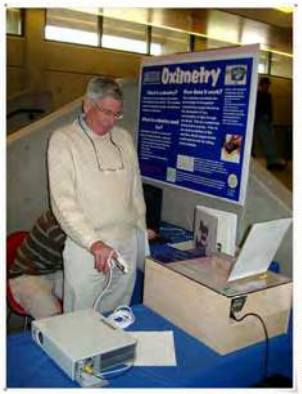
Interactive Exhibits and Programs for the General Public via a Modern Science Center

Joseph Andrade*, Seung-Jae Kim, Michael Anderson, Bioengineering, University of Utah, Salt Lake City, UT.

INTERACTIVE EXHIBITS

The Utah Science Center (USC) opens in 2008 as part of The Leonardo— on Library Square in Salt Lake City. USC will be the first science center to take interactivity and personal involvement to an entirely new level. Targeted to all who will respond to being treated as adults, USC will involve and empower its visitors to be participants – helping to enhance its exhibits and improve its programs. You are more than a visitor – you are more than a participant; you will help create, enhance, and design the experience.

The Utah Science Center emphasizes creative and active exploration of the worlds of science and technology. Visitors to the Utah Science Center will question, learn and invent through personal, hands-on experimentation and exploration. The goal is to facilitate personal Eureka! moments – personal scientific revelations.



DEVELOPMENTS

The Utah Science Center utilizes exhibits and related activities designed, developed, and tested by University of Utah students involved in course projects, thesis projects, and as volunteers.

Students and faculty from
- Architecture and Planning
- Bioengineering,
- Chemistry,
- Geography,
- Geology,
- Materials Science,
- Mechanical Engineering,
- Meteorology,
- Pharmaceutics,
- Physics,

have been involved to date--with additional Departments and programs becoming involved.

Project types have included

- freshman undergraduate laboratory course team projects,
- individual independent project courses,
- Masters degree level thesis projects, and
- high school interns.

SOME OF THE PROJECTS

Active:
- YOU are the Experiment!--the Bioengineering Team
- The Body Electric--more Bioengineers;
- Weather in the West--the Meteorology Team

Developing Projects:
- Chemistry and Chemicals: sophomore organic chemists
- Raging Hormones--Pharmaceutics Team
- Flight!--Mechanical Engineering Team
- PlanetPlace--the Geography Team

SERVICE LEARNING CREDIT

Although some of the activity has been documented and 'blessed' as service learning--much of the work and involvement has not received any formal 'service learning' credit or recognition. The 'credit' or 'recognition' comes in the participants seeing their work used in a public facility committed to science and technical awareness, education, and literacy.

These activities are planned to continue, to expand to many other departments and programs, and to involve greater numbers of students and faculty.

For more details on these activities;
www.utahsciencecenter.org
www.theleonardo.org
(or email to*) **joeandrade@uofu.net**

Bioengineering Education via Projects and Activities for an Interactive Science Center: the University of Utah Experience

Joseph Andrade*, Seung-Jae Kim*, Douglas A Christensen
Dept. of Bioengineering, University of Utah, Salt Lake City, UT 84112 and
*The Leonardo, 210 East 400 South, Ste. 14, Salt Lake City, UT 84111

www.bioen.utah.edu

www.theleonardo.org

The Public

A functioning democracy requires a literate, aware, educated, involved, and responsible electorate. Because the general public is very interested in bioengineering and medical technologies, these subjects provide an avenue for enhanced and expanded activities related to scientific and technical literacy.

The Science Center

Science Centers provide opportunities for the general public to experience, participate, and learn a wide range of science and engineering fields. Most major urban areas have a science and technology center—see www.astc.org

The Leonardo -- opening in 2010 in Salt Lake City as a major center for science, art, and culture—takes interactivity and personal involvement to an entirely new level—using measurements and activities designed and developed by Bioengineering and by other students.

The University

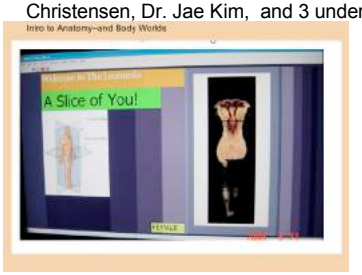
The University's research and education mission extends to its general public and community. Most Universities have 'outreach' and related programs with which to involve, inform, and educate the greater community. Service learning and related community involvement activities are generally fostered and encouraged.

University of Utah programs already involved with The Leonardo include:

Bioengineering	Biology	Chemistry	Geography
Informatics	Meteorology	Pharmacy	Physics

Projects, Exhibits, and People:

A Slice of You?!--a proximity sensor-based human anatomy sectioning experience using the NIH's Visible Human data sets; developed by Prof. D. Christensen, Dr. Jae Kim, and 3 undergraduate students.



Projects, Exhibits, and People:

Is it REALLY Diamond!--Molecular Fingerprinting via fiber optic Raman spectroscopy (unit donated by Process Instruments, Salt Lake City), implemented by summer BioE intern T. Bird and by Drs. J Kim and J Andrade.

Seeing YOUR Insides—personal blood vessel visualization using Bard Access' Site-Rite system (Salt Lake City); implemented by summer intern Alex dePoix and J Andrade.

Private Statistics —a dynamic statistical histogram activity using weight and height sensors; developed by J Abernathy, D Bartholomeusz, Y Al-Sheikh, and J Kim.

Balance Plate —an interactive, competitive balance activity using a Neurocom Balance System (Clackamas, OR); implemented by J Kim and Neurocom.

Bioengineering Students

Bioengineering students have access to undergraduate and/or graduate courses which provide opportunities and environments to design, implement, and test interactive exhibits for a general public audience. The successful exhibits are implemented in ongoing and future programs and activities, such as:
http://www.utahsciencecenter.org/lows/

Current suitable courses at the University of Utah include:
BioE 1102: Intro to Bioeng II (freshman team semester projects)
BioE 5020: Interactive Science Exhibits (independent projects)
BioE 6060/1: Scientific Presentations (beyond PowerPoint!)
BioE 6920: Internship Program

Bioengineering Profession

Bioengineering is a subject of great interest to the general population—as all individuals are involved in various and personal ways with medical technologies, the health care system, etc.; bioengineering, biotechnology, and related topics are among the most popular science/technical topics in magazines, newspapers, and other media. Such intrinsic interest provides an opportunity to involve and educate the public about the field and the profession.

AIMBE (www.aimbe.org) has recognized the potential for encouraging interactions and cooperation between academic bioengineering departments/programs and their local science centers or museums. AIMBE, University of Utah, and The Leonardo have initiated a project:

Enhancing Public Understanding of Bioengineering Research and Applications

This poster serves as an initial report of that preliminary project.

Presented at Third Biomedical Engineering Education Summit Meeting
St. Charles, IL June 16, 17, 2008