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REVIEW ARTICLE

Review of Designing an Information Processing Ware for a Diabetic Chip

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Abstract

Miniaturization of clinical chemistry analyzers can empower research conducted to better understand, diagnose, manage, and cure diseases such as diabetes. For the last decade, we have been working on the design and development of miniaturized clinical chemistry devices, including a Diabetic Chip (diabetiChip). These devices measure a small array of analytes, are small, portable, fast, easy-to-operate, and inexpensive. The chosen analytical method for the diabetiChip uses bioluminescence, which is highly sensitive and specific, and is based on photon counting and specific enzymatic reactions. Bioluminescent reactions were intentionally chosen for analyzing metabolic reactions because they use some of the central nodes of metabolism, such as adenosine triphosphate. Operations of the diabetiChip's information processing ware are the focus of this paper; we show the feasibility of using a set of kinase-containing enzymatic reactions of a firefly bioluminescence-coupled glucose assay in designing the diabetiChip. We have developed and tested the feasibility of the glucose assay; the assay's analytical detection limits (before sample dilution) were 5–185 μM. Uncertainty associated with reporting a 100 μM concentration was about ± 5 μM. The results show that an FFL bioluminescent-coupled glucose assay is promising in terms of reducing sample volume and cost. The concept of GlucoFaces™ in visualizing measurements of the diabetiChip is also discussed.

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Introduction

Miniaturization of clinical chemistry analyzers can empower ongoing research in better understanding, diagnosing, managing, preventing, and curing diseases such as diabetes. For the last decade, we have been working on the design and development of clinical chemistry labs-on-a-chip (ChemChips), including a diabetic chip (diabetiChip), that measure a small array of analytes, are small, portable, fast, easy-to-operate, and inexpensive.[1,2] In this article, we intend to provide background on the chip fabric (ChipWare) and the chemical analytical principle (ChemWare) of the diabetiChip; the reader is advised to refer to the cited references for further details regarding chip fabrication and assay lyophilization and immobilization. The focus of this paper is the development of the diabetiChip's information processing ware (InfoWare).

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Abbreviations: (ATP) adenosine triphosphate, (ChemCD) clinical chemistry CD, (FFL) firefly luciferase, (PMT) photomultiplier tube, (RLU) relative light unit

Keywords: assay calibration, bioluminescence, Diabetic Chip, GlucoFaces™, information ware, lab-on-a-chip, simulation, visual display

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Chip Ware

Analytical processes of the diabetiChip are carried out within a micro-scale, multi-well chip, where chemical assays are deposited, the sample is processed and transported, and photons emitted from the chemical reactions are detected and counted. Chips can be manufactured in large quantities at a relatively low cost. A variety of microfabrication techniques can be used to build arrays of detection wells, where each well contains an independent bioluminescence-based analytical assay. Each unique assay is deposited and stabilized (via lyophilization) in small quantities in individual detection wells. Commercially available silicon photodiodes, avalanche photodiodes, and photomultiplier tube (PMT) arrays designed for low-light detection are ideal for producing small size-detectors to measure signals simultaneously from multiple analytical channels. Our group has focused on PMT-based detectors during the chip development processes. We also examined various sample processing methods and products, such as top-loading, through-flow, hydrogels, plasma separation membranes, and microfluidic channels. We then developed a total micro-analytical chemistry system (ChemCD), which integrates all of the wares.[3] ChemCD can empower the clinical chemistry chip in measuring analyte concentrations in different biological samples and beyond the analytical detectable limit via automatic filtering and dilution of those samples.[3]

Chemistry Ware

The analytical principle of the diabetiChip is the measurement of the number of photons emitted from a bioluminescent chemical reaction coupled to an enzymatic reaction specific to the metabolite of concern.[1,2] A reaction that produces photons has many advantages: the problems associated with color perception or wavelength separation are eliminated, as in the case of reflectance colorimetry; no light source is needed, as in the case of fluorescence spectroscopy; no electrodes susceptible to contamination are used, as in the case of electrochemical analyzers; and less sample volume is required, a major concern for the patient. Bioluminescence is light produced by compounds undergoing specific oxidation reactions catalyzed by enzymes. Bioluminescence-based measurements are efficient for analyzing multiple metabolites in the micro to sub-microMolar concentration range over a short time (about a minute or less), due to their high sensitivity (100 to 1,000 times more sensitive than common spectroscopic or colorimetric methods).

Generally, 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 naturally occurring luciferases and luciferins with at least 30 different known bioluminescent reactions. The yellow-green (580 nm wavelength) bioluminescence of fireflies is based on the enzyme-catalyzed oxidation of firefly luciferin using adenosine triphosphate (ATP) as a highly specific co-reactant (key light inducer). Interactions between the reactants and ATP result in light emission. Moreover, a calibration curve can be obtained for various ATP initial concentrations or any metabolite coupled to ATP via an enzymatic reaction.[2,4,5] Since ATP is one of the central nodes of metabolism, a large number of metabolites can be measured by coupling a metabolite-specific reaction that also involves ATP (usually enzymatic reactions using a kinase) to the bioluminescence platform of firefly luciferase (FFL).[2] In such coupled reactions, light emission will also depend on the type of the kinase reaction, i.e., whether it produces ATP or competes for ATP. In either case, whether ATP is consumed or produced, the amount of emitted light (or photons) can be correlated to the metabolite concentration by generating a calibration curve for the coupled assay.[2,4,5]

Tight control of blood glucose levels close to normoglycemia is necessary for reducing the frequency of the short-term complications of diabetes (hypoglycemia and hyperglycemia) and delaying long-term complications. Therefore, developing a glucose assay is the cornerstone for the diabetiChip. Establishing the glucose analytical assay began with searching the metabolic pathways for enzymatic reaction(s) that use glucose and could be coupled to the FFL bioluminescence platform via ATP. Then, preliminary assay protocols were obtained from the literature.[6-10] Glucose assay simulation, experiments, and calibration are discussed later, in the Assay Data Ware section.

Information Processing Ware

InfoWare refers to mathematical models and tools for simulation of chemical assays, data analysis, assay calibration, estimation of analytical errors, and the presentation of multianalytical data in simple visual patterns that are easily interpretable.[11,12] InfoWare is further divided into two complementary units:

1. Assay data processing ware (DataWare): First, configured assays are simulated to examine assay feasibility and optimal physical and chemical parameters, such as medium acidity and enzyme concentration. Also, mathematical and statistical methods for the design of experiments are used to minimize the number of experiments required in assay validation. Then, experimental data and calibration curves are analyzed for optimal assay calibration. Finally, a general analytical calibration curve that encompasses the effects of other metabolites is created with the aim of minimizing the number of experiments required to validate and implement assays of related metabolites onboard the chip.
2. Data visualization ware (VisWare): Data are represented via simple, but significantly informative, visual patterns. These visual patterns can ease interpretation and provide new insights for understanding disease etiology and pathophysiology.

Assay Data Ware

Firefly Bioluminescence-Coupled Glucose Analytical Assay - Simulation

The main goals of simulation are examining assay feasibility and estimating optimal concentrations for reagents. The FFL bioluminescence reaction requires FFL, luciferin (LH_2), oxygen (O_2), and ATP in the presence of magnesium ions (Mg^{2+}). Adenosine triphosphate uses Mg^{2+} and binds rapidly and reversibly on LH_2 to form the complex luciferase-luciferyl-adenylate "FFL-LH_2-AMP" (steps 1-2 in **Figure 1**). Molecular oxygen oxidizes the complex to produce oxyluciferin (L_{oxy}), AMP, carbon dioxide (CO_2), and light (step 6 in **Figure 1**). The light emitting reaction is rate-limited by three steps; the first two occur before oxidation:

1. A proton abstraction from luciferin (step 4 in **Figure 1**).
2. A conformational change of luciferase (step 5 in **Figure 1**).
3. Dissociation of the luciferase-oxyluciferin complex "FFL-L_{oxy}" (step 7 in **Figure 1**).[4,5]

If the reagents were mixed rapidly for about 5 ms, a lag phase of 25 ms would occur before light was emitted (**Figure 2**). The two rate-limiting steps preceding oxidation, mentioned above, are responsible for this delay. The maximum intensity of the emitted light (I_{max}), which is proportional to the ATP concentration for

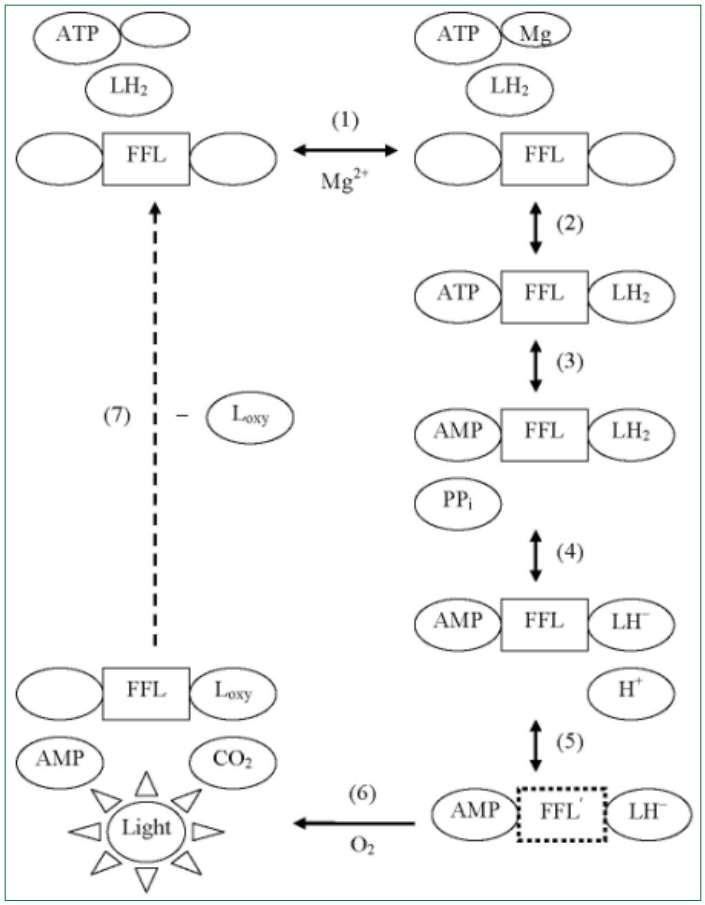

Figure 1. Schematic representation of the detailed steps in an FFL bioluminescence reaction.[4-5] Adenosine triphosphate firsts bind magnesium ions, which facilitates binding on the enzyme FFL (Step 1). ATP and luciferin (LH_2) get bound to FFL (Step 2). Adenosine triphosphate loses orthophosphate and turns into AMP (Step 3). A proton is abstracted from luciferin and a conformational change in FFL occurs (Steps 4-5). Steps 4 and 5 are responsible for the 25 ms lag phase in light emission. Oxidation of the deprotonized luciferin leads to the release of carbon dioxide (CO_2), AMP, the complex FFL-oxyluciferin, and light (Step 6). Steps 6 and 7 contribute to another limitation on light emission: the more oxyluciferin (an inhibitor) released, the less light emitted.

values less than 1 μM, is reached in less than 1 s, but the time necessary to reach half of this value ($I_{max}/2$) is constant (about 100 ms) and independent of the ATP concentration. A steady-state light signal is obtained only for ATP concentrations lower than 10 nM. As the ATP concentration is increased beyond this value, the peak light intensity increases. The light's decay rate also increases due to the noncompetitive inhibition of luciferase by oxyluciferin, the concentration of which increases when the ATP concentration is raised.

A non-steady-state kinetics, proposed by Deluca and McElroy, and modified by Gandelman, was used in simulating the ATP assay with Gepasi Biochemical Simulation (**Figure 3**, **Table 1**).[4,5,13] Based on simulation, optimal concentrations for ATP, luciferin, and FFL were

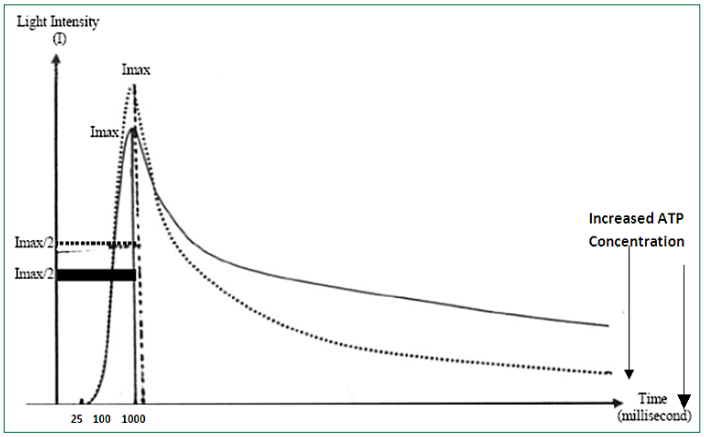

Figure 2. Light emission profiles of the FFL bioluminescent reaction. The solid curve corresponds to ATP concentrations less than 10 n*M*. The dotted curve corresponds to ATP concentrations higher than 10 n*M*. One half of the light intensity is independent of ATP concentration and is always reached after about 100 ms. One the other hand, maximal light intensity depends on ATP concentration. There is a lag phase of about 25 ms in emitting light. The lag phase is mostly due to the conformational change in the firefly luciferase enzyme. The light emission decay rate also depends on the concentration of the reaction product oxyluciferin, which is a noncompetitive inhibitor and its concentration is directly correlated with ATP concentration.

Table 1.
Values of the Kinetic Rate Constants Used in Simulating the FFL Bioluminescent Reaction and Bioluminescence-Kinase Coupled Reactions.[4-5]

Kinetic Rate Constant	Value	Unit
$K_{m(Analyte)i}$	Specific to the Metabolic	Molar
K_{cat}	Kinase Activity	μmole·mg^{-1}·minute^{-1}
K_m (ATP)	250×10^{-6}	Molar
K_m (Luciferin)	2×10^{-6}	Molar
K_i (Oxyluciferin)	$\pm 23 \times 10^{-8}$	Molar
K_1	$(10 - 30) \times 10^3$	Molar^{-1}·second^{-1}
K_{-1}	$(4 - 8) \times 10^1$	Second^{-1}
K_2	$(5 - 15) \times 10^5$	Molar^{-1}·second^{-1}
K_{-2}	$(5 - 15) \times 10^1$	Second^{-1}
K_3	$(20 - 40) \times 10^1$	Second^{-1}
K_4	$(7 - 13) \times 10^1$	Second^{-1}
K_5	$(7 - 13) \times 10^{-2}$	Second^{-1}
K_{-5}	$(8 - 12) \times 10^5$	Molar^{-1}·second^{-1}

Rows above the double line represent kinetic rate constants of the kinase-specific reaction that is coupled to the FFL bioluminescence reaction; the rows below the double line correspond to the kinetic rate constants of the FFL bioluminescent reaction.

$$FFL + ATP \underset{K_{-1}}{\overset{K_1}{\longleftrightarrow}} ATP - FFL \underset{K_{-2}}{\overset{K_2}{\longleftrightarrow}} ATP - FFL - LH_2 \xrightarrow[-AMP,-PP_i]{K_3, O_2}$$

$$FFL - L^*_{oxy} \xrightarrow[-Light]{K_4} FFL - L_{oxy} \underset{K_{-5}}{\overset{K_5}{\longleftrightarrow}} FFL + L_{oxy}$$

Figure 3. The kinetic model for the FFL bioluminescence platform (or ATP assay).[4-5] The first two steps and the last step are reversible and rapid. Rate constants for backward reactions are denoted by minus signs. Adenosine triphosphate binds to the enzyme FFL in the first step. Firefly luciferase binds luciferin (LH_2) in the second step. Luciferin is oxidized and converted to the energetically excited oxyluciferin (L_{oxy}), and AMP and oxygen are released in the third step. The energetically excited oxyluciferin becomes energetically unexcited and releases the excitation energy in the form of light in the fourth step. Finally, oxyluciferin is dissociated from FFL in the fifth step.

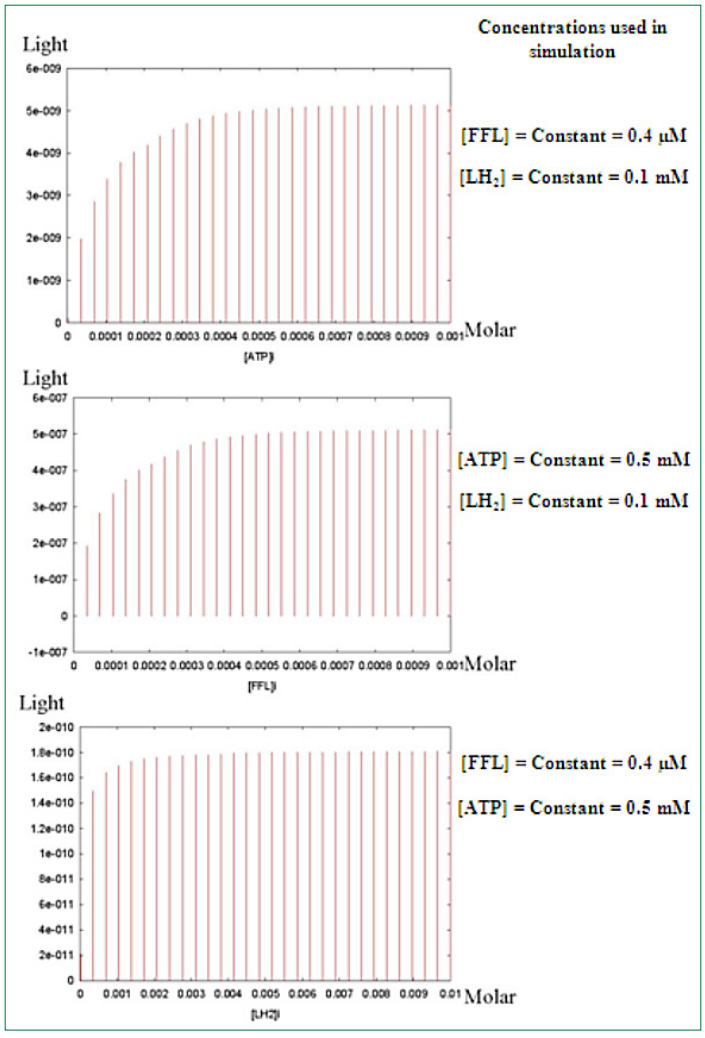

Figure 4. Optimal (minimal) concentrations of ATP, FFL, and luciferin (LH_2) for the FFL bioluminescence platform, found from reaction simulations.

about 650, 1000–3000, and 400–500 μM, respectively (**Figure 4**).

The enzymatic reactions involving glucose convert glucose and ATP to glucose-6-phosphate and ADP via the glucose kinase enzyme (**Figure 5**). In **Figure 6**, we present the general kinetic model "AL-sheikh's Kinase-Kinetic Model" for the enzymatic reactions that convert an analyte (denoted by i) and ATP to another analyte (denoted by j) and ADP via a kinase enzyme (denoted by ij). These kinase reactions can be coupled with the FFL bioluminescent reaction model, discussed earlier, by simulating the bioluminescence-kinase coupled reactions for the glucose assay. This model may be very useful in coupling the kinase reactions of multiple metabolites to the FFL bioluminescent platform, which may improve our understanding of metabolism and the metabolic origin of diseases.[14]

$$ATP + Mg^{2+} + \text{D-Luciferin} + O_2 \xrightarrow{\text{Firefly luciferase}} AMP + Mg^{2+} + CO_2 + \text{Pyrophosphate} + \text{Oxyluciferin} + Light$$

$$Glucose + ATP \xrightarrow{Glucose\ Kinase} ADP + Glucose-6-Phosphate$$

Figure 5. Reactions of the firefly bioluminescence-glucose coupled assay. Glucose and D-luciferin compete for ATP and, thus, the light signal is decreased.

$$(Kinase)_{ij} + (Analyte)_i \xleftrightarrow{k_{-1},k_1} (Kinase)_{ij} - (Analyte)_i$$

$$K_{ij} + x_i \xleftrightarrow{k_{-1},k_1} (K_{ij} - x_i)$$

$$(K_{ij} - x_i) + ATP \xleftrightarrow{k_{-2},k_2} (K_{ij} - x_i) - ATP$$

$$(K_{ij} - Analyte_i) - ATP \xleftrightarrow{k_{-3},k_3} (K_{ij} - Analyte_j) - ADP$$

$$(K_{ij} - x_i) - ATP \xleftrightarrow{k_{-3},k_3} (K_{ij} - x_j) - ADP$$

$$(K_{ij} - x_j) - ADP \xleftrightarrow{k_{-4},k_4} (K_{ij} - x_j) + ADP$$

$$(K_{ij} - x_j) \xleftrightarrow{k_{-5},k_5} K_{ij} + x_j$$

$$(K_{ij} - x_i) + ATP \xleftrightarrow{k_{-2},k_2} (K_{ij} - x_i) - ATP \xrightarrow{k_3} (K_{ij} - Analyte_j) - ADP$$

$$I\alpha([ATP]_0 - [ATP])$$

$$d[ATP]/dt = -k_2[(K_{ij} - x_i)][ATP] + k_{-2}[(K_{ij} - x_i) - ATP]$$

$$d[(K_{ij} - x_i) - ATP]/dt = k_2[(K_{ij} - x_i)][ATP] - (k_{-2} + k_3)[(K_{ij} - x_i) - ATP]$$

$$[(K_{ij} - x_i) - ATP] = \frac{k_2}{k_{-2} + k_3}[(K_{ij} - x_i)][ATP]$$

$$d[ATP]/dt = -k_2[(K_{ij} - x_i)][ATP] + \frac{k_{-2}k_2}{k_{-2} + k_3}[(K_{ij} - x_i)][ATP]$$

$$d[ATP]/dt = \left\langle \left[-k_2 + \frac{k_{-2}k_2}{k_{-2} + k_3} \right](K_{ij} - x_i) \right\rangle [ATP]$$

$$d[ATP]/dt = \left\langle \left[-\frac{k_2k_3}{k_{-2} + k_3} \right](K_{ij} - x_i) \right\rangle [ATP]$$

$$d[ATP]/[ATP] = \left\langle \left[-\frac{k_2k_3}{k_{-2} + k_3} \right](K_{ij} - x_i) \right\rangle .dt$$

$$[ATP] = [ATP]_0 e^{\left[-\frac{k_2k_3}{k_{-2} + k_3} \right](K_{ij} - x_i).t}$$

$$I\alpha\left([ATP]_0 - [ATP]_0 e^{\left[-\frac{k_2k_3}{k_{-2} + k_3} \right](K_{ij} - x_i).t} \right)$$

$$I\alpha\left(1 - e^{\left[-\frac{k_2k_3}{k_{-2} + k_3} \right](K_{ij} - x_i).t} \right)[ATP]_0$$

Figure 6. AL-sheikh's General Kinase-Kinetic Model for kinase reactions that can be coupled to the FFL bioluminescent reaction.

Firefly Bioluminescence-Coupled Glucose Analytical Assay - Experiment

Adenosine triphosphate and glucose assays were examined experimentally. The ATP assay was prepared by mixing 100 μL of 1.2 μM FFL with 100 μL of a mixture of 300 μM luciferin and 15 μM magnesium sulfate ($MgSO_4$), all prepared using a glycine-glycine buffer at pH 7.8. Adenosine triphosphate samples, each of 100 μL, at various concentrations (0, 0.3, 3, 30, and 300 μM) were also prepared with a glycine-glycine buffer at pH 7.8. The final concentrations of the ATP assay are listed in **Table 2**. For the FFL bioluminescent-coupled glucose assay, the assay cocktail was prepared by mixing 50 μL of 2.4 μM FFL with 50 μL of a mixture of 600 μM luciferin and 30 μM $MgSO_4$, 100 μL of 6 μM glucose kinase, and 50 μL glucose samples at various concentrations (0, 150, 600, and 1200 μM). The assay reactions were then initiated by adding 50 μL of 3 mM ATP. All chemicals were prepared using a glycine-glycine buffer at pH 7.8. The final concentrations of the FFL bioluminescent-coupled glucose assay are listed in **Table 2**.

In each experiment, five assays for each analyte concentration were run for 60 s using a TD 20/20

Table 2.
Final Concentrations for the Reagents Used in the ATP and Glucose Assay Cocktails.

Reagent	ATP Assay	Glucose Assay
Glycine-Glycine Buffer	pH = 7.8	pH = 7.8
Firefly Luciferase	0.4 μM	0.4 μM
Magnesium Sulphate	5 μM	5 μM
Luciferin	100 μM	100 μM
ATP	0, 0.1, 1, 10, 100 μM	500 μM
Glucose		0, 25, 100, 200 μM
Glucose Kinase		2 μM

Luminometer (Turner BioSystems Inc., Sunnyvale CA). Data points of the ATP and glucose assays were normalized by dividing each data point by the maximal value of the data set. Data points at each second were averaged. Each experiment was repeated three times. We present here results of one experiment performed to validate each of the ATP and glucose assays (**Figures 7** and **8**).

Firefly Bioluminescence-Coupled Glucose Analytical Assay - Calibration

A measurement is a set of operations performed to determine the value of a quantity, such as the concentration of blood glucose. Calibration is a measurement aimed toward comparing an analyte concentration from a patient sample with a calibrant that has a known value for the same analyte. Calibrants are either directly prepared from a pure substance via simple procedures or indirectly via a series of comparative procedures when the substance is impure. Thus, the measurement procedure on the patient sample constitutes the last step in a series of comparisons, in which each comparative step adds to the uncertainty of the final result.

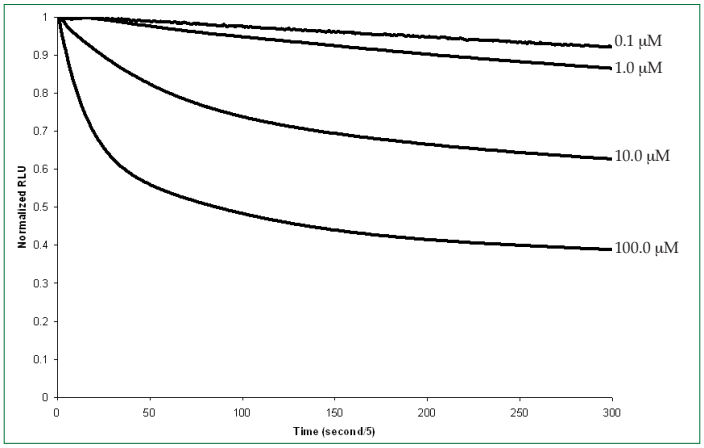

Figure 7. Adenosine triphosphate assay light emission profiles. The concentrations to the right of the curves are of ATP.

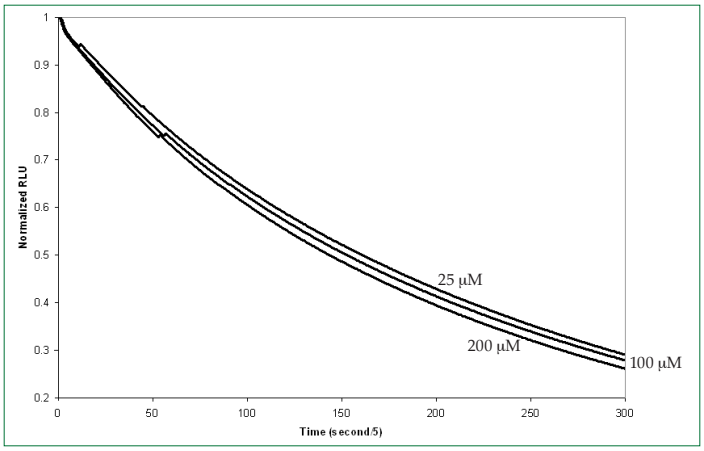

Figure 8. Normalized light emission profiles of the glucose assay: The top curve corresponds to 25 μM glucose, the middle curve corresponds to 100 μM glucose, and the lowest curve corresponds to 200 μM glucose.

Analyte concentration is usually correlated to the level of signal detected by a clinical laboratory analyzer; the signal is scaled by calibration parameters for conversion into concentration units. In the case of point-of-care devices, such as the glucometer, each batch of testing strips has a calibration code that adjusts the scaling parameters to account for variation among batches. However, calibration codes do not account for variation among testing strips within a batch, changes due to storage conditions, or changes in the sensor's accuracy. Measuring the signals from analytical calibrants (or standards) can account for these variations.

Onboard calibration engages treatment of the unknown, unaccounted-for, random interferences attributable to the patient sample (or biomatrix effects); this calibration validates the chip's analytical performance and estimates the total analytical error. Onboard calibration incorporates a number of analytical standards to optimize calibration constants. The more calibration constants in the mathematical equation describing the calibration curve(s), the more standards are required. For the purpose of onboard calibration, we make use of one of the advantages of microarrays, i.e., the availability of a large number of analytical wells. Also, we make use of microfluidics to treat and distribute biological samples in certain wells, and not to distribute them in other wells.

There are various means for assay calibration such as internal standards, standard additions, and spikes. However, by using multianalytical arrays we were able to employ the analytical calibration technique "standard additions" for onboard calibration. In addition to the blank, standards with known analyte concentration were mixed with the patient sample (**Figure 9**). By this, most of the interferences and systematic errors, such as biomatrix effects, inter-batch variability, and signal-to-concentration correlations, are accounted for.

Different methods can be used for generating calibration curves: integrating total or partial area under the curve, slope determination, and end-point value (**Figure 10**). An off-board calibration curve for the glucose assay was generated by integrating the area under the curve

of normalized relative light units (RLUs) (**Figure 11**). The calibration curve was fitted using the Matlab-Curve Fitting Toolbox[15] (**Figure 12**, **Table 3**). The fitting model was a quadratic polynomial model. A good fit was obtained, with $R^2 = 0.99$. The assay's analytical detection limits were 5–185 μM, which was expected from the fast and competitive FFL bioluminescence-kinase coupled reactions. Uncertainty associated with reporting a 100 μM concentration was about ± 5 μM. The results showed that an FFL bioluminescent-coupled glucose assay requires sample dilution and is promising in terms of reducing sample volume and cost.

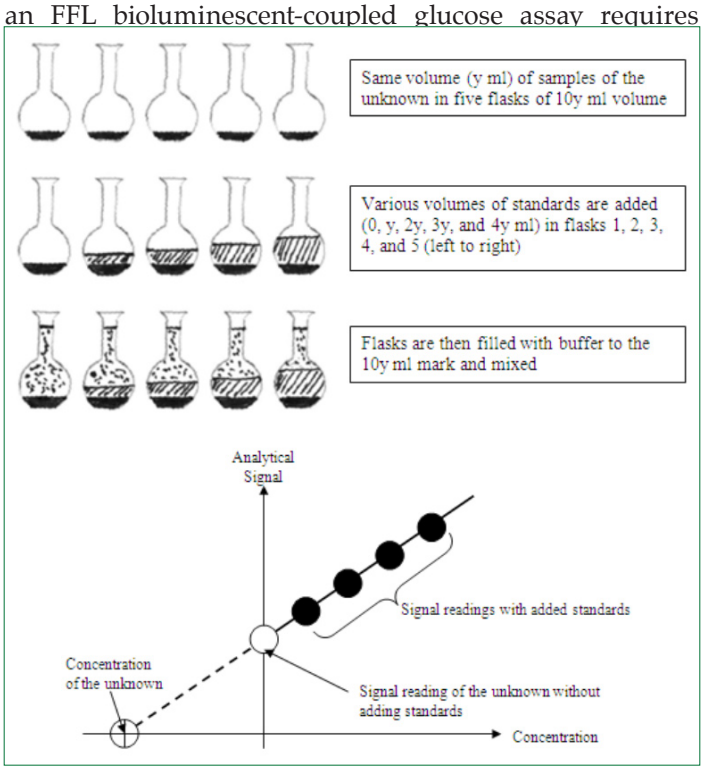

Figure 9. Demonstration of the standard additions assay calibration method.

Table 3.
Fitting Model and Parameters and Goodness of Fit for the Glucose Assay Calibration Curve.

Fitting Model	Quadratic Polynomial $f(x) = p_1x^2 + p_2x + p_3$	x is normalized by mean 81.25 and standard deviation 89.85
Fitting Parameters		Coefficients (95% confidence interval)
	p_1	1.209 (0.9026, 1.514)
	p_2	-5.453 (-5.678, -5.227)
	p_3	164.1 (163.8, 164.4)
Goodness of Fit	SSE	0.0005988
	R-square	0.9998
	Adjusted R-square	0.9999
	RMSE	0.02447

Data Visualization Ware

High dimensional data visualization projects n dimensional data onto a 2D physical medium.[16-22] Multianalytical clinical chemistry data can be displayed via simple patterns, providing ease of interpretation and enabling healthcare providers or patients to rapidly make decisions about treatment.[22,23] Simple visual displays have been the major theme of our philosophy regarding data representation.[22,23] We have emphasized the representation of diabetic states, symptoms, and measurements with simple facial (or emotional) iconic data displays in previous publications, such as "Public Adventures in Diabetes: Personal Interactivity in a Modern Science Center."[1,23] Using common visual cues to communicate clinical data is especially effective with children and the medically uninformed. Therefore, we designed a grid or a set of emotional iconic displays,

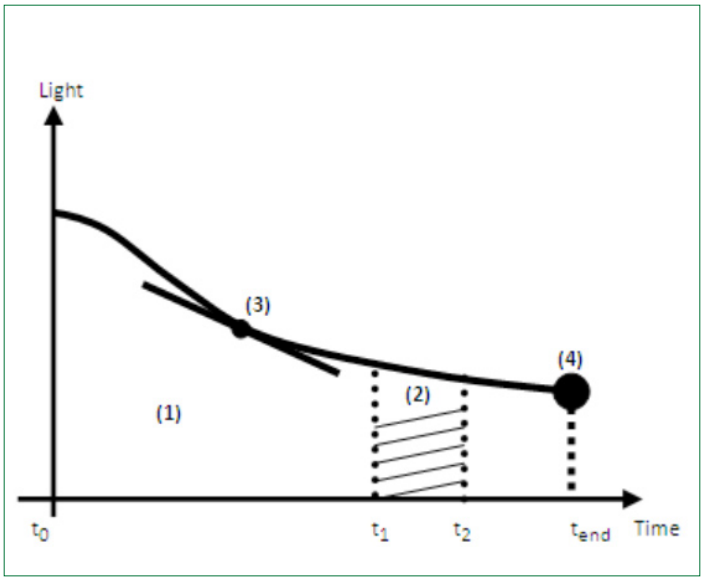

Figure 10. Different methods for calibration curve analysis and parameterization. Four methods are shown: Total area under the curve (1), area under the curve between two time points (2), slope value (3), and end point value (4). Method (1) is the easiest as the number of emitted photons, which constitute the area under the curve, can be directly obtained from the light detector. However, if the calibration curve was not graphically examined, potential systematic errors may not be revealed, thus, the total number of emitted photons may not be a valid means for assay calibration. Method (2) can solve ambiguities of method (1). Method (3) may be useful if a change in analyte concentration can result in a noticeable change in the light emission profile. Method (4) resembles method (1) in ease (the last signal reading of the instrument is considered) and in not recognizing systematic errors. Taking into consideration the pros and cons of these methods, that the experimental light emission profiles agreed with the kinetic model, and that no significant systematic errors were noticed, we used the easiest methods (integrating total area under the curve and end point analyses).

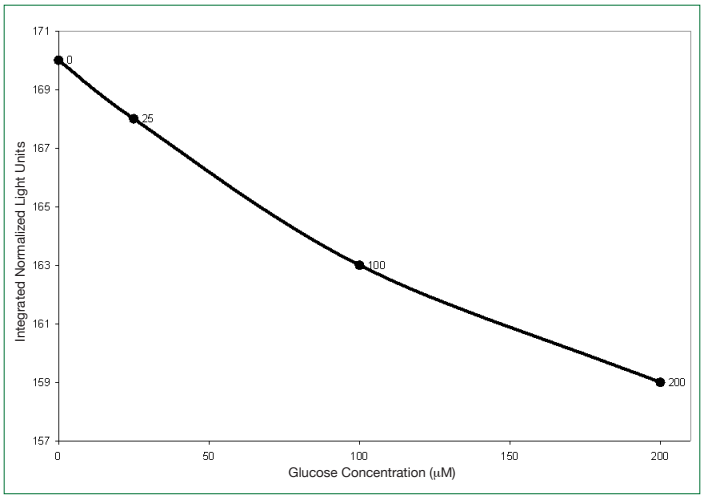

Figure 11. Glucose assay calibration curve.

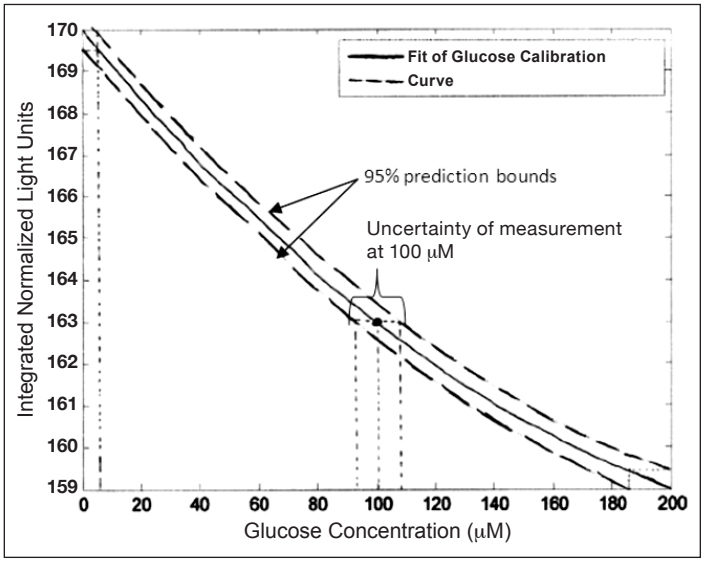

Figure 12. Fitted glucose assay calibration curve. The assay's analytical detection limits were 5–185 μM. The uncertainty associated with reporting a 100 μM concentration is about ± 5 μM.

"AL-sheikh's GlucoFaces™," to represent the diabetiChip's glucose measurements.

The concept of the GlucoFaces is simply to correlate glucose measurements with facial features and emotions. For example, low glucose values can be represented via the degree of tilting of the head, high glucose values can be represented via the amount of shading of the pupils of the eyes, normal glucose values by the amount of smile, which refers to happiness. Also, all glucose measurements are represented on the nose (a longitudinal bar scaled from 0 to 600 mg/dl). Color attributes were also added to the bar to alarm the patient of hypo- and hyperglycemic states via the conventional alarming colors

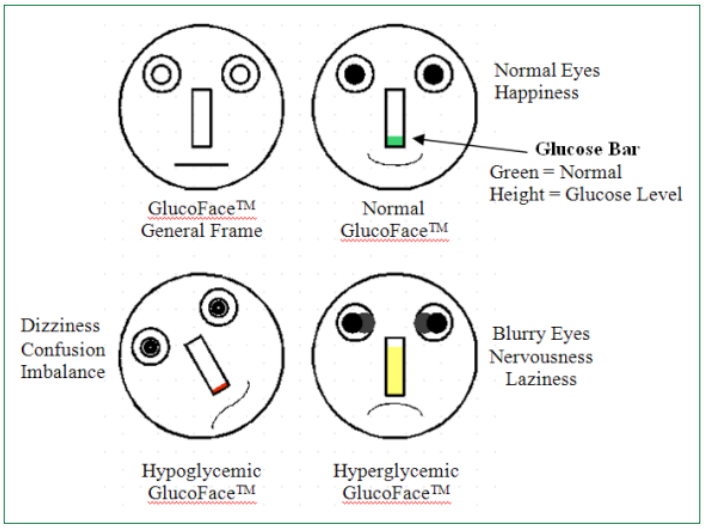

Figure 13. Using simple facial and emotional iconic displays (AL-sheikh's GlucoFaces™) to present results of the diabetiChip.[1,23] The concept of the GlucoFaces is to simply correlate glucose measurements with facial features and emotions. All glucose measurements are represented on the nose (a longitudinal bar scaled from 0 to 600 mg/dl). Color attributes are also added to the bar to alarm the patient of hypo- or hyperglycemic states via the conventional alarming colors (green for normal range, yellow for hyperglycemic range, and red for hypoglycemic range). The general frame for the GlucoFaces shows no emotions (upper left). The normal GlucoFace expresses a happy face with clear eyes (upper right). The hypoglycemic GlucoFace expresses dizziness, imbalance, and confusion (lower left). The hyperglycemic GlucoFace expresses laziness, blurry eyes, and nervousness (lower right). Other glucose measurements are variations of these three fundamental GlucoFaces.

(green for normal range, yellow for hyperglycemic range, and red for hypoglycemic range). Only the general frame of the GlucoFaces and the three fundamental GlucoFaces (normal, hypoglycemic, and hyperglycemic) are shown in **Figure 13**. All glucose measurements are variations of the three fundamental GlucoFaces.

Moreover, we extended our data visualization designs and developed a software program, Multi-Analytical Chemistry-Recognizer of Optical Patterns (MACROPatterns®), to simultaneously visualize the measurement results of multiple analytes on simple visual displays. A detailed discussion of MACROPatterns is not within the scope of this article. However, both GlucoFaces and MACROPatterns along with their interactive educational manuals will be available soon on the digital creativities section within the Diabetes Info Portal "DIP" (http://www.DiabetesInfoPortal.org/Creativities/Digital.html).[24]

Though our ChemChips Project has lasted for over ten years, with efforts from tens of researchers, we believe much additional work needs to be done. Our current work is extending the use of GeneChips with the various

developed ChemChips, including the diabetiChip, to generate information useful in studying the diabetic genome, "diabeteome". Modeling and artificial intelligence techniques are also being used to build hybrid (physiological-probabilistic) diabetes-individualized ("diabetualized") models along with insulin-adjustment electronic protocols and tools.[25-27]

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