Development of a Point-of-Care Device for the Quantification of Bilirubin in Cerebral Spinal Fluid

Fred R. Beyette, Jr., Member IEEE, Blaine Booher, James Drennan, Lee Carraher, Josh Butler, Peggy Bowman, Joseph F. Clark and Philip A. Wilsey, Senior Member, IEEE

Abstract—In North America, an estimated 30,000 patients annually experience an aneurysmal subarachnoid hemorrhage (SAH). In approximately five percent of these patients, the hemorrhage is not visible on computerized tomography scans due to the inability to image blood at time intervals greater than 12 hours post symptom onset. For these patients (many of which have experienced a sentinel hemorrhage that is a precursor to a more significant rupture) a method is needed for accurately analyzing cerebral spinal fluid (CSF) for evidence of SAH. Further, it is necessary to differentiate blood associated with the SAH from blood associated with the spinal tap procedure. This paper presents the development of a point-of-care device that is capable of performing such an analysis. The stand alone prototype device uses commercially available embedded system components to implement a hardware platform that is capable of collecting and analyzing optical absorbance spectra. A mathematical model for the hemorrhagic CSF sample is then developed using a PLSR based regression methodology that is able to differentiate between SAH and blood associated with the spinal tap. This differentiation is achieved by quantifying bilirubin (associated with the breakdown of old blood) in the CSF. Initial testing on the prototype device suggests that the device is able to quantify bilirubin in the presence of hemoglobin over concentrations ranges that are clinically relevant to the patient population of interest.

I. INTRODUCTION

The estimated number of aneurysmal subarachnoid hemorrhages (SAH) in North America is 30,000 per year. In approximately five percent of these SAH patients the hemorrhage is not visible on computerized tomography (CT) scans. This is predominantly associated with the fact that approximately 12 hours post SAH, blood becomes much less visible on the CT scan. For those individuals with a sentinel (or warning) hemorrhage, detection within 12 hours is critical. Unfortunately, for a variety of reasons, (including the patient’s own delay in seeking medical attention) a significant number of patients are not evaluated in this initial diagnosis window where CT scans are most effective.

At present, clinicians possess neither the tools nor the diagnostic modalities sufficient for assessing patients with suspected aneurysmal SAH and normal CT scans. Over the last several years, the authors of this paper have been engaged in a research effort focused on the development a spectrophotometric device that is suitable for identification of patients with suspected SAH [1, 2]. The operation of this point-of-care device is founded in the detection of bilirubin, and concomitant blood products produced within the CSF following SAH [3].

Importantly, the quantification of bilirubin is critical to differentiating SAH from a traumatic spinal tap because the production of bilirubin relies upon the time dependent up-regulation of the heat shock protein heme oxygenase-1 (HO-1) [4]. Therefore, a traumatic spinal tap contains relatively little bilirubin compared to the hemorrhagic CSF following a SAH. Thus, the quantification of bilirubin and hemoglobin will provide a sensitive and rapid mechanism for the diagnosis of SAH and for its differentiation from a traumatic spinal tap.

To date, biochemical verification (using a porcine model) of this diagnostic technique has been completed.[5] In the last few years, a study to further refine the signal processing algorithm and demonstrate the potential for implementation of a point-of-care device based on commercially available computing hardware, was completed.[6] The critical remaining need is the development of a stand-alone prototype instrument that meets the needs of clinicians who provide acute neurologic care in the emergency department and neuro intensive care unit.

In this paper we describe the development of such a device. Section II describes the hardware system and device packaging developed for the point-of-care device. Section III details the collection of absorbance spectra using the hardware platform described in section II. Section IV describes the signal processing algorithm developed to quantify bilirubin in the presence of hemoglobin. Finally,
section V summarizes some of our early testing results and suggests future work that is currently underway.

II. DEVICE HARDWARE DESCRIPTION

At the core of the device hardware is an Overo Air embedded computer available from Gumstix Inc. [7] This computer on module (COM) is based on the ARM Cortex-8 OMAP3503. The processor core runs at 600 MHz and contains 256 MB of flash and 256 MB of RAM. Additional memory can be acquired through the use of the on-board microSD slot. Currently 2 GB or 4 GB microSD chips are being utilized from Kingston. Also included with the COM are the TI TPS65950 IC for system power management and the W2CBW003, which provides both 802.11(b/g) and Bluetooth wireless communication, from Wi2Wi which is based on Marvell’s 88W8686.

The Overo Air COM is connected to a Chestnut43 daughterboard to provide power and allow for convenient peripheral connections. The Chestnut43 comes equipped to attach a 4.3” LCD touch-screen. A Samsung LCD panel with a resolution of 480 by 272 pixels and capable of supporting 8 color bits per Red, Green, Blue channel is currently being used. The built in touch-screen controller on the Chestnut43 allows the touch-screen to be used as an input device (such as a mouse or a keyboard). The daughterboard also includes three USB ports: a USB standard A for USB Host, a USB mini-AB with on-the-go (OTG) signals, and a USB mini-B connector with USB for serial console. The USB high-speed enabled host found on the Over Air allows for fast USB 2.0 transfers between the COM and attached peripherals such as the Ocean Optics USB4000 USB Miniature Spectrometer used to capture transmission spectra through a CSF sample. A 10/100 base Ethernet jack is also included on the Chestnut43. The Ethernet, USB serial console, and wireless technologies are used to communicate with the COM and allow for data flashing, software upgrades and development, and data retrieval. The daughterboard also includes many other wire connections as well as stereo audio in and out jacks that are currently unused.

The Overo Air COM board and the USB 4000 Miniature Spectrometer are housed in a prototype package that consists of four separate pieces printed using an SLA (stereo-lithography apparatus) rapid prototyping process. SLA is an automated process consisting of a liquid photopolymer resin that cross-polymerizes as it is struck by a laser to create a solid 3D object with geometries specified by a CAD model. Each of the four parts—top, bottom, and the two inserts—are printed separately and designed to fit together snugly, using screws for assembly. Generally, the design consists of platforms, anchors, and external ports to accommodate the electronics and air flow features to maximize utility of the cooling fan. Figure 1 shows images of the assembled prototype device and an exploded assembly diagram of the packaging including the internal electronics and one of the packaging inserts. When assembled, the completed prototype device has external dimensions of 7” (depth) by 9” (width) by 3.5” (height).

III. ACQUIRING OPTICAL ABSORBANCE SPECTRA

As suggested in Section II, the prototype device uses an Ocean Optics USB4000 USB Miniature Spectrometer to collect spectral data. The USB4000 is a small and powerful device with many factory-enabled configurations and user-programmable parameters. A Toshiba TCD1304AP Linear CCD array in the spectrometer is configured by the manufacturer to be sensitive to a wavelength range required for our component analysis. The detector is capable of reading from 200nm – 850nm. The light source we use is a tungsten halogen bulb which allows for effective photon penetration from 200nm – 2000nm. The light source has a shutter which is software controlled to allow for the reading of dark noise data. We interface with the USB4000 through a USB cable and an API provided by Ocean Optics. We have developed a custom USB driver to control the spectrometer and light source.

A. Wavelength Concerns

Our current configuration uses a device grating that allows for effective data collection through the wavelength

![Fig. 1. a) Expanded assembly diagram of the prototype device showing external packaging, internal electronics (including Overo board, touch screen and USB 4000) and the packaging insert which has been designed to shunt liquid spillage away from internal electronics. Not shown is a second package insert designed to manage cabling that distributes power and provides USB connection between the Overo board and the USB 4000. b) Photograph of the assembled prototype device.](image-url)
range that is known to be important in the analysis of Hemoglobin and Bilirubin; namely 350nm – 625nm. A small subset of the wavelength range from 460nm - 510nm, corresponding to the largest absorption region for bilirubin, is referred to as the “critical region”. This wavelength region is used for the analysis of bilirubin and hemoglobin concentrations in the current generation of our algorithm (described in section IV). To the left of the critical region from about 370nm – 430nm is the peak wavelength range of hemoglobin absorption. The absorption peak can be fully acquired on concentrations of hemoglobin that are less opaque but quickly exceed the measurement capabilities of the USB4000 as concentration increases. It is for this reason that our critical region does not extend into the hemoglobin peak; we can obtain spectra from samples with saturated hemoglobin peak spectra while maintaining good data inside the critical region.

B. Data Collection

When collecting a spectral analysis of a sample there are three important data pieces that must be acquired. These three components are light reference (L), dark reference (D), and sample spectra (S) for all wavelengths of interest. The light reference is a measure of how many photons were “shot” at the sample during the time that the detector shutter is open and collecting. The dark reference is a measure of how many photons were falsely registered by the detector during the same period of time. The sample spectra represents how many photons successfully penetrated through the sample. In order to get the “True” sample spectra and light spectra we take the acquired value registered by the detector and subtract the dark reference value from each. Because the dark noise varies by virtue of random interference, we take multiple readings and average them. This also gives us a statistical measure of variance and standard deviation of dark noise (which is the primary source of variation in optical measurements) to give an idea of sample error. With the use of averaging and statistical analysis we are able to obtain, with error measurements, absorption calculations with data very close to the noise floor. The absorption of a sample is given as:

\[
\text{Absorbance}_i = -\log_{10} \left( \frac{S_i - D_i}{L_i - D_i} \right) \text{ for every } i \in \text{Wavelengths}
\]

C. Typical CSF Spectra

Figure 2 shows a set of typical absorbance curves for varying concentrations of bilirubin mixed into 0.1 g/dL of hemoglobin. The shaded band in the spectra represents that critical region. We can see the effect on the spectra through the critical region as the concentration of bilirubin increases. Further, at this “less opaque” concentration of hemoglobin we are able to see the entire hemoglobin absorption peak from 370-430nm. Notice that as the concentration of bilirubin increases, the total absorbance near the hemoglobin peak exceeds the measurement capabilities of the spectrometer and the spectra becomes saturated and noisy. We can already see hints of noise in the top-most spectral curve as the higher concentrations of bilirubin increase the absorption amount (in Optical Density, which is essentially a unit-less log10 ratio of how many photons were absorbed).

IV. PLSR BASED ALGORITHM DEVELOPMENT

As suggested in the previous section, our signal processing algorithm is built from various samples of bilirubin prepared with concentrations appropriate for diagnosis of SAH. In addition, various biologically relevant levels of hemoglobin have been added to make the sample set and subsequent model more robust in dealing with variation in human hemoglobin. Our critical wavelength region for determining bilirubin concentrations lies between 440 nm and 510 nm. This range allows us to avoid device saturation for higher concentrations of hemoglobin which peaks slightly below our range around 410 nm. The upper boundary at 510 nm, prevents modeling of unpredictable variance in spectra of different hemoglobin species (e.g. oxy-, deoxy-, carboxy-hemoglobin, etc.).

The algorithm used to build a mathematical model for extracting bilirubin and hemoglobin concentrations from spectra is a partial least squares regression (PLSR). The device uses this model to then quantify bilirubin from a spectra with unknown concentrations. PLSR is a form of regression that is similar to principle component regression in that it transforms the data into...
a set of descriptor vectors (concentrations) that explain the maximum variance of the predictor variables (spectra) of a dataset. It differs however in that it also takes into account the covariance of the predictor variables and the observed variables (concentrations). This has the effect of more accurately weighting concentration changes in respect to observed spectra changes. The goal of a PLSR model is to find the latent variable space projection of the predictor variables (spectra) that maximizes the variance of the descriptor variables (concentrations).

A sample PLSR decomposition is displayed in figure 3. The wavelength range exceeds our device range in order to better visually illustrate the effects of PLSR’s ability to extract sample component spectra. In the prototype device we use a much shorter wavelength range in which PLSR’s modeling works better for a wider range of concentrations and hemoglobin variability. From figure 3 we can see that PLSR is able to extract the two primary variants in our dataset, hemoglobin and bilirubin spectra.

The general equation for evaluating a PLSR model is:

\[
X = TP^T
\]

where T is the score matrix corresponding to the impact of each component of P in the loading matrix. Implementation of this expression in the prototype device has the advantage that the evaluation consists only of a few small matrix multiplies. While the more complicated regression required to generate the loading matrix can be done off-line, thus limiting the requirements of the on-board processing system. Furthermore the data requirement consists of only the T and P matrices which are respectively a square degree matrix (5), and wavelength range(300) by degree(5).

V. CURRENT RESULTS AND ONGOING WORK

To date we have used the prototype device to collect and analyze ~300 CSF samples. The “control” CSF used in these experiments has been collected from various human subjects. All samples are de-identified and then stored as frozen CSF in an archive that is maintained by the University of Cincinnati with support from the Point-of-Care Center for Emerging Neuro Technologies (POC-CENT) [8].

A variety of algorithm assessment experiments have been run using the spectra from the samples collected to date. In these experiments a portion of the collected spectra are randomly selected as the “training set” used to generate the loading matrix described in the previous section. This loading matrix is then used against the remaining spectra (those not used to generate the loading matrix) to evaluate effectiveness of the PLSR model in quantifying bilirubin and hemoglobin from the “spectra under test”. Preliminary analysis of these experiments suggests that this approach can be used to quantify bilirubin to less than approximately ±20% relative accuracy over a bilirubin concentration range from 0.2-8.0 mg/dL while in the presence of hemoglobin in a concentration range from 0.7 g/dL.

Work is currently on going to build a larger database of CSF absorbance spectra with concentration targets that are more appropriately distributed over the readable range for the prototype device. It is anticipated that this larger database will enable the generation of a much more accurate loading matrix that will be capable of producing more accurate bilirubin quantification over a larger portion of the spectrometers usable range.

Once the “population” of this larger database is completed in spring of 2010, testing will be initiated to evaluate the quantification accuracy and detectability limits of the prototype device. Results from these performance evaluation experiments will be reported upon completion of evaluation testing of the prototype device.

REFERENCES


[8] Information regarding the Point-of-Care Center for Emerging Neuro Technologies can be found at http://www.ece.uc.edu/POC-CENT.