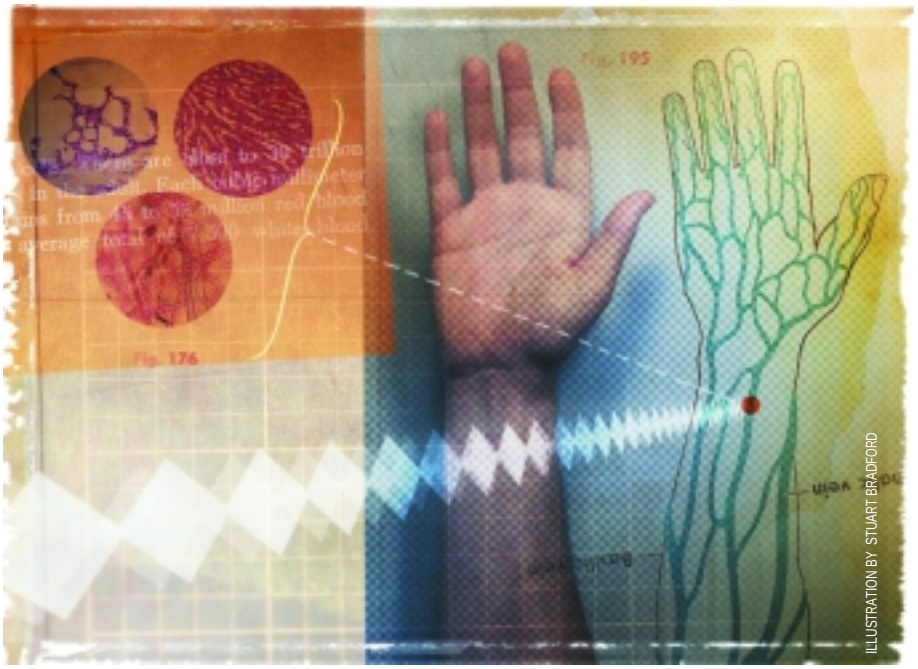
NIR spectroscopy allows doctors to monitor substances in blood noninvasively.



Getting under the Skin

By Russell Abbink and Craig Gardner, InLight Solutions Inc.

Given the choice, most of us would prefer noninvasive testing to having our blood drawn. Noninvasive testing eliminates painful needle sticks, blood loss, and the handling of infectious blood waste. Noninvasive measurements enable continuous monitoring that allows clinicians to speed up therapeutic response times. Yet the technical challenges associated with most noninvasive measurements are significant. Skin is chemically and morphologically very complex. For use in hospitals and doctors' offices, the instruments must be stable, compact, and inexpensive. Using near infrared (NIR) spectroscopy, our company is developing systems that measure blood constituents without pricking the skin.

Two noninvasive applications have successfully overcome the technical challenges of noninvasive measurements and achieved widespread use in medical practice: pulse oximetry, which measures oxygen saturation in a patient's blood, and bilirubinometry, which measures the bile pigment bilirubin to check liver health.[1,2] In both cases, the substances being measured have large absorption bands in the visible spectrum, which makes these applications ideally suited to noninvasive absorption spectroscopy. But there are many clinically important analytes such as glucose and ethanol that do not have absorption features in the visible spectrum. Fortunately, they have distinct IR absorbances.

In general, we've found that the NIR region of the spectrum best balances the need for absorption band strength and light penetration depth necessary to measure small analyte changes. Each blood analyte has an absorption spectrum whose magnitude is proportional to concentration. In the 2- to 2.4-μm wavelength range, several analytes of interest show vibrational absorption spectra quite distinct from the major absorbers in skin tissue. Common analytes include urea, for monitoring dialysis; glucose, for monitoring blood sugar; and ethanol, for measuring blood alcohol levels. Each analyte has a unique spectral signature that allows development of a spectroscopic calibration that relates changes in the absorbance spectra (ΔA) to changes in analyte concentration (Δc) based upon Beer's law:

$$\Delta A(\lambda) = \Delta c \varepsilon(\lambda) l$$

Here, ε is the absorptivity spectrum of an analyte and l is the path length of light through the medium. Using

IR spectroscopy to measure these analytes in chemically complex blood-serum samples in vitro produces clinically relevant accuracy.

Translating these in-vitro measurements to noninvasive in-vivo measurements requires us to overcome several obstacles. First, skin scatters light strongly due to differences in the refractive index between formed elements (such as collagen) and their surroundings. The net result of scattering is that light travels a distribution of path lengths through the tissue, making direct application of Beer's law unsuitable. Still, measuring analytes in simplified scattering solutions or "tissue phantoms" produces clinically relevant results.

A second obstacle in real tissue is the fact that the distribution of analytes is not uniform. Glucose, collagen, and water, for example, have different local concentrations within blood vessels, interstitial fluid, and skin layers. Producing quantitative measurements in samples with both variable light path-length distributions and absorber inhomogeneities is a complex problem. Absorber variations also contribute to disparities between the NIR results and the gold-standard blood reference measurements taken by an in-vitro clinical chemistry analyzer.

a complex strategy

Clinically relevant measurement of small analyte concentrations in tissue requires a multi-pronged technical strategy. First, instrument designers must address the geometry of light delivery to and from the tissue, and accurately capture the spectral response. Second, developers need to minimize the effects of scatter on the quantitative absorption measurement by applying algorithms to the raw spectral data. Finally, analytical chemists have to model the inherent complexities of the sample using cutting-edge calibration methods.

Several signal characteristics bear heavily on instrument design. Between 1.3 and 2.5 µm, for example, most of the analytes of interest display distinguishing absorption features that do not suffer excessive water attenuation. In this spectral region, scattering and absorption in the tissue limit the usable optical path lengths to a few millimeters or less, making reflection measurements, or backscattering, more effective than transmission measurements. Calibration schemes must either avoid or carefully account for changes in path-length distribution, which can be introduced by physiological changes in tissue scattering properties or by changes in the way the instrument launches and collects light from the tissue.

Another issue is that the spectral absorbance features of many compounds of interest overlap with the absorbance spectra of other interfering materials. Glass and ambient air, which are visibly transparent, can contain significant infrared absorbers. Given all these issues, we need to measure a full spectrum covering hundreds of points—as compared to the wavelength pairs used in blood oximetry—to separate and quantify the spectral signature of a particular analyte.

We have developed a noninvasive measurement system that performs tissue sampling on the forearm (see figure 1). The illumination system contains an incandescent lamp inside an integrating sphere. This provides a highly stable angular and spatial light distribution that does not change as the system components age. Bundles of high-numerical-aperture fused silica optical fibers transfer light to and from the tissue. At the sampler head, the fibers are arranged in groups containing both illumination and collection fibers to allow light from several illumination fibers to reach each collection fiber after traveling through the tissue. To restrict collection to light that has traveled through the analyte-bearing region of tissue, as opposed to just across superficial layers, the design maintains a minimum separation distance of about 0.5 mm, edge-to-edge between each collection fiber and the nearest illumination fiber. The fiber groups are spread out over an area of a few square centimeters to average over tissue inhomogeneity.

The next element in the signal chain is the spectrometer. We

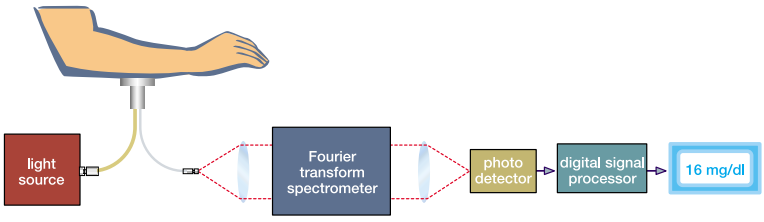


Figure 1 Noninvasive measuring system for the forearm consists of a light-source fiber coupled to the measurement head, a tissue sampler, an interferometer, an InGaAs photodetector, processing components, and results.

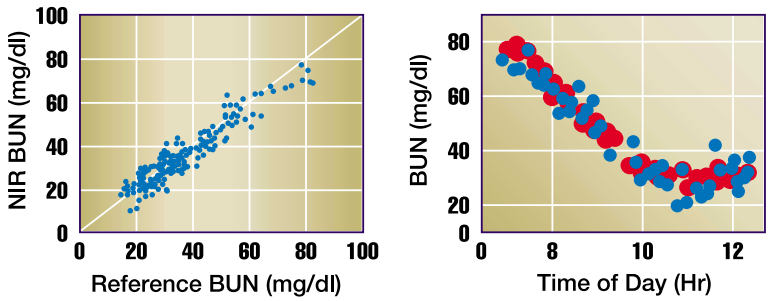


Figure 2 Tests of the instrument on a forearm yield well-behaved data (left). Data captured during dialysis treatment (right, blue) compares well to reference data (right, red).

chose a Fourier-transform interferometric spectrometer (FTS), partly because of its ability to provide higher throughput and better spectral resolution in a small physical package than other technologies, such as grating spectrometers. It also has excellent spectral stability directly derived from a stable reference laser that allows the system to meet the stringent demands of a multivariate calibration model highly sensitive to this parameter. A further advantage is that the FTS measures all wavelengths simultaneously with a single detector element. Because all the light falls on this single element, the output signal level is relatively high, providing excellent signal-to-noise ratio. We selected a thermoelectrically cooled indium gallium arsenide (InGaAs) element for its good specific detectivity (D^*) and excellent amplitude linearity over the large dynamic range required for the interferogram signal. Cost of this single element is considerably lower than that of the array detectors often used in grating spectrometers.

But the FTS poses a significant challenge to producing cost-effective consumer medical instruments. Expensive, laboratory-grade FTS instruments offer more versatility, higher spectral resolution, and larger spectral range than necessary for our measurement. To lower the cost, we built an application-specific FTS based on a Michelson interferometer configuration with retroreflectors. The result is a small, rugged, permanently aligned and desiccated component that contains the input and output optics, the IR detector, and the laser reference. Using center-burst modulation efficiency as a figure of merit, we achieve a thermal stability of around 0.2%/°C, compared to numbers as large as 6%/°C for some high-end commercial instruments. Such thermal stability is necessary to maintain calibration outside the well-controlled environment of a clinic.

A 24-bit delta-sigma analog-to-digital converter digitizes the signal. A digital signal processor then performs signal conditioning and Fourier transformation, identifies bad data, and computes the concentration of the desired analyte.

processing the data

We wanted to process raw spectra to make the data better suited to multivariate calibration, for example by minimizing the effects of scattering to make Beer's Law more applicable. Tissue optics researchers have developed numerous techniques for separating scattering from absorption.[3] They use instruments that measure back-scattered intensity of a pulsed source as a function of time, a modulated source as a function of frequency, or a CW source as a function of spatial or angular position on the sample surface. We find that the spatially resolved approach yields useful information about scattering for the lowest instrument cost.

There are numerous algorithms that process the data and separate absorption from scattering. One popular method is the diffusion approximation to light transport, but that is not applicable in most of the infrared region because scattering is not the dominant light interaction with skin. Monte Carlo simulation is a very flexible algorithm that traces rays through the medium according to its absorption and scattering properties. Using Monte Carlo simulation, we can model any combination of absorption, scattering, and measurement geometry. Because of the time such simulation takes, the method is typically used to generate an approximate, empirical relationship between the measured intensity and the underlying sample absorption.

Noninvasive medical instruments require two kinds of calibration. The first is the empirical development of a multivariate model, which mathematically extracts the response of the instrument to the analyte while teaching it to ignore variation in other analytes or sources of interference. The second is the individual instrument normalization, enabling the instrument to use a standardized multivariate calibration model.

Multivariate calibration and estimation is the process by which we convert a measured spectrum to an analyte concentration c. Mathematically, this often means multiplying the processed spectrum A by a vector of calibration coefficients b and summing the result over all wavelengths i:

$$c = \sum_i A_i b_i + \text{constant}$$

This equation holds true for linear calibration methods such as partial least-squares regression. For nonlinear methods (such as those involving artificial neural networks), we replace the above summation with some function of the processed spectrum and the calibration coefficients.

We must determine and fix the vector of calibration coefficients beforehand using a calibration experiment in which spectra are collected for varying amounts of analyte concentration. The design of the study is very important in developing a robust calibration. Users must take care to eliminate any correlations between the analyte concentration and other sources of variation.[4] Multivariate calibration methods model complex systems quite well, and can account for nonlinearities between spectral and concentration changes that occur in tissue due to scattering. Many multivariate algorithms exist, and we recommend trying several.[5]

We measured urea noninvasively in four subjects and found good correlation between the NIR and reference methods, with an accuracy sufficient for monitoring the dialysis procedure in real time (see figure 2 on page 20). We've shown that NIR spectroscopy can measure blood chemistry noninvasively and produce medically relevant results. Making it work requires close attention to system design, light propagation through skin, and multivariate modeling methods. With continued progress, portable, noninvasive systems to measure blood constituents will become common in hospitals, clinics, and homes. **oe**

Russell Abbink is a senior engineer and Craig Gardner is a senior research scientist at InLight Solutions Inc., Albuquerque, NM. Phone: 505-272-7408; fax: 505-272-7021; e-mail: russell.abbink@inlightsolutions.com or craig.gardner@inlightsolutions.com.

Acknowledgements

The authors wish to gratefully acknowledge Amy Gardner, William Radigan, Stephen Vanslyke, Art Jacobs, and Jim McNally for their technical and editorial contributions.

References

1. A. Jubran, Crit. Care 3, R11-R17 (1999).
2. A. Robertson, et al., J. Perinatol. 22 [1], 12-14 (2002).
3. F. Bevilacqua et al., Appl. Opt. 38 [22], 4939-4950 (1999).
4. M. Arnold, Anal. Chem. 70, 1773-1781 (1998).
5. S. Brown, Appl. Spec. 49 [12], 14A-29A (1995).