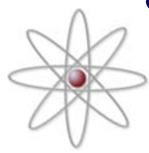

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# Photonic Molecular Probe™

# FAQ and Responses

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## 1. Provide a description of the principle of operation of the Photonic Molecular Probe (PMP).

While initial investigations are with transmission measurements of Optical Rotatory Power (OR) (also known as ORD, where D is for dispersion indicating it is a frequency dependent phenomenon) and Circular Dichroism (CD), reflection measurements utilizing OR and CD are equally viable options/complements.

As early as 1811, Jean Baptist Biot discovered OR in turpentine. Arago, Fresnel, Herschel, Pasteur, Le Bel, van't Hoff and Boltzmann. Faraday in 1864 discovered magneto-optical activity. Lord Kelvin in 1884 in his Baltimore lectures introduced the term *chiral*. André Cotton discovered CD in 1896. Collectively ORD and CD are known as the Cotton Effect. The history, theory and experiments of both phenomena can be found in many texts[1] and papers in the literature.

In the 20th century, great fundamental theoretical advancements in the undying physics were made including the quantum mechanical descriptions of these phenomena. However, only until recently with the rapid explosion of electronic and optical high technologies have scientists been able to exploit the richness of ORD and CD information[2].

Consider first the creation of monochromatic elliptically polarized light by reflection from a planar, or slightly curved, surface. Unless incident at the Brewster angle the light will be reflected with an elliptical polarization. The orientation and ellipticity of the ellipse is a function of the wavelength and incident angle. Repeat the same procedure N-times with N-different wavelengths to obtain N-elliptical "basis states". Each polarization ellipse will respond differently upon traversing an optically active molecular sample. Each polarization ellipse will carry its own information about the structure of the molecule upon exiting the sample. The collection of this information is fundamentally much richer than that which would be obtained with linear absorption spectroscopy using the same wavelengths.

An extension of this idea is to superimpose all the different frequency ellipses in one beam. Reflecting a polychromatic beam of light from a planar, or slightly curved, surface can do this. The result is a beam of partially polarized polychromatic light. The "spikes" are, in fact, the remnants of each polarization ellipse describe above.

While there is no complete quantitative theory of optical rotatory power and circular dichroism employing partially polarized polychromatic radiation interacting with matter, the phenomena have been known for over two hundred years and extensive theoretical work on monochromatic radiation has been done over the last seventy years. The basis for at least a qualitative understanding of chromatic polarization is well established. Thus, the device is essentially empirically based and a comprehensive, quantitative "principles of operation" statement at this time is not possible, which is fertile ground for several theoretical Ph.D. theses.

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[1] See references 1-3.
[2] This is evidenced by Biotools' (http://www.btools.com/chiralir.htm) commercialization of a device called the Chiral*ir* in 1997, which identifies different enantiomers.

**2. Provide a brief definition of some terms that are used in describing the principle of operation of the Photonic Molecular Probe (PMP).**

**Chiral:** Referring to the handedness, or helicity, of an asymmetric molecule.

**Optical Rotatory Power:** Rotation of polarized light upon traversing a chiral sample. Classically this effect is due to a phase shift of the two opposite circularly polarized beams making up the basis states of the polarized light[3].

**Circular Dichroism:** The change of ellipticity of elliptically polarized light upon traversing a chiral sample. Classically this effect is due to the differential absorption of the two opposite circularly polarized beams making up the basis states of the elliptically polarized light[3].

**Quantum Mechanical Explanation of Optical Rotatory Power and Circular Dichroism:** The asymmetry of chiral molecules allows for the coupling of the electric and magnetic dipole moments[1, 4]. This in turn is responsible for the rotational and distorting properties of polarized light traversing a chiral sample[5].

**Optical Activity:** A molecule or crystal that exerts some form of ORD and CD on polarized light is said to be optically active. Chiral substances are optically active.

**3. The PMP's primary path appears to be the same construct as a conventional polarimeter. How does it differ from a conventional polarimeter, if indeed it does?**

Conventional polarimetry dictates the dual use of a polarizer/analyzer pair. Indeed, the specific state of the polarization emerging from the initial polarizer into the sample is "plane polarization." Conventional use would be to characterize the structure of the sample by observing optical rotation of the plane-polarized light as a function of individual frequencies. The polarizer conventionally used is a strong function of frequency and of high quality assuring that near exact plane-polarized light is emergent. However, the emergent beam from the initial polarizer in the PMP is "segmented partially polarized polychromatic light" not plane polarized light. The implication is subtle but very significant to the operation of the PMP.

Using a not so high quality polarizer[6], wherein a small range of unaltered partially polarized polychromatic light traverses the polarizer and emerge as segmented partially polarized polychromatic light. There are projections of all other wavelengths but this is more or less noise on the prepared segmented signal. The importance of this procedure lies in the fact that the incident beam is made up of partially polarized light whose frequency dependence is a function of angular displacement about the propagation direction of the beam. This is due to how the light is initially polarized. Given this angular dependence a data acquisition and signal processing scheme has been developed.

[3] See Appendix A in US Patent Nos. 5,871,442 and 6,236,870.
[4] See Appendix B in US Patent Nos. 5,871,442 and 6,236,870.
[5] Linear/achiral absorption involves only the square of the electric dipole moment.
[6] A "leaky polarizer" is perhaps better termed a "segmenter".

Succinctly, the use of a "segmenter" to allow a small range of unaltered partially polarized polychromatic light to traverse the polazier enables the PMP to engage the physical mechanism of circular dichroism as well as optical rotation, which is not possible by use of a conventional polarizer.

**4. How does the instrumentation sketched in the Patents[7] identify and measure the specific dichroic properties of the target molecule?**

The PMP as sketched in Figure 4 of the patents[7] measures: ORD and CD, which correspond to a particular single frequency polarization ellipse; and, the collective effects of ORD and CD for multiple frequencies in a selected bandwidth of the partially polarized polychromatic light[8] (See Q&A 3).

A polarimeter-like setup characterizes the light before it enters the sample and characterizes the light after exiting the sample. By carefully constructing the input polarized beam to include the range(s) of frequencies (be it a polychromatic beam or a series of single frequency elliptical basis states), which interact strongly with the target molecule a complete picture can be obtained. By inputting partially polarized polychromatic light, stepping the first polarizer, spinning the analyzer and synchronizing both to the readout electronics the PMP essentially is looking at frequency segments and their relative changes do to ORD and CD.

If linear absorption were the measurement, there would be one data point per frequency—L frequencies, L data points. Now, if just elliptically polarized light of a single frequency is used and the initial polarizer characterizes the input beam with M rotational increments and after traversing the sample the analyzer characterizes the exit beam with N rotational increments there would be a total of N x M data points. If L frequencies were used, then there would be a total of L x M x N data points. The advantage in the volume of data collected over a system using linear absorption is obvious[9]. The selection of appropriate bandwidths and the data correlation between the polarizer and analyzer and the signal processing are company proprietary and are the subject matter of various pending patents.

**5. Claims have been made about the PMP's accuracy in sensing glucose concentration. On what basis are these claims made? Have studies/tests been performed to establish this? If not, please explain how this likely range was extrapolated or surmised. Over what range of concentrations is this accuracy likely to prevail? You have already observed that other non-invasive technologies/products suffer grave limitations relative to accuracy – why is the PMP likely to be better?**

The claims referred to are based on preliminary experimental results and deductive estimation.

Routinely in the literature *in vitro* experiments of one type of chiral substance report ORD and CD measurements to within ± a few percent of the known concentration. For mixed species of chiral substances, the accuracy of determining the corresponding concentrations depends critically on the sophistication of the signal processing used. Under the best conditions the numbers reported are

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[7] US Patent Nos. 5,871,442 and 6,236,870.
[8] See Question & Answer number 3 above.
[9] In addition, to this add the quality of the data, i.e. ORD plus CD versus linear absorption.

within about ± 5%. It is our contention that the signal processing that we will employ (See Q&A 7), which has not previously been employed in these types of measurements, will greatly increase the accuracy of our extracted measured results as it has done in other fields. Given this and the fact that we have made *in vitro* measurements on physiological levels of glucose to within ± 5% with a severely limited proof of principle device leads us to believe that measurements made *in vivo* can be done to within ±5%.

Typically, when linear absorption measurements give correct results, *in vitro* measurements range from ±4 – ±7%, and i*n vivo* from ±6 – ±12% in relative error[10].

Current invasive measurements by home diagnostic devices in which blood is drawn, put on a paper tab and inserted into a scanner can vary as much as ± 15% and are still considered accurate and medically acceptable.

Compared to an accepted laboratory reference method for serum glucose, the PMP should yield results meeting the following objectives:

| **Glucose Range (mg/dL)** | **Error Criterion** | **Percentage of Results Meeting Criteria** |
|---|---|---|
| ≤ 25 | NMT +15 mg/dL<br>NMT -25 mg/dL | 99% values < 40 mg/dL |
| 25 to < 60 | NMT ±15 mg/dL | 95% |
| 60 | NMT -20 mg/dL<br>NMT +9 mg/dL | 99% values > 40 mg/dL<br>95% |
| >60 to < 500 | Ideal NMT ± 10%<br>Acceptable NMT ± 15% | 95%<br>95% |
| 500 | Ideal NMT ±10%<br>Acceptable NMT ±15% | 95%<br>95% |
| 600 | NMT -15%<br>Display HIGH code if > 500 mg/dL | 99%<br>99% |

Notes: NMT means No More Than
Below computed values of 25 mg/dL, instrument will display LOW
Above computer values of 500 mg/dL, instrument will display HIGH

These limits can be met. The PMP is more sensitive than current technologies using linear absorption spectra and is much richer in information content. Looking at the academic literature, and some recent industrial devices, to see the advances being made in molecular structural diagnostics/characterization with new technologies employing various forms of ORD and CD can be clearly seen over past optical techniques.

**6. Is the Signal to Noise Ratio of the PMP's detected signal adequate to yield high accuracy at low blood concentration levels?**

[10] See for example, reference 4.

We believe so, even though we have estimated S/N ratios for some of our measurements that appear to be very good the information needed to do an accurate calculation/measurement is not available with the present proof-of-principle, semi operational device. Far too many assumptions must be made about the noise in the optics and electronics to obtain a high confidence number. This can only be done when a fully operational breadboard device that has been appropriately optimized for the measurement at hand is built. That is, it must be part of the R&D program.

In addition, it may be that S/N will have to be redefined, as it must be for new superlattice IR detectors, to be a meaningful figure of merit. This is because the operational concept of the PMP device is very unlike the more traditional optical devices such as lens/mirror systems, spectrometers and interferometers, there is not any one single, scalar, S/N number which can characterized the device as operational or not. First, one has to define what is meant by a signal. The device is polychromatic. Data sets are collected by setting the polarizer and stepping the analyzer and the process is repeated for various polarizer angles, with perhaps several narrow bandpass filters being used. Then there are several possibilities for combining the data sets to track the chromatic information in and out. In the least, one winds up with sets of second rank tensors whose elements are individual S/N numbers for whatever particular chromatic information that it may correspond to.

No one number, or a series of numbers, representing the best of all will give the operational capabilities of the devices. In a very real sense the sum of the parts is greater that the whole. The PMP uses all the information, good and not so good in order to arrive at the desired result.

The S/N ratio can be defined using the magnitude of the total integrated signal through water with no glucose and estimate the fluctuation about that signal level. The fluctuation is the noise for a 0 signal that can be expressed as % of signal or if the zero glucose signal is also 0, then as a +/- fluctuation in au (arbitrary units). Then give what the signal for a 100 mg/dL level is for comparison. And this may indeed be the way to obtain a meaningful S/N ratio. However, it will depend on how the individual data is acquired, the input/output signals combined and processed and that is still to be determined.

**7. What are the exact features of the "secondary peaks" that represent glucose concentration…their amplitudes? With what precision will these peaks have to be measured to achieve what precision in glucose concentration? If multiple secondary peaks occur, which will be used to compute glucose concentration?**

The secondary peaks are indeed there and they are real. They contain an enormous amount of structural information about the target molecule. However, to try to capture each peak is not a very efficient way of obtaining information about the target, which in some sense is analogous to looking at a single system in an ensemble. What the PMP does, with a few clever "tricks," is to look at an average of all peaks in a small selected frequency bandwidth—this would be the ensemble. One way to accomplish this is by using a "leaky" polarizer (See Question & Answer 3). Then each increment of the polarizer allows a small band of wavelengths through having the average properties of all frequency spikes. The resolution can be controlled by the quality of the polarizer and the size of the rotational increments in which it is possible to overlap frequency intervals[11]. Here, a fused silica

[11] A somewhat analogous setup can be found in the literature, see reference 5.

plate compressed to create relative phase retardance between the fast and slow axes is placed after the initial polarizer. The strained plate creates highly eccentric elliptically polarized light, which is transmitted through the sample onto an analyzer. Interestingly, the experiment is done with all three optical components fixed in a preset position.)

**8. What difficulty do you anticipate having in discriminating against overlapping responses from other substances?**

The direct answer is very little, as the PMP is designed to extract signature information from the frequency content of the signal. While amplitude or intensity information is present, it is not the last word. Relying only on the intensity information would undoubtedly confuse the signals of various substances, especially of those having similar achiral linear absorption or transmittance in a particular spectral range. As a result, linear absorption devices presently being developed rely very heavily of signal processing techniques to identify the glucose signature spectrum. In fact, signal processing techniques used with the linear absorption devices appear to be a very problematic area.

Separation of signature information due to other chiral substances, which may be present, such as saccharides or proteins is not important. What is important is the separation/identification of the glucose signature from all the other signals. That is accomplished by multiple frequencies or polychromatic light (see Question & Answer 1 & 3) along with clever data collection and reduction techniques, ala the PMP, possible wavelet transforms and sophisticated statistical analysis.

The specific wavelet construct to be used in the signal processing will depend on the target molecule and the data acquisition. Nevertheless, a general approach to the PMP signal processing/discrimination has been developed, which is based on a conditional probabilistic reduction method known as Bayes' Rule. Using a Bayesian based approach allows the incorporation of very precise physical models directly into the method. For example, atomic form factors, which characterize the electronic structure of various target molecules can be used for cellular image enhancement. Alternatively, "structure factors" (analogues to those used in x-ray diffraction) can characterize the signature of various concentrations of target molecules in solution. The physical models can be all empirical, all theoretical, or a combination of both. The Bayesian method for discrimination/image enhancement is not generally well known but has been employed very successfully in satellites and heat seeker missiles[12]. The Bayesian method has not been previously used in the physiological arena. The unique approach to the PMP data collection and signal processing is the subject matter of pending patents.

**9. What other substances in human tissue will also produce a signature in the PMP? How do you know? Have tests already been performed? With fat, water, or protein? What about enzymes?**

Any optically active chiral substance should produce ORD and CD spectra and hence is should be measurable by the PMP. Certainly, this includes biological molecules such as proteins, peptides, and DNA. The discrimination is a matter of collecting *in vitro* laboratory data under various circumstances, building a database and structural models ala the Bayesian Method and programming

[12] See references 6 and 7.

it into the onboard electronics. No *in vivo* tests have been made yet (we do not have the resources and that is why we are looking for a partner). However, it is not necessary to look far in the literature to uncover ORD, CD, and variants of, *in vitro* characterization measurements of literally thousands of substances. Within the last ten years, there has been an virtual explosion in the field. ORD, CD, and variants of, are now common tools for biochemists, geneticists, the pharmaceutical and medical instrumentation industries (for example, BioTools[2] and Beckman Instruments).

Molecules, which are not chiral, such as ethanol, can be detected by the PMP, by resonantly RF tweaking the molecule to induce a pseudo-chirality.

**10. How will the instrument be calibrated, i.e., how will the effects of varying finger thicknesses, color, structure, and contamination be accounted for? Do you anticipate that the PMP will require specific calibration per person?**

**Glucose Calibration:** The secondary path allows for a real-time comparison of known to unknown concentrations of the target specimen. In other words, placing a calibration sample in the path and coding the appropriate software into the PMP, allows for the possibility of self-calibration, which is essential for a diagnostic instrument. In addition, the secondary path is a reference path. It is made up of a 1/4 wave plate, a reflecting mirror, and an incremental stepwise rotating polarizer. The quarter wave plate is used in the reference beam path to control the ellipticity of the beam and also may be used for interference alignment if coherent light is used.

**Finger Thickness/Structure:** From about 760 to 1200 nm the absorbance due to water is about one to two orders of magnitude smaller than that of pure glucose, with some stronger water interactions at about 900 nm in tissue. The "therapeutic window" is centered around 1.0 micron. Absorbance due to water is still only about 5 – 10% of the total light directed to a surface of tissue, such as the finger, in this NIR range, and, indeed, this effect may become the calibration means. In fact, this is the approach that reference 8 uses in making oximetry measurements through a human finger.

**Color:** Should have no effect on calibration. It may cause some unwanted absorption and reflection, but again preliminary testing indicates this is not be a problem.

**Contamination:** Certainly whatever part of the body, which the measurement is being made on should ideally be as clean as possible and indeed it may be good practice to cleanse that part before the measurement is made. However, under normal circumstances the polarized IR light should not be disrupted from adequate transmission by small amounts of grease or dirt (see Question & Answer 10).

**Specific calibration per person:** If the studies with the reference channel show that a properly designed reference sample can compensate for finger or tissue effects we don't presently know about, then no specific calibration may be needed. Second if the analytic glucose in solution studies show that the machine calibration is valid for tissue, we are home free. Worst case would be an infrequent calibration against an invasive method.

**Issues in general:** Blood glucose vs. interstitial fluid glucose. Recent research corroborates the fact that the intracellular and interstitial concentrations equilibrate with extreme rapidity, especially for

glucose, so the precise ratio of these two compartments traversed by the beam is irrelevant. This is true for the upper part of the body or where blood perfusion is rapid: eyes, ear, tympanic membrane, forearm, fingers and hands. The delay is still on the order of 4 min lag behind on increasing glucose and 4 to 8 min behind when the glucose level is falling. The latter is very useful to give extra time to detect falling glucose levels before they become critical – the under the skin area is emptied first.

Calibration begins with developing a minimal signature in vitro: e.g. glucose in distilled water. Various contaminants may be added to that *in vitro* experiment to develop the signature further and ensure correlation with concentration of only glucose. After that *in vivo* runs are done and compared to known accepted assay techniques. Again, the signature is further developed (lengthened) if necessary until satisfactory clinical correlation is achieved.

**11. Scattering effects introduce errors in knowledge of the path length over which concentration is sensed. Are scattering effects significant with the PMP? If so, how will they be accounted for?**

Diffusive scattering tends to depolarize the light, but does not completely depolarize the beam and information is still carried[13]. This reduces the intensity of the polarized light in the forward direction where the detection system is. However, the degrees of ORD and CD are not intensity dependent. Whatever polarized light is left responds to a chiral medium in the same way as it did before its intensity was reduced. If polarized light of intensity I rotates 6 degrees then light of I/2 will also rotate 6 degrees. The issue is simply will enough polarized light get through in order to make meaningful measurements, and the answer is believed to be yes.

Preliminary experiments with human blood and through the finger also gave encouraging results, although these tests were severely limited by the apparatus and resources available. Significantly, enough of the polarization character of the input signal was maintained to yield positive results. In addition, polarization experiments with the PMP where successfully conducted through a sample containing 20% whole milk, i.e. through fat globules, a very highly diffuse scattering medium.

The propagation of polarized light through a solution of D-glucose in which latex microspheres are in suspension has been demonstrated, wherein the microspheres act as a diffuse scattering mechanism (bone and turbidity for example) tending to depolarize the light[14]. Even when the density of microspheres was great enough to block transmission of the visible light large optical rotations were observed.

Other researchers have reached the same conclusions that we have concerning the use of optical rotatory power and circular dichroism to investigate a chiral target specimen within a diffusive and highly scattering medium:

"There has been much research into the use of polarization data to characterize highly scattering turbid materials such a biological tissues, turbulent flows, and fog. This has resulted in recent discoveries of correlation and order-like effects in the light scattering from random heterogeneous

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[13] See references 9, 10 and 11.
[14] See reference 9.

media traditionally believed to completely "scramble" the polarization state, coherence, and direction of the incoming beam. The fact that multiply scattered photons exhibit partial polarization preservation suggests a novel way to probe the characteristics of optically dense scattering media."[15]

And,

"For example, it has been noted that circularly polarized light (note added—a special case of elliptical polarization used in the PMP) preserves it's polarization better than linearly polarized light (note added—used in all present attempts at in vivo glucose monitoring that we are aware of, except for the PMP) in a medium composed of anisotropic (forward-directed) Mie scatterers. This type of scattering is particularly relevant in biomedical optics, as it applies to mammalian tissues in the red to near-IR part of the electromagnetic spectrum."[16]

In order to overcome the strong scattering nature of tissues, a commonly used measure is to use red or near infrared light to take advantage of the low scattering and hence higher tissue penetration at these wavelengths. Tomographic imaging of a human finger using two near IR wavelengths, 715 and 1064 nm has been done. The results were compared to T1 and T2 MRI imaging and x-ray CT imaging. The 1064 nm compared most favorably with the T1 MRI image.[17]

Additionally, the backscatter signal can be used as a source of redundant or additional information. In fact, ORD and CD can be exploited in reflection spectroscopy[7] as well as transmission measurements. Right now we look at the transmitted signal. One possible use of the backscattered signal would be a determination of path length, should that ever be desirable or necessary.

## 12. How significant are temperature variations in the subject's finger or environment to the accuracy of the PMP? Have you tested in this area yet?

Small variations in the temperature of the body part in which the measurement is being made will have no measurable effect. A change of temperature from 98 F to 102 F is approximately 2 x $10^{-4}$eV. This is hardly enough to affect the vibronic states—it certainly will not affect the pure electronic states, which have energy on the order of eV.

As far as the effects of temperature variation on the device go, the specifications have yet to be worked out. However, it is assumed that the device will be used within "reasonable limits" of room temperature.

[15] See reference 10.
[16] See reference 11.
[17] See reference 12.

**13. Heterodyne detection techniques are discussed in the patents[7]. Will heterodyne methods be used in the ultimate product? If so, why are they required?**

Heterodyning is not needed for the proposed measurements of *in vivo* blood glucose. This is an optional/complimentary mode of operation that has been experimented with. However, it is specifically disclosed in the patents[7] in order for the patent to be as broad as possible. In general, topics discussed but not claimed in the patent have been extended and are claimed in various pending continuation and continuation-in-part patents.

**14. Please give some details about the instrument you used to make measurements, including size, cost, needed modifications/improvements for clinical trials and projected cost of a marketable device.**

The measurements were made with a $250 US proof-of-principle device constructed to sit on an 8 by 8 inch platform—i.e. it is almost miniaturized.

The PMP is a new and unique optical device. The efficient optimization and systems integration of all its optical and electronic components will require the expertise and experience of specialists working in the field. MetroLaser (http://www.metrolaserinc.com), a recognized as a leader in Optics R&D, in Irvine, California, has been identified to take the lead on the device development. The proposed strategy is for MetroLaser to take the lead on the building and testing of the device including the optical systems analysis, optimization of optical components, etc. International Diagnostic Technologies' (http://www.idtscience.com) technical team will work with MetroLaser acting as consultants in the areas of device modification, data acquisition, signal processing, construction of physiological models (theoretically and empirically), preparing samples, building a sample library, pre-clinical and clinical studies (including setting up an independent clinical review board). The full R&D program, including pre-clinical studies, is estimated to be approximately 14 months.

The projected retail cost will be less than $1000 US per portable unit, probably $500 to $700 US or less. Mildly invasive units (based on electrophoresis in which the skin is tweaked by a small electric current to release tissue fluid, not blood, from which they determine blood glucose levels, or a weak laser that vaporizes the upper tissue layer that is collected and then chemically analyzed) scheduled to be on the market in the near future have reported price tags of $1,500 to $2,000 US.

**15. To use the PMP device to measure and track the changes in glucose levels in a subject undergoing rapid changes up or down in glucose level, how can the device be configured?**

This answer requires breaking the question into several parts:

1. When the glucose level in a subject rapidly changes, where do these changes appear in the vascular and tissue structures?

2. What is the relationship between the changes monitored in selected tissues and the vascular glucose level in the body?

3. How can the PMP device be configured to monitor those changes?

Traditionally, glucose levels in the body are monitored, by taking either of two types of blood samples. One is from a venipuncture in the arm, and the other by taking a shallow lance, fingerstick on the hand. In the first case, the blood comes from a major vein in the arm, which carries a slightly depleted load of glucose since the arterial system has perfused the body with nutrients. The venous system carries out the wastes and the leftover nutrient supply. The fingerstick sample is from the capillary bed, which lies near the base of the epidermis layer of skin. The capillary blood exchanges nutrients with the interstitial fluid, which surrounds the tissue cells. In animals and humans, this capillary blood is the primary source of cellular nutrients.

In the typical human body, the upper parts of the torso are more rapidly perfused with blood than the lower parts. In general, the head, arms, hands, and chest at or above the heart level are well supplied with blood while the lower chest, abdomen, and legs are less rapidly perfused by blood. For clinical purposes, glucose levels in blood sampled from the arm or finger represent the best estimate of the blood glucose for the body.

When you cannot use a blood sample to assess glucose levels, what can you use and what is the relationship of the glucose level in that fluid to that of capillary or venous blood?

Much literature has been published in the last 10 years on the use of implanted glucose sensors, microdialysis or ultrafiltration sampling loops, and sampling interstitial fluid from various locations in animals or humans. Briefly, if the sampling point is in the rapidly perfused portion of the body and near a capillary bed of microvessels, the time it takes for capillary blood glucose to diffuse into the interstitial fluid is on the order of 4 to 8 minutes. Interstitial fluid samples from tissues such as the dermis layer (below the epidermis), or from the fatty tissues in the abdominal area have shown that the glucose exchange time lag is even longer.

There are several clinical situations for which blood glucose samples are taken, and the relationship to glucose in the interstitial fluid would be different according to the situation.

First, is the case of fasting glucose levels. Here the body is at a relatively static situation and the glucose levels in the blood and surrounding tissue interstitial fluid are at equilibrium. The glucose levels in the capillary blood and the interstitial fluid would be the same.

Second, there is the case of rapidly rising glucose levels such as during an oral glucose tolerance test in which a 75 g dose of glucose is rapidly ingested, or following a high caloric meal. The blood glucose level will rise rapidly as the body tries to absorb the glucose from the digestive tract. The nutrient exchange between the capillary blood and the immediately surrounding interstitial fluid is relatively fast while more distal interstitial fluid exchanges take much longer by diffusion. The best evidence is that a 4 to 8 minute time lag is typical for the glucose in the interstitial fluid in the epidermal layer to reach the same level as the capillary glucose level. This lag continues until the blood level glucose peaks and begins a relatively slow decline. The body stores the excess glucose temporarily in the interstitial fluid until the liver can process the glucose into other metabolites or eventually fat.

In the situation where the blood glucose levels are falling rapidly due to a high insulin dose, the reverse situation occurs. The blood glucose is depleted by passing through the liver and peripheral tissues while the reservoir of glucose in the skin epidermis is now back diffusing into the blood as the capillary blood glucose level falls. Again, the time difference is on the order of 4 to 8 minutes with the interstitial fluid level falling faster than the capillary blood level. Since the epidermal layer glucose is not being replenished, it falls below the capillary level and this depletion precedes the fall in capillary glucose by about 4 to 8 minutes. In the case of a diabetic patient beginning to go to hypoglycemia, measuring the glucose level in the epidermal interstitial fluid would be an advantage. In both the rapidly rising and falling blood level glucose situations, the brain is adjusting hormonal levels to try to regain the normal glucose range as rapidly as possible. In the case of severely depleted blood glucose levels, the brain adjusts glucose usage in the body to preserve the blood supply level of glucose as long as possible.

There is one clinical condition in which knowing the interstitial glucose levels can provide some critical information not presently available to physicians. In the case of severe hypoglycemia, both the tissue interstitial fluid and the blood glucose levels are very low. Conventional treatment in this emergency is to infuse glucose such that the level in the blood remains in the normal range (90 to 120 mg/dL) until the patient recovers. This can take hours. The infusion rate is not higher to prevent overshooting the target level and causing hyperglycemia with shock. If the infusion is interrupted before the interstitial fluid levels have also returned to the normal range, the blood glucose level will rapidly fall since there is no readily available source of glucose from the interstitial fluid to replenish the blood supply.

If the interstitial fluid glucose levels were monitored, the healthcare providers would know when it would be safe to stop the infusion saving patient time and money. When the interstitial fluid glucose returns to the level of the blood glucose during an infusion, the patient is well on the way to recovery. In the future, continuous monitoring of interstitial fluid glucose levels would also permit a higher level of glucose infusion without the danger of overshooting the target glucose level, thereby shortening the patient's time in the dangerous clinical condition. The very long lag time for the interstitial fluid glucose level to recover to the normal blood glucose level after a severe hypoglycemic episode is called hypoglycemic hysteresis.

From the discussion above, there are two types of glucose monitoring needs. One is for measuring glucose under the fasting condition, such as in health fairs or walk-up clinics for which a quick test is used to screen people for further testing for the diabetic condition. Since nearly 50% of Type 2 (non-insulin dependent diabetics) have not been diagnosed, a quick and painless test would be useful to identify people for further tests to catch the disease in the early stages.

The second is monitoring needs of the diagnosed diabetic, plus testing at the physician office, in hospital wards, and in the emergency room/critical care situation. Here the need is to accurately track the rapid changes in glucose level to prevent hyper and hypo glycemic episodes with their attendant complications.

How can the PMP device be configured to address these needs? The basic PMP device, which analyzes the bulk fluid properties of the sample in the light path, would be useful for the fasting glucose tests. The system would look through the bulk tissue of the finger or other appropriately thin tissue and report the glucose level averaged over the bulk. Contributions from cartilage, bone,

fat, ligaments, etc. would be negligible, as would the intracellular glucose levels since that level is essentially zero.

For the second situation to monitoring rapid changes of glucose level in the blood, these changes are only seen in the capillary blood and the surrounding interstitial fluid. Using the light that probes the bulk tissue of a finger would average the rapid changes of glucose in the epidermis layer over the relatively unchanged levels of glucose through the rest of the finger. A large change in epidermal glucose, reflecting the capillary blood glucose level, would not be measured if the entire finger tissue were used.

These levels could be monitored using the epidermal glucose levels in the rapidly perfused tissues of the body. Since the skin layer extends at most 1.5 mm deep (including the epidermis, dermis, and underlying support tissue), the PMP need only sample at most a few hundred microns into the tissue to see the epidermal and uppermost dermis layer. The capillary bed is at the junction of the epidermis and dermis. At near infrared wavelengths, the tissue is sufficiently transparent. The measurement can be made by reflection optics, or other well-known methods to send and retrieve light a short distance into a tissue medium.

In the patent for the PMP in column 6 line 40 there begins the section Reflection and Backscatter. This section enables the patent in these areas. In addition, note that we specifically state the use of the PMP to analyze different types of vascular tissue such as the finger, toe, ear lobe, scrotum or labia.

A suggested method to use reflected light was to reflect the signal through a window (which complicates things a bit due to the refraction) against which the finger is pressed to give a more or less flat surface. It is further possible to take advantage of what is known as frustrated interference at a near Bragg angle reflection at a refractive interface. This is a "fuzzy" waveguide effect wherein the imperfectly reflected signal picks up some information in the translucent surface of the finger. This area requires work to be completed with MetroLaser.

## 16. Give an Intellectual Property summary covering the Photonic Molecular Probe.

Issued patents include: US Patent No. 6,236,870, issued on May 22, 2001, US Patent No. 5,871,442, issued on February 16, 1999; and, Australian Patent No. 715949, issued on May 25, 2000.

Pending patents include: Canadian Patent Application No. 2,265,624; European Patent Office (EPO) Patent Application No. 97941667.4, which elected *Belgium, Switzerland, Germany, France, Italy, Luxembourg, Netherlands, United Kingdom, Ireland, Denmark, Spain, Finland, Greece, Portugal, and Sweden*; Israel Patent Application No. 128,873; and, Japanese Patent Application No. 128,873. In addition various US Continuations and Continuations-In-Part based on US Patent No. 5,871,442 are pending.

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