Discovering the Near-Infrared Window into the Body and the Early Development of Near-Infrared Spectroscopy

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ABSTRACT

Extension of optical monitoring of intact tissues from the visible and ultraviolet to the near-infrared (NIR) range (700–1300 nm) was first undertaken in 1977 for the purpose of monitoring the redox behavior of Cytochrome c oxidase (cyt c ox) in vivo. Soon it became evident that the much greater NIR translucency of skin and bone made it possible to reach brain and muscle tissue without surgical intervention. The presence of hemoglobin absorption led to complications forcing the construction of algorithms to separate the signals of the two molecular entities. It was also realized, however, that the hemoglobin signals provide information regarding the source of oxygen in the tissue, while the cyt c ox signals indicate the intracellular availability of oxygen for oxidative phosphorylation. This ability of recognizing the source/sink relationship greatly enhances the value of NIR spectrophotometry (NIRS) for research and clinical purposes. © 1999 Society of Photo-Optical Instrumentation Engineers. [S1083-3668(99)01404-5]

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I want to start by thanking Professor Okada for his kind invitation to the First International Symposium on Medical Near Infrared Spectroscopy (NIRS) and also for giving me the opportunity to briefly relate my very first attempts at performing NIRS on intact tissues which led to the discovery of the optical window into the body in a limited region of the NIR. I feel much honored by his request to provide a brief historical introduction of our topic.

It was early December 1976 when a special request for applications (RFA) issued by the National Institutes of Health (NIH) came across my desk. It asked for proposals to perform exploratory research in cardiac physiology or medicine with emphasis on novel techniques toward novel goals. This request truly addressed my interests. I have always considered myself more an explorer than a scientist, but very little funding was and is available for “Blue Sky Research.” In fact it is frowned upon by peer reviewers and funding authorities alike—although there are exceptions as I will show further on. But here was a rare chance to indulge myself in my predilection for so-called “fishing expeditions”—and get funded for it!

Now for a long time I had suspected that Cytochrome c oxidase (cyt c ox) (or Cytochrome aa₃ cyt aa₃), reacted differently in vivo than was to be expected from in vitro experiments. Ever since I learned the techniques for optical monitoring of intact tissues as a postdoctoral in Britton Chance’s lab, I had concentrated on following intracellular events (pH, Ca⁺⁺, redox state of the cytochromes, etc.) during physiological challenges to a tissue—either excised or in situ with intact circulation. Most of the information that led to my suspicion had come from transillumination of various excised, but otherwise intact tissues specialized for ion transport (gastric and intestinal mucosa, kidney, carotid body, etc.). Reflectance spectrophotometry and surface fluorescence were used for larger solid organs such as the brain and showed similar disparities with in vitro results using purified enzyme preparations. In all the transport tissues, but not in skeletal muscle, the redox state of the respiratory chain was more reduced than in their isolated mitochondria studied in the test tube. The most outspoken difference is in the steady state of cytochrome c oxidase. In mitochondria studied in vitro hemes a and a₃ are
a few percent reduced at any state of metabolism, except of course during anoxia or hypoxia. In functioning transport tissues, however, they are 40% or even 50% reduced and become more oxidized when oxidative metabolism is increased rather than the opposite as is seen in preparations of isolated mitochondria. In excised skeletal muscles the enzyme’s redox state approximate the in vitro levels but in strips of cardiac tissue the cyt aa₃ redox state is again high, although not as high as in the transport tissues.

Commonly, optical measurements are made on various components of the respiratory chain, since their light absorption peaks depend on their level of oxidation or reduction. Most peaks are located in the visible part of the spectrum and classically have been the indicators of the redox behavior of the respiratory chain components—ever since the visual measurements of Professor David Keilin in the 1920s.

What I had wanted to do for some time was to study all four redox centers of cyt c ox, the terminal enzyme of the respiratory chain: hemes a and a₃ as well as the two copper atoms that absorb in the near infrared region of the spectrum. It was my hope that this approach might provide better clues to the unexpectedly high reduction state of the enzyme in situ and of the respiratory chain in general. But up to this time I had no funds to allow me to explore the NIR region. It was considered a “fishing expedition” even though in open-skull, animal preparations we had studied the heme a component (absorption peak in the orange colored region at about 605 nm) and I had had some very limited success in observing the reactions of heme a₃. The latter has a large and redox-dependent absorption peak in the violet region (approximately 445 nm) but hemoglobin absorbs even more intensely in the violet and it was clear that convincing data on the in vivo state could only be obtained from intact organs with normal blood circulation.

Although the experiments in the violet region strongly hinted at a similarly high reduction state of a₃ in vivo, it was clear that we would face a daunting task in convincing ourselves, let alone biochemists, that the extracted enzyme had been affected by the isolation procedure: that its in vitro behavior might be an artifact. Studying the relative absorption strengths of hemoglobin and cytochrome oxidase it seemed that the NIR region was more promising. However I did not have the funds to equip the laboratory properly for a thorough study and the idea was too controversial to expect approval by NIH study sections that included biochemists.

Then along came the RFA inviting the research community to do some Blue Sky research—go on a fishing expedition! And so I started to prepare a grant application for the January 1 deadline, a grant to probe the exposed heart with NIR spectrophotometry. In the process of organizing my application I realized that the NIR photons would penetrate much more deeply into the tissue than those of the visible range since the wavelengths are longer. Thus comparison of the NIR results with previous ones using visible light would be difficult. Then the question arose of course from how deep we might be deriving our signals and a new thought struck me one day at dinner. On December 28, 1976 our family menu featured a grilled chuck roast, the poor academic’s substitute for steak. This very American cut of beef still contains part of the shoulder blade of the steer; a flat piece of bone perhaps 3 or 4 mm thick, about the same as the human skull. I asked my 14 yr old son Paul to clean all the muscle tissue from the bone. When he had done so we held the pink object up against the light and noticed that the shadow of a finger could easily be noted in the diffuse red light coming through the bone. If red light could, then certainly NIR light at the longer wavelengths would penetrate the human skull and provide access to the brain. Possibly other tissues could be monitored too in a minimally invasive way. We had discovered the possible existence of an optical window into the body ... if we could now prove its practicality for obtaining significant information about the ongoing metabolic reactions!

A few days later the grant request was mailed in. It emphasized cardiac studies in the exposed heart but hardly mentioned possible noninvasive, atraumatic monitoring of metabolic reactions and circulatory parameters without surgical exposure. Monitoring the brain through scalp and skull was well beyond the topics of interest of the RFA.

In January 1977 we stopped my ongoing experiments to explore the possible existence of a NIR window into the body. We scraped together equipment to test the possibility of the novel idea for noninvasive cerebral monitoring. Borrowing parts from other equipment my collaborators; Ben Comfort, electronics technician; Hans Keizer, labtech chief; and Ron Overaker, instrument maker, put together a make-do system. In the last week of January we managed to obtain NIR signals through a cat’s head from temple to temple and in mid February through mine, the thickest head around.

After this success two unexpected developments occurred: one positive, the other negative. The positive one was the provision of extra money by the National Institute of Neurological Diseases and Stroke (NINDS) of the NIH from which I had a current grant. It allowed me to buy photon counting equipment, enabling us to make human measurements more reproducibly. The most remarkable aspect is perhaps that this extra funding was in place within a few weeks after one phone call to my NINDS program manager and a simple letter of request. Truly an exceptionally wise man!

Writing patent applications on the new discovery was the not-so-happy other development. A visit to the Dean to plead for the continued employment of
Dr. Mike Rosenthal, my most valuable collaborator, brought this about. It appeared to me that his past scientific productivity in optical monitoring of the exposed brain and the need for his continued involvement in our new and important monitoring technique should be reason enough to grant him tenure. (This was not to be; tenure was denied and he left for the University of Miami where aside from a distinguished research career he also functions as a dean in the Miami School of Medicine.) However our dean insisted that the University should apply for NIRS patents. Looking back, it now seems that this has resulted in an unwarranted delay in the practical development of the technique until others, outside the USA, were successful in developing devices. The American company that took out an exclusive license did not realize the time required for the research and development (R & D) to produce a clinical device. Our group wrote and obtained several more patents but the company shelved the project time and again and it was never brought to fruition.

But at our Duke lab we proceeded at breakneck speed. We realized that the digital, photon counting technique could and should be replaced for clinical applications by analog measurements. The first prototype of a commercial, bedside instrument was completed by the Fall of 1980—a mere half year after receiving commercial support. The optical design—including the use of laser diodes, at the time a novel light source—was my brain child as was the design of the algorithms. The electronics were designed by Ben Comfort and Jim Meyer, a former lab technician, who was by then running his own electronics shop. The electronics layout and construction were by Mr. Comfort. The product was debugged and fine tuned by Brian Dodge, the electronics technician of the medical center’s Research Instrument Shop. It is worth noting that none of the electronics team were formally trained as biomedical or electronic engineers. Two were products of two year, junior college programs; the third was mainly self taught. Their system is still the basis of our present day instrumentation and compares favorably in cost and performance with the other much more complex, commercial designs.

Ronald Overaker, head of that amazingly effective Research Shop, took charge of the fiber optics manufacture and all mechanical development. Later he also took my son Paul under his wing as an apprentice and taught him the art and science of instrument making. Jim Meyer taught him the practical fundamentals of electronics circuitry. By programming our NIRS instruments and obtaining a doctor’s degree in Physiology from the Scripps Institution, Paul continues to contribute to further NIRS development.

While the instrument was under construction Hans Keizer and I concentrated on obtaining data to compose algorithms that would, among other objectives, cleanly separate the information about cytochrome \( c \) oxidase from that of hemoglobin in the tissue being monitored. This is not a minor task since the hemoglobin spectra dominate the entire region in which the copper bands of cytochrome \( c \) oxidase occur. Proof of the appropriate separation was provided by manipulations in which the Hb and Hb\(_2\) traces and the cytf \( c \) ox trace responded in opposite ways during animal experiments.\(^1\) Later we added tests in which the hemoglobin was removed by exchange perfusion with fluorocarbon-containing, artificial blood. If the algorithms were correct the hemoglobin traces fell without affecting the cytochrome trace. The best example was published somewhat later by Claude Piantadosi\(^2\) showing the accuracy of our algorithms. It should be noted that these algorithms calculate the changes in the three components (Hb, Hb\(_2\), and the cytf \( c \) ox copper component) not in concentration terms but as fractions of their total signals. I chose this expression since actual concentration terms would require knowledge of the path length traversed by the photons. But by multiple scattering in the tissues, the path length is elongated to an unknown extent. My unusual parameter has served well in monitoring physiological events in the lab and clinic.

Soon we raised the interest of several clinicians at Duke University who realized the potential value of cerebral monitoring of tissue oxygen sufficiency. Dr. Elisabeth Fox, anesthesiologist and Dr. Jane Brazy, neonatologist, were in the very forefront of clinicians who saw the potential benefit of the new technique. Their enthusiasm and energy resulted in novel clinical insights and in technical improvements in the patient/machine interface. Many of these are still used in the clinical applications of NIRS. Later we were joined by Dr. Claude Piantadosi from pulmonary medicine who also became quite active in some more experimental aspects of the NIRS technique. However the most important contributions, in the form of constant encouragement from the earliest stages on, came from Dr. Herbert Saltzman, professor of Pulmonary Medicine. Well before my discovery of the NIR window into the body he had promoted my visible and UV optical monitoring efforts and spoke many times and in many places about the need to monitor metabolism \textit{in situ} in compromised tissues.

Incidentally, my RFA grant application to explore cytochrome \( c \) oxidase in the NIR earned the very best critical review I ever received. Yet my request was “approved but not funded.” This seemed an impossible contradiction. In a telephone conversation the program manager emphasized the excellence of my idea and the fact that it had received the best priority score of all. The problem was, he explained, that some one higher up in the NIH had withdrawn the entire program since he had “found a better use” for those earmarked funds. I bet it was not for exploratory research. Nevertheless, this RFA had spurred my interest in truly noninvasive monitoring with the NIR and so had fulfilled its purpose.
The early development of NIRS was not concluded by the first article appearing in Science magazine in late 1977, nor by our several short papers, early reviews, and contributions at various symposia. Two of the most important verifications of the NIRS opportunities for noninvasive monitoring were provided by the study of Mook et al. of the correlation between the NIRS signals and the cerebro-cortical $O_2$ tension measured by multiple surface $O_2$ electrodes, while the other important verification was the study of Colacino et al. comparing blood flow measurements with NIRS and simultaneous $^{133}$Xe measurements. The correlation was found to be excellent but with the NIRS being much more rapid, radio-isotope free, and less expensive. (It was not requested that I provide an all inclusive bibliography of the flood of early reports on NIRS activity, but that I recount my personal experiences in opening the field. Therefore the bibliography is more a sampling than an exhaustive listing and I apologize to those collaborators and colleagues whose contributions I may have underreported; in my heart and mind they are not undervalued.)

The end of the period of legitimizing the new field is perhaps best marked by the 1984 meeting of the International Society for Oxygen Transport to Tissues (ISOTT) held in Nijmegen, the Netherlands. This was a significant location for me since that old city was the birthplace of my father. I wished that he would have been able to be present at this event, if only for all the tribulations I caused my parents by my rejections of the fetters of a standard education.

In the early 1980s the Rome group that had visited my lab some years before started publishing significant NIRS articles. It was a pleasant surprise to find Marco Ferrari at the ISOTT meeting where he presented a significant paper. Several other groups, including the London one of Dawood Parker and David Delpy, also visited to learn about the new technique but they did not publish until later.

In Nijmegen our group presented several basic papers and two clinical ones providing insight into the in vivo reactions of cyt $c$ ox, the NIRS technique, and into the contributions it could make to patient monitoring. Perhaps the three most important basic findings were:

(i) progress to analog, reflectance spectroscopy instead of the digital, transillumination technique used initially;

(ii) the analysis of the NIR absorption band of cytochrome $c$ oxidase as consisting of the confluence of two separate bands; and

(iii) the ability to separate the contributions of hemoglobin from the cytochrome signal.

I believe that at that point (Nijmegen in 1984) we had advanced our discovery sufficiently well to warrant acceptance of medical near infrared spectroscopy (mNIRS) as a promising technique. And now, here in Tokyo in 1998, I stand in awe of all the sophisticated efforts and significant findings of my many colleagues. I am most grateful for the important advancements they have made in the science and for their intellectual courage in applying it to the benefit of their patients. No explorer could wish for more.

REFERENCES


