Early development of near-infrared spectroscopy at Duke University

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Abstract. Optical monitoring of living tissues in the near-infrared (NIR) region of the spectrum (700 to 1300 nm) was first demonstrated some 30 years ago by Professor Frans F. Jöbsis of Duke University. Jöbsis had intended to study the oxidation-reduction (redox) behavior of the copper band (CuA) of cytochrome *c* oxidase (*cyt* a, a_3) to understand certain anomalies in the behavior of the mitochondrial respiratory chain in the ultraviolet and visible regions between living tissue and isolated preparations of mitochondria. Instead, he discovered a new window into the body—for NIR light penetrates deeply into living tissues. Jöbsis's pioneering studies proved it was possible to interrogate hemoglobin absorption and saturation and to assess the redox state of vital organs such as the brain directly through skin and bone. He and his collaborators had also recognized that the tissue hemoglobin signals provided valuable information about the oxygen (O_2) content of the tissue, and *cyt* a, a_3 signaled the availability of cellular O_2 for oxidative phosphorylation. The ability to noninvasively monitor the O_2 delivery-uptake relationship has made NIR spectroscopy a unique tool for the assessment of tissue oxygen sufficiency in health and disease. [DOI: 10.1117/1.2804925]

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For quite a number of years, Professor Frans F. Jöbsis (1929–2006) of Duke University—Dr. J his friends called him—had wondered why the terminal member of the mitochondrial respiratory chain, cytochrome *c* oxidase (cytochrome aa_3), behaved so differently *in vivo* than expected from *in vitro* observations. Often Frans reminded himself and those working in his laboratory of this discrepancy. This was part of his mindset, a legacy it seems, he owed to his days as a graduate student in zoology at the University of Michigan in the late 1950s under Professor Dugald Brown.

Indeed, much of Dr. J's uniqueness as a scientist came from his unusual background. Born in Batavia, now Jakarta, Indonesia, his mother had been trained in internal medicine at the University of Leiden, and his father was a banker and self-taught economist. His parents got him interested in field biology, and he dreamed of becoming an explorer of rain forests. The family, however, had returned to Holland by 1939, just before the Japanese occupation of Indonesia but just in time for the German occupation of Holland. After the war, and his freshman year at the University of Leiden, he had an opportunity to spend a year at the University of Maryland. He had intended to study agriculture, a remnant perhaps of his experience in the Dutch famine of 1944–45, but he remained in the United States to complete both his undergraduate and graduate training in zoology. In his unpublished autobiographic sketch, he noted, however, that he felt it "ironic that I ended up spending my professional life in a dark optical laboratory watching flickering monochromatic lights until my foray into the NIR put an end to even that."

Frans Jöbsis had emerged from his graduate training with a strong conviction about physiology: "Study Nature, not bricks." What he meant by bricks was pretty clear—the little things that make up intact tissues—genes, proteins, organelles, and cells. The qualities of these various components—the isolated bricks—were never the same after they had been extracted from a living tissue and were therefore only faintly reminiscent of the complex physiological behavior they had supported in their parent system. "If the goal is to understand physiological function, then keep the system as intact as possible," he would say. According to Dr. J, the nature of the bricks must be provided by other biological disciplines but understanding the function of Nature's edifice is the nature of physiology.

Ever since Frans learned the techniques of optical monitoring of intact tissues as a postdoctoral fellow in Britton Chance's laboratory at the University of Pennsylvania, he had worked on monitoring important intracellular events—pH, calcium, cytochrome redox state, during physiological challenges—either in excised perfused tissues or *in situ* with

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circulation intact. In Philadelphia, he had devoted himself to the noninvasive study of intracellular reactions, mostly using optical techniques.[1] To quote him again: "Check on your bricks, know your bricks, but do not confuse them with the architecture of the building. No cathedral is a pile of bricks."

Most of the information that made him skeptical of *in vitro* "mitochondriology," and that it did not have much to do with "cellular vitology" (the term by which his laboratory came to be known), had come from studies of transillumination of various thin, excised, but otherwise intact tissues specialized for ion transport—gastric mucosa, carotid body, and kidney.[2–5] Reflectance spectroscopy and surface fluorescence, used for large solid organs such as the brain, showed similar disparities compared with *in vitro* data from isolated mitochondria or mitochondrial complexes. In each of 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 a cuvette. Why?

I was first sent to Dr. J's laboratory in 1980 from the clinic by Frans's close friend and colleague, Dr. Herbert A. Saltzman, because I was looking for a spot to do a postdoctoral fellowship on oxygen. Dr. J ushered me through dark laboratories flush with dark red walls and beset by heavy black drapes, packed with operating tables, stereotaxic devices, chart recorders, and peculiar but wonderful optical instruments with light pipes and analog devices for mixing electronic signals. One time, he set up a projector and showed me a pale 8-mm film clip of the mitochondria of living cells that had been taken painstakingly through a light microscope near its limit of resolution. If one paid close attention, tiny mitochondria could be seen, flitting about through the cytoplasm like a flock of songbirds in a field, first in one direction, pausing briefly at one spot, then moving on to another, where they again paused, and so forth, like a horde of tiny tireless workers holding the cell together. I was hooked too—these tiny endosymbiotes were the mortar of the cathedral of physiology.

When I arrived on the scene, the development of near-infrared spectroscopy (NIRS) in Jöbsis's laboratory at Duke was already well underway. Dr. J's sole-author paper, now a classic, had been published three years earlier in *Science*,[6] and a prototype NIRS spectrophotometer using four solid state lasers of different wavelengths, fiberoptic cables or optodes (or optrodes), and photomultiplier tubes had already been built in Ron Overaker's instrument shop in the Department of Physiology—the Niroscope it came to be called. The problem for Dr. J was how to develop algorithms that could deconvolute the overlapping near-infrared (NIR) spectra of deoxyhemoglobin, oxyhemoglobin, and the oxidized CuA band of cytochrome oxidase in real time. The problem for me was to get up to speed in optical monitoring and the nature of the bricks (mitochondria) as quickly as possible so that I could be of some use to him.

Frans was a wonderful scientific mentor to all students and fellows in physiology, including me. He was brilliant, patient, flexible, and creative but most especially, inveterately inquisitive. I came to learn his history at Duke, which was quite fascinating. He had been recruited to the Department of Physiology by Professor Dan Tosteson in 1964 and came to be viewed more as an optical pioneer than a "lab cat" as he sometimes referred to the rest of us. The rest of the story is one of thinking outside the box—although relevant to the genesis of NIRS at Duke—its period of gestation began before his arrival in Durham.

It so happened that as a graduate student, Frans Jöbsis had presented data on muscle physiology at his first national meeting as Britton Chance prowled the audience looking for a "muscle man." Chance offered Frans a postdoctoral position, and Jöbsis, still sans PhD, packed up and left Ann Arbor for Philadelphia. He then learned the first principles of optical monitoring from Chance at the Johnson Foundation. There he developed an interest in energy turnover during ion transport and cerebral cortical activity using the oxidation-reduction (redox) state of the respiratory chain as an indicator. Out of this came his idea that cytochrome *c* oxidase might be more directly involved in ion transport than simply by providing adenosine triphosphate. He was determined to study the enzyme in all its optical glory, including the NIR region of the spectrum. Eventually it became clear that NIR light penetrated into tissues much more deeply than visible light—even passing through skin and bone with ease—though it too lost its beam characteristics through scattering. This is the short story of how Dr. J. discovered a new window into the body.

I have, however, gotten ahead of myself a bit, so it is fitting to quote Dr. J again, this time from his address in Tokyo in 1998 on the invention of NIRS:[7] "In mitochondria studied *in vitro* hemes *a* and a_3 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 approximates the *in vitro* levels but in strips of cardiac tissue the *cyt* aa_3 redox state is again high, although not as high as in the transport tissues."

Dr. J had longstanding plans to study all four redox centers of cytochrome *c* oxidase: hemes *a* and a_3 and both copper atoms that absorb in the NIR region. He thought this approach might provide better clues to the unexpectedly high reduction state of heme *a in situ* that had been described in considerable detail by his group—Myron Rosenthal, Joseph LaManna, Frank Hempel, Tom Snow, and Avis Sylvia—in the 1970s at Duke. These investigators were successful at monitoring heme *a* at 605 nm *in vivo*, but had more limited success in observing the a_3 heme because even though it has a large and redox-dependent absorption peak in the Soret (violet) at 445 nm, hemoglobin absorbs there even more intensely. Dr. J was convinced that meaningful data on the *in vivo* redox state could only be obtained from intact organs circulated with normal blood. Based on the absorption intensity and maxima of hemoglobin compared with cytochrome *c* oxidase, the NIR seemed more promising than the Soret.

Dr. J had been taught by Chance of the complex scattering behavior of light in tissues and the qualitative nature of the information derived even when using differential methods. He always viewed NIRS as a qualitative tool—"a trend monitor," as he said. He thought, the calibration especially where the relatively weak signals from mitochondria are concerned, is best done from *in vivo* data, not phantoms or models, a conviction he held throughout his life. Indeed, during the NIR stage of his career, he began to publish under his full name,

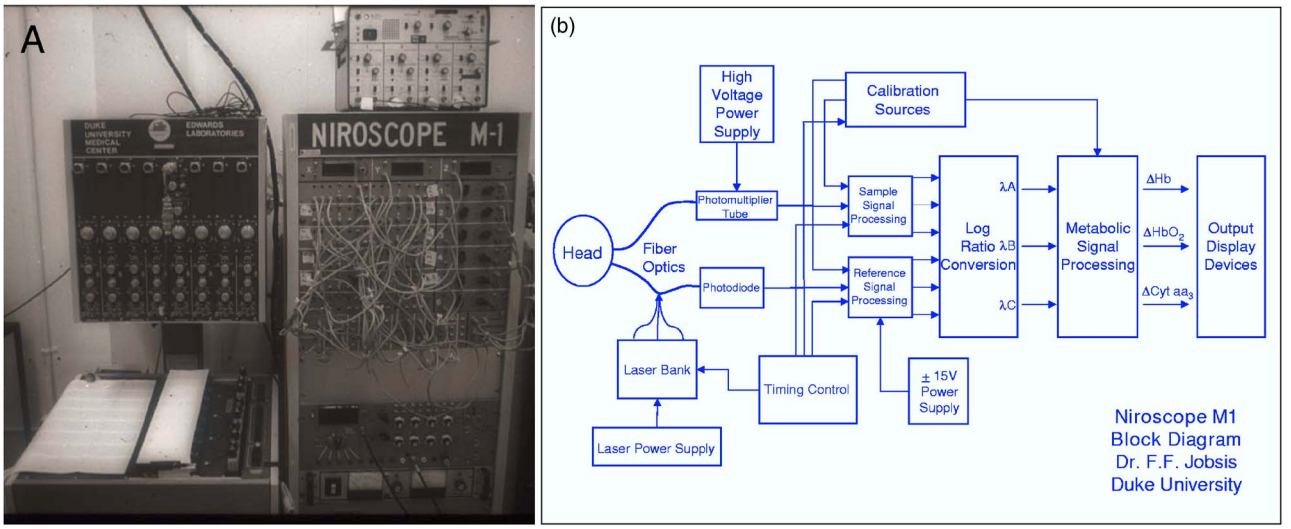

Fig. 1 (a) Photograph of the first working prototype of the Jöbsis Niroscope circa 1982. (b) Early block diagram of a three wavelength instrument for monitoring a single tissue. The actual Niroscope prototype was a four wavelength instrument capable of monitoring two separate tissues simultaneously.

Jöbsis-vanderVliet—partly in recognition of his Dutch heritage and partly to express the qualitative aspect of NIRS. Instead of strict units of optical density (Δ O.D.) by an absorber measured at multiple wavelengths in highly scattering tissue, he preferred relative variations in density (Vd). Later, however, he came to accept to a first approximation, quantitative derivations of mean optical pathlength and the calibration of the oxy- and deoxyhemoglobin signals in the brain.

For the development, testing, and validation of algorithms for use in the NIR region, we did many laborious and difficult *in vivo* experiments using extensive exchange transfusions with highly scattering, oxygen-carrying fluorocarbon emulsions, trying to remove the last vestiges of hemoglobin while still maintaining cytochrome oxidase in its oxidized form.[8] Many of these fluorocarbon transfusion experiments were conducted inside a hyperbaric chamber, where the tissue PO_2 could be raised to hundreds of Torr. These and other experiments at Duke, along with those done by Marco Ferrari's group in Rome, including in human volunteers,[9] provided the earliest experimental basis for *in vivo* validation and practical feasibility of the approach.

When optical measurements are made on the components of the respiratory chain, the light absorption depends on their level of oxidation or reduction. Most of the absorption and the most intense peaks are located in the visible part of the spectrum, and classically—dating from the spectrograph measurements of David Keilin in the 1920s and 1930s—have been the indicators of the redox behavior of the respiratory chain.[10] For cytochrome *c* oxidase, the intensity of the visible peak of the reduced hemes at 605 nm is some 13 times as great as the broad peak of oxidized CuA in the 820-nm region. Thus despite the greater penetration and the weaker hemoglobin absorption of NIR light in living tissue, the copper band(s) of the enzyme *in vivo* remained rather elusive. However, it has certainly proven possible to measure changes in the CuA band semiquantitatively *in vivo* in the presence of physiological concentrations of hemoglobin.

Setting CuA aside for the time being, NIRS has over the years come to be an important noninvasive monitoring tool for tissue oxygenation. Dr. J recognized its enormous clinical potential very early on, as he discussed in his Tokyo Symposium talk of 1998.[7] He established several collaborations in the early 1980s with investigators from the Departments of Anesthesiology (Elisabeth Fox) and Pediatrics (Jane Brazy) and from the Departments of Surgery (Herbert Proctor) and Anesthesiology (Kenneth Sugioka) in Chapel Hill to prove clinical feasibility and develop potential applications.[11,12] However, all that remains of his first clinical prototype—the Niroscope M1—is a few photographs and block diagrams (Fig. 1).

Dr. J was delighted by the early validation studies of the NIRS for noninvasive monitoring of tissue oxygenation.[7] He was especially pleased with studies on NIRS and blood flow and oxygen tension. He often quoted Colacino's comparison of simultaneous cerebral blood flow measurements with NIRS using indocyanine green dye and ^{133}Xe measurements.[13] The correlation was excellent and NIR was quicker, isotope-free, and less expensive. Another study by Mook found that the intensity of the oxidized CuA band in the NIR correlated well with cortical O_2 tension measured by surface O_2 electrodes[14] and was not dissimilar to the behavior of the 605-nm band of reduced cytchrome oxidase.[5] Finally, the ability to use NIRS noninvasively in skeletal muscle, where the blood supply can be temporarily occluded by tourniquet to obtain the total labile signal for purposes of calibration has had clinical utility.[15,16] Some years later, Boushel demonstrated the feasibility of using NIRS to quantify skeletal muscle blood flow in humans.[17]

Finally, to return to the *in vivo* measurement of the NIR band of the oxidized CuA. This measurement is possible, although confounded by hemoglobin crosstalk with changes in blood flow, blood volume, oxygen extraction, pH, and water content. These variables have always made the accurate inter-

pretation of the CuA signal difficult—requiring a high level of sophistication with handling optical data.[18,19] Moreover, the behavior of the CuA redox center differs from that of the heme centers, at least *in vitro*, as it is spatially oriented to the cytochrome *c* side of mitochondrial complex IV.[20] However, the potential to use cytochrome *c* oxidase as a regional intracellular redox signal *in vivo* remains as attractive today as it was 40 years ago. This enzyme complex is the terminal electron acceptor of the respiratory chain and the sink for more than 90% of the oxygen reduced by living tissues. Its concentration and redox behavior are faithful indicators of the overall balance between tissue oxygen supply and the status of oxidative phosphorylation in health and disease. Dr. J was ever hopeful that in the 21st century, we would learn to use NIRS to monitor it more quantitatively and at high spatial resolution.

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