

In situ measurements of brain tissue hemoglobin saturation and blood volume by reflectance spectrophotometry in the visible spectrum

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Abstract. Before the development of near-infrared spectroscopy (NIRS) for monitoring of hemoglobin and cytochromes *in situ*, the Jöbsis laboratory designed a visible light reflectance spectrophotometer. The method was not as useful for cytochrome oxidase measurements, which stimulated the search for a better method that culminated in NIRS. Visible light reflectance spectrophotometry was, however, usefully applied in several experimental applications, such as the study of brain capillary hemoglobin saturation during changes in inspired gas mixtures in awake and anesthetized animals, and to record transient increases in total hemoglobin (blood volume) after local neuronal activation by direct cortical electrical stimulation, demonstrating a response that is fundamental to functional magnetic resonance imaging blood oxygen level-dependent methods. A third application of the instrumentation was for brain capillary red cell mean transit time analysis, estimated by recording the passage of a red cell-free bolus through the cerebral cortical optical monitoring field. Taken together with his previous application of fluorescence detection of nicotinamide adenine dinucleotide, the visible and near-infrared spectroscopy demonstrate that Frans Jöbsis was a pioneer in the application of optical techniques to the study of intact organs *in situ*. These methods have been used to illuminate the basic function of the cerebrovascular and metabolic pathways in both physiological and pathological conditions. © 2007 Society of Photo-Optical Instrumentation Engineers. [DOI: 10.1117/1.2804184]

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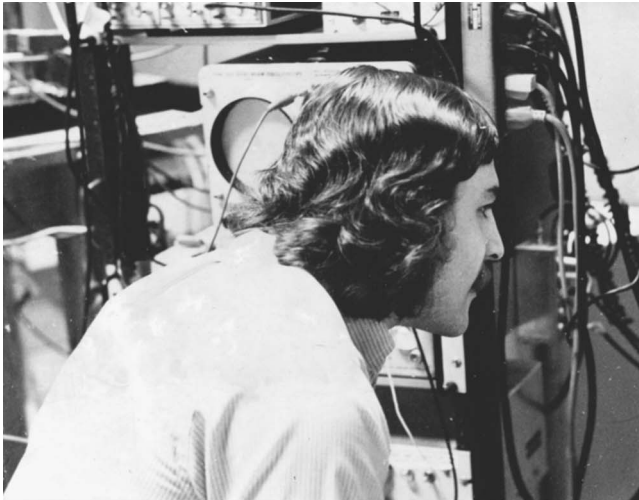
This is a short review paper in honor of the contributions of Frans Jöbsis as a pioneer in biomedical optics. The story of the development of near-infrared spectroscopy (NIRS),¹ for which Frans is rightfully best known, has been told.² However, Frans's contributions go beyond the NIRS method, or rather predate it. His expertise was adapting the quantitative optical instrumentation that had been developed at the Johnson Foundation to intact organs *in situ*. The first application was the detection of the nicotinamide adenine dinucleotide (NADH) fluorescence signal from the exposed brain surface.³ The microfluorometric method was then further developed by O'Connor^{4,5} and Rosenthal.⁶⁻⁸ Outside of the Jöbsis lab, the method was being used by several others at that time.⁹⁻¹³

In the early 1970s, Frans had the idea that the redox state of the mitochondrial cytochromes could be measured *in vivo* using visible wavelengths spectrophotometry. The task was to adapt the double beam spectrophotometer of Chance¹⁴ to the

microscope optics designed for the NADH fluorimeters. This task became the basis of my PhD thesis project (Fig. 1) under Mike Rosenthal's direction.¹⁵

The layout of the instrumentation included narrow pass optical filters, a chopping wheel, and a fiber optic Y-bundle that was handmade by Ron Overaker to approximate a randomized distribution. The optics included a 3.8× Leitz Ultrapak objective, providing just over 3-mm field of view. The light was detected by the EMI9698 extended red end window photomultiplier tube, which was relatively new at the time, in the barrel of a microscope. The instrumentation and technique were described in a methods paper.¹⁶ In one notable experimental variation, a special double slit apparatus on the half-meter Bausch and Lomb monochromator allowed "triple" beam studies, where two reference wavelengths, one above and one below the signal wavelength, were used to correct for wavelength-dependent light scattering effects.¹⁶ A version of this instrument was used by T. R. Snow (Fig. 2) for *in situ* measurements of the beating dog heart.¹⁷

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(a)



(b)

Fig. 1 (a) This is the author working on his thesis research at Duke University in this 1973 photograph taken by Hans Keizer. (b) Frans Jöbbsis and the author attending the Annual Meeting of the International Society for Oxygen Transport to Tissues in Curacao, 1991.

Frans Jöbbsis brought back Warburg's idea of the "action spectrum"¹⁸ as a way of using a very sensitive two wavelength instrument to obtain spectral information. He also used the term "equibestic" wavelength to indicate a reference wavelength where the absorption differences between hemoglobin (Hb) and HbO₂ were the same as at the sampling wavelength used to detect the cytochrome redox state.¹⁷ This term was based on the use of the phrase "isosbestic point" to indicate a wavelength of light at which two absorbers are equal and derived from the Greek isoj="equal," and sbest-oj="extinguished." (Note: the Jöbbsis term should probably have been "equisbestic" to be consistent with the Greek derivation, or perhaps "equideminutio" to keep to Latin roots.) The usefulness of the concept has faded with the growth of multiple wavelength analyses and full spectrum instrumentation.



Fig. 2 From left to right: Tom Snow, the author, Akos Koller, and John Haselgrove. The photograph was taken in 1980 in Budapest, Hungary, during the meetings of the International Society for Oxygen Transport to Tissue and International Union of Physiological Sciences.

At that time, the high quality narrow bandpass filters available along with the high sensitivity and low noise photomultiplier tubes meant that multiple wavelength instruments were preferred over the rudimentary spectral scanning instruments. The value of spectral scanning had been demonstrated previously by Lübbers's "rapidspectroscope."^{19,20} Multiple discrete wavelength instruments could also be designed to be less costly to build and operate.²¹ This led directly to the choice of individual wavelengths and the required "algorithm" for the NIRS instruments, despite long discussions on the promise of wide spectral detectors. A spectral scanning instrument based on a Cohu silicon intensified target (SIT) television camera and a new Jarrel-Ash monochromator design was built in the laboratory by L. J. Mandel (with technical assistance from Jim Meyer) using a DEC PDP-8E for analysis.^{22,23} Later, more refined spectral scanning designs were accomplished by T. J. Sick (with technical assistance from S. M. Pikarsky).²⁴

In experiments that made use of the NADH fluorometer, it was customary to display, on the strip chart recorder and in publication figures, the trace for the raw fluorescence signal (450 nm), the trace for the reflected excitation light (366 nm), and the subtracted weighted difference between the two. Changes in the reflected light trace were interpreted as changes in blood volume and changes in tissue light scattering properties. As a carry over, the practice for the double beam experiments was to display the reference wavelength signal and the subtracted sample wavelength minus the reference wavelength trace. Here the changes in the reference trace were considered to be overwhelmingly due to changes in total hemoglobin and soon were labeled as "blood volume."

Despite the disadvantages, the visible reflectance spectrophotometer nevertheless offered instrumentation that could be exploited to explore new aspects of cerebral vascular and metabolic physiology. An important insight imparted to the field by Frans Jöbbsis was that energy metabolism and vascular coupling had to be studied *in situ* for full understanding. The multiwavelength visible reflectance spectrophotometer proved to be less than useful for dynamic measurements of cytochrome oxidase,²⁵ however, this was possible with spectral

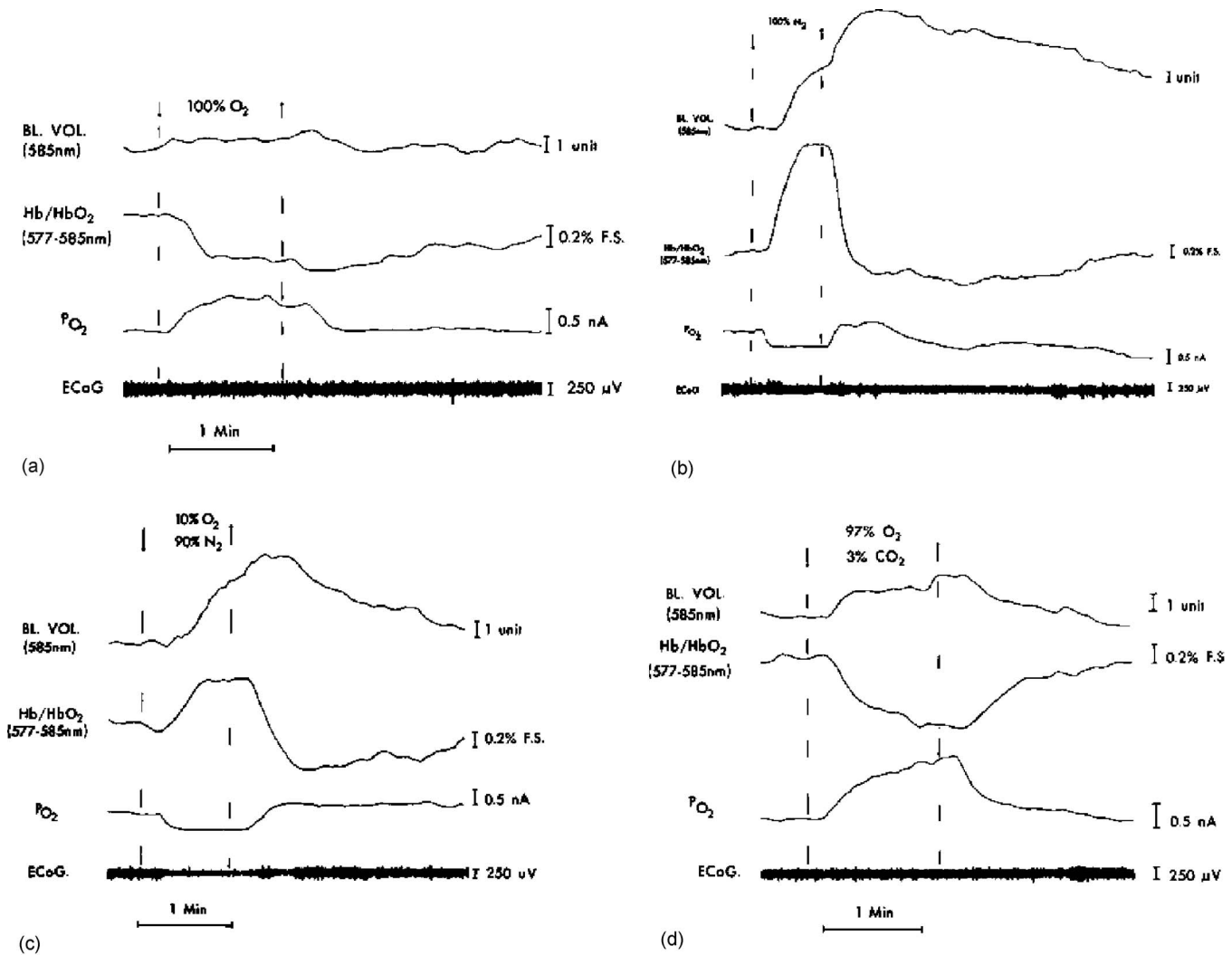


Fig. 3 Local tissue responses of blood volume, hemoglobin saturation, oxygen tension, and electrocorticographic activity recorded from the cerebral cortical surface of a pentobarbital anesthetized rat brain to transient changes in inspired gas mixtures. (a) 100% oxygen. (b) 100% nitrogen. (c) 10% oxygen (balanced nitrogen). (d) 97% oxygen, 3% carbon dioxide. Data were recorded during the experiments reported in Ref. 34.

scanning instruments, with computer analysis of spectra,²⁶ or used with a blood-free²⁷ or frozen brain.^{28,29} The former, however, could be used to detect local tissue blood volume and capillary hemoglobin saturation with great sensitivity and excellent time resolution in the intact, fully functional, cerebral cortex.

The earliest experiments, presented at the 1974 Benzon Symposium, reported data from the cat cortex preparations for cytochromes a, b, and c in addition to hemoglobin saturation.³⁰ But it soon became clear that the wavelength range below the hemoglobin absorption peak at 577 nm would be nearly impossible to study. That paper reported increases in blood volume, monitored at 585 nm, during pentylenetetrazol-induced seizures and spreading depression. Capillary hemoglobin, monitored as the difference between the signal at 577 nm and the reference at 585 nm, became desaturated during the seizures but slightly more saturated during the spreading depression.

The basic experimental preparation at that time was the

cerveau isolé exposed cat brain cortex, observed through an open craniotomy, but it was possible to monitor the cortex in unanesthetized rabbits through a chronically inserted plexiglass window.³¹ Changes in the capillary hemoglobin saturation were detected through the cortical window in awake rabbits exposed to hypercapnia, hyperoxia, and hypoxia, and an *in vivo* hemoglobin saturation curve was generated.³¹ Cortical windows have been used during optical monitoring in dog preparations,³² and comparisons of so-called “closed skull” versus “open skull” have demonstrated the value of less invasive methods.³³

The exposed cortex preparation does allow the simultaneous use of electrodes and cannulae. One such set of experiments, previously unpublished, is illustrated in Fig. 3. These data were collected as part of larger study by N. R. Kreisman et al. in the late 1970s.³⁴ Methodological details can be found in that paper. These data show that the tissue oxygen tension and capillary hemoglobin saturation are rapidly and reversibly altered with changes in the inspired gas mixture. Figure 4,

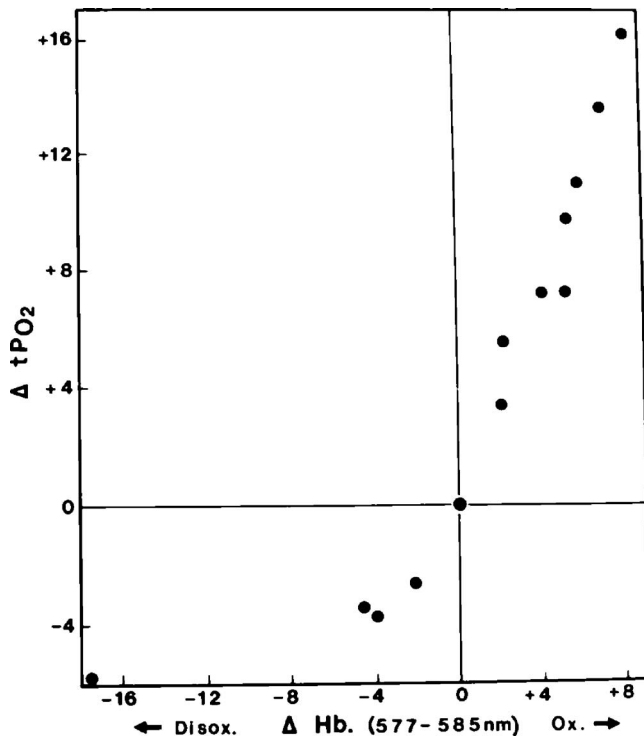


Fig. 4 Graph mapping the relative changes in local capillary hemoglobin saturation measured by visible light reflectance spectrophotometry and local tissue oxygen tension measured by platinum polarographic microelectrodes. Data are from Ref. 34.

from the same experimental series, shows the tight direct relationship between local capillary hemoglobin saturation (as the ratio of Hb to HbO₂), measured by the double beam spectrophotometer, and tissue oxygen tension as determined by a platinum microelectrode. It is interesting to compare the data from the less invasive NIRS- and blood oxygen level-dependent (BOLD)-based functional magnetic resonance (fMRI) methods with the electrode data already recorded in these types of experiments. A comparison would also be useful in studies with direct electrical stimulation of the cortex, which elicits transient increases in blood volume³⁵ that can be modified by, for example, loss of the cortical microvessel noradrenergic innervation by lesion of the locus ceruleus.³⁶⁻³⁸

Hemoglobin deoxygenation and blood volume decrease was shown in transient global ischemia in cats³⁹ and dogs³² with overshoots in both blood volume and capillary hemoglobin oxygenation on reperfusion.

The dependence of the visible optical signal on hemoglobin makes it especially adapted to experimental paradigms that involve hemodilution. One such application is in the determination of the erythrocyte capillary mean transit time.^{40,41} Mean transit time in seconds represents the quantitative relationship between local blood volume and local blood flow, where $MTT = BV/BF$ —the central volume principle derived from the Stewart-Hamilton equation.⁴² If any two of the three variables can be determined quantitatively, then the third variable can be calculated with confidence.⁴³

Although most of the current attention is on NIRS, there has been a continued interest in visible reflectance spectrophotometry (e.g., Refs. 44-46). New instrumentation and

computer analysis have made it possible for more detailed and quantitative development of techniques and concepts related to intrinsic optical properties of intact brains.⁴⁷⁻⁵¹

It is likely that some of the future advances made in these areas will be based on or strongly influenced by the approaches pioneered by Frans Jöbsis using visible wavelength reflectance spectrophotometry.

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