

7: General Conclusions

Chapter 7 summarizes the conclusions drawn throughout the body of this dissertation. Since the objectives of this dissertation involved both experimental research and instrumentation development, Chapter 7 is divided into two sections: The hardware development section covers the salient points, conclusions, and lessons learned from investigations in optical source and detector selection, source encoding, anesthesia, biomonitoring, and stimulation. The experimental measurements section then presents conclusions drawn from the rodent somatosensory cortical measurements, including observations concerning the effects of variations in the forepaw stimulus current and the stimulation frequency.

7.1 Hardware Development

Optical Sources

The two most common optical sources used for DOT include laser diodes and light emitting diodes (LEDs). LEDs are optically stable and are available in a wide range of wavelengths, however their spectral bandwidth is wide enough (~ 20nm to 40nm FWHM) to complicate blood oxygenation calculations.

- The most effective way to employ LEDs for DOT measurements is to mount them directly to the optode assemblies.

Laser diodes are powerful and are easy to couple to fiberoptics. Index guided laser diodes produce a 2-4nm wide output spectrum and don't mode-hop, so they provide better power stability than single mode gain-guided lasers.

- Multimode laser diodes are better for DOT applications.

Optical Detectors

The three most common optical detectors used in DOT instrumentation include silicon PIN photodiodes, silicon avalanche photodiodes (APDs), and photomultiplier tubes (PMTs).

PIN photodiodes are very inexpensive (\$5), very linear, and have a very large dynamic range, but they are not very sensitive. PIN photodiodes are best suited for measurements where penetration depth and sensitivity are not a problem.

- Photodiodes are best used in CW instruments for small animal and neonate measurements, where optode separations are small and detected signal levels are relatively high.

APDs and APD modules (APD + power supply and preamp) have high quantum efficiency over the 600nm to 900nm band like PIN photodiodes, but they are more sensitive and have a much higher gain-bandwidth product. However they have less dynamic range and are more expensive than photodiodes.

- Silicon APDs are better than photodiodes in CW instruments designed for adults.

PMTs and PMT modules are comparable in sensitivity and cost to APDs below ~800nm and they have a very high gain-bandwidth product, however they are very sensitive to supply voltage and stray magnetic fields (an issue when MRI magnets are nearby), they have a strong spectral gain dependence,

and their linearity is poor. Some PMTs have the unique ability to perform RF (i.e. nonlinear) mixing internally.

- PMTs are best suited for RF and time-domain DOT systems.

Source Encoding

Encoding provides a means by which the light from each source can be uniquely identified by the detection circuitry. In order to measure the signal from all optode pairs simultaneously, some form of source encoding is required. Domains in which source identity can be encoded include **time**, **frequency**, and **phase**.

- With DOT, the number of **source** optodes can directly affect both the demodulation complexity and the amount of source power that each detector receives. However the number of **detector** optodes has no effect on demodulator design or source power.
- The **maximum** achievable SNR of any optical detection system is determined by the number of source photons detected (i.e. the photocharge collected) during each measurement.
- Both time and frequency encoding can provide similar shot noise-limited sensitivity, which decreases as a function of the square-root of both the frame rate and the number of source optodes.
- At the shot noise-limit, **time-encoding offers a greater dynamic range than frequency-encoding**, since there is no self-generated background flux with time-encoding.

Temporal Skew

Temporal skew represents the time lag between the first and last optode pair measurements collected within a single “frame” of data. It can introduce significant blood volume and oxygenation errors, since optical measurements vary both spectrally and spatially with time.

- Pulse-TDM and FDM encoding provide the least temporal skew (best case) and switched-source TDM provides the most (worst case).

Individualized Gain Control

The static optical attenuation between optode pairs is relatively stable over time and is of little experimental value. If it can be removed from the measurement, the dynamic range can be preserved for hemodynamic signals of interest.

One solution is to dynamically adjust the gain of the preamplifiers to compensate for the unique static attenuation between each optode pair. Although individualized gain control requires additional circuitry, it should enable a DOT system to more closely approach the theoretical limits of performance, simultaneously capturing both local and distant optode information to provide higher quality tomographic reconstructions. Regardless of whether demodulation is performed in hardware or in software, IGC must be implemented prior to demodulation to be effective.

- A 40dB improvement in dynamic range was achieved with no penalty in crosstalk or linearity by adding individualized gain control to a time-encoded DOT system. This was confirmed through both electrical and in-vivo measurements.
- The detector and its preamplifier must still operate over the entire dynamic range spanned by the optical signals. Therefore significant effort should be spent in optimizing the detector and preamplifier circuitry to perform well over this dynamic range.

Anesthesia

The volatile halocarbon agents (Halothane, Isoflurane) provide excellent anesthesia, but they suppress a number of normal homeostatic processes: thermoregulation, respiratory and cardiac function, hemodynamic autoregulation, etc. and so cannot be used during DOT measurements.

The haloalcohols (a-chloralose, chloral hydrate) provide sufficient analgesia in the post-surgical period during experiments, yet only weakly perturb hemodynamic function. For this reason, a-chloralose was used for the DOT measurements discussed further below.

a-chloralose must be administered parenterally for these measurements, and its unique pharmacokinetics made analgesic management difficult during DOT experiments. The onset of action is delayed by about 20 to 30 minutes, so “feedforward” dosing was used to compensate for this delay.

An anesthetic delivery and ventilation system was designed and constructed specifically for use with rodents. It contains a pressure-cycled, time and demand-triggered positive-pressure ventilator with an internal air pump. Volatile halocarbon agents (HAL or ISO) are entrained in the gas flow through saturated vapor dilution, and the vapor concentration can be controlled over the standard 0 to 5% range. Additional features included apnea and obstruction detection, variable inspiratory assist trigger level, and automatic crossover from continuous induction mode to cycled mode. These features were found to be quite helpful in a number of ways.

- Automatic mode crossover allowed the tracheotomy tube to be installed and removed without interruption, facilitating an often precarious procedure.
- The apnea alarm alerted on tracheal occlusions within about five seconds, allowing the successful clearance and resumption of data collection on at least three rats.
- The adjustable assist threshold acted to stabilize the anesthetic depth, thus minimizing the total amount of anesthetic required.

This is important for DOT, since residual hemodynamic effects from volatile agents can interfere with the subsequent experiments. It also allowed the health of the animal to be assessed, since any changes in ventilatory demand were readily apparent.

- A “breath accumulator” allowed the collection of a sufficient volume of expired breath for stable and reasonably accurate capnometry.

This was vital, since it allowed the use of a real-time capnometer, thus obviating the delays and sparse sampling inherent to using a remotely-located blood gas analyzer.

Biomonitoring

Any parameters which can affect cerebral hemodynamics in real-time should be recorded concurrently, so that their confounding effects can later be removed from the data. In order to acquire and record all of this physiological information, a complete biomonitoring system was assembled. Heart rate and rhythm, MAP, respiration rate and depth (pneumogram), body temperature, volatile anesthetic concentration, and end-tidal CO₂ measurement were all displayed in real-time and were also available in analog electrical form for recording.

- The real-time nature of this physiologic information was found to be vital for both surgical anesthesia and for maintaining adequate and stable analgesia during the DOT measurements.

Stimulation

Since many DOT experimental paradigms for rodents include electrical forepaw or hindpaw stimulation, a suitable stimulator was designed and built. It was important to both control and monitor the delivered current in real-time, so a custom stimulus delivery, isolation, and monitoring unit was

constructed. The main design objectives included galvanic isolation to minimize interference and prevent stray electrode currents, charge-conservative pulse delivery to prevent electrochemical tissue damage, and the ability to monitor the current pulses in real-time to confirm proper current delivery. On one occasion the stimulator lead broke internally, resulting in the loss of an entire day of measurements. In response, a “delivery” indicator was added to display when current was actually being delivered to a resistive load.

It was discovered that once adjusted, the current amplitude remained quite stable over many hours, and typically required no adjustment even after 8 hours of stimulation. The current delivery sensor quickly detected intermittent clip connections on a couple of measurements.

7.2 Experimental Measurements

Diffuse optical tomography (DOT) can image spatial variations in highly scattering media, and offers great promise as an adjunct to fMRI for simultaneous real-time quantification of total hemoglobin concentration and oxygen saturation.

We used an inexpensive and portable continuous-wave DOT system (CW1) to perform cortical absorption measurements on rodents following electrical forepaw stimulation and presented preliminary results showing that DOT is capable of quantitatively imaging both Hb and HbO₂ concentration changes during brain activation in a rat model.

We also used a more advanced frequency-encoded CW imager (CW4) to acquire DOT images of rat brain function following electrical forepaw stimulation. The main purpose of these measurements was to perform a quantitative examination of the temporal correlation between fMRI and DOT. We also sought to explore the effects of stimulus repetition rate, duration, and magnitude, both to determine the robustness of this preparation and to explore the physiology underlying the neurovascular response.

- Both DOT [HbR] and [HbO₂] compared well to fMRI BOLD during stimulation but their profiles differed in the post-stimulus region, thus different hemodynamic mechanisms might be responsible for the post-stimulus responses of the [HbR] and [HbO₂] signals.
- The DOT [HbT] time courses followed the 6 and 30 second fMRI CPV time courses reasonably well. The similarity of the DOT and MRI hemodynamic measures suggests that the vascular sensitivity of the two methods is qualitatively similar, but not identical.

In principle, both DOT and fMRI should exhibit near linear proportionality to changes in the blood volume fraction, provided the hematocrit remains constant during the measurement, however it is possible that blood plasma volume and hematocrit may vary following cerebral activation. Although the transient peak observed in the DOT [HbT] data was not present in the averaged fMRI data, it was observed in individual rats.

- The magnitude of the [HbO₂] signal increased sublinearly (i.e. compressed) with increasing stimulus current. If correct, this implies that a saturation in neural activation occurs at a stimulus current near 2mA.
- The [HbO₂] signal magnitude also decreased in approximate inverse proportion to stimulus frequency.

This trend was also observed by others, who attributed it to a progressive reduction in the neuronal recovery time.

- The FWHM appeared to peak at 3Hz and then decreased in inverse proportion to stimulus frequency.

This could result from frequency-sensitive activation of different neural pathways within the cortex, each of which may elicit different hemodynamic responses. Similar research on rat whisker barrel cortex has revealed frequency-selective or “tuned” neural responsivity vs. stimulus frequency, which appeared as a variation in the spatial extent of activation.

- Signal amplitude was also observed to vary with electrode placement (data not shown).

Although total stimulus current is easy to quantify, the actual current distribution within the forepaw itself is not easily measured. In future experiments, this aspect will need to be addressed more carefully, perhaps by using monitor electrodes to directly assess the degree of neural activation.

7.3 Suggestions for future work

This dissertation stemmed from the desire to understand precisely how cortical CW DOT signals relate to neural activity. Since fMRI measurements on rodents with good temporal resolution had recently been performed under conditions which were easy to emulate with DOT, the rodent forepaw preparation was the ideal means by which the vascular sensitivities of fMRI and DOT could be compared and contrasted.

In order to perform these hemodynamic measurements with DOT at a temporal resolution comparable to fMRI, significant instrument development efforts were required in a number of areas. This led to investigations into improving optical source encoding strategies, better anesthesia and biomonitors equipment, and more precise stimulus delivery hardware. The results of these efforts were fruitful in many ways:

- A new source encoding technique (Pulse-TDM) was developed which offered a combination of features unavailable with other techniques in use at the time.
- The use of individualized gain control was shown to significantly improve the useful dynamic range of DOT measurements over a wide optode spacing range.
- An anesthesia/ventilator system was constructed as a testbed for the development and evaluation of features (pneumometry, combined time/demand-triggering, inspiratory and expiratory hold, etc.) currently unavailable on similar commercial designs. Many of these features were found to be useful in preserving hemodynamic stability, providing feedback on metabolic status, and reducing the surgical workload.

Future DOT design directions

There are two somewhat complementary design philosophies concerning modern instrument design. The *computer-centric* philosophy advocates shifting the workload from the hardware domain into the “digital” domain. Signals are sampled and digitized as early as possible in the signal chain, thus simplifying the hardware and reducing its overall cost, weight, and power dissipation. This results in lower hardware design costs, which are somewhat offset initially by higher software design costs. The main benefit of this technique is the flexibility in signal processing. Disadvantages include the large volume of raw data which must be acquired and processed, leading to sometimes lengthy data processing delays. Other disadvantages include the added risk of “crashes” and other computer-related problems, which reduces reliability and can lead to subtle and often irreproducible artifacts in the data. Computer-centric systems are well suited for laboratory and research environments, where the need for flexibility often outweighs the need for speed and reliability.

The *hardware-centric* view advocates maximizing the amount of work performed in hardware or firmware, such that only processed waveforms are actually recorded and stored in digital form. This generally leads to equipment which is large, complex, and power-hungry, yet provides repeatable, reliable performance with nearly instantaneous feedback to the user. Advantages include stable and

predictable performance, low digitization rates and data volume, and a short power-up time. Hardware-centric designs are ideal in life support situations, since they can be designed to recover nearly instantly from power interruptions and electrical faults. Disadvantages include the design, operation, and maintenance of large, heavy, and complex equipment and the loss of flexibility which results from preprocessing data within the instrument.

Certain signal processing steps (synchronous detection) are easily performed in hardware while others (complex high-Q and multi-pole filters) are better performed in software. The CW4 and CW5 instruments employ frequency encoding, which is easier to demodulate in software, hence the choice to digitize the raw analog signal stream. NIRS3 uses Pulse-TDM encoding, which is more easily demodulated in hardware, hence the greater circuit complexity and the availability of the demodulated and filtered analog waveforms in real-time.

So what is needed is the following:

- A thorough examination and assessment of each encoding technique (frequency encoding, time-switching and Pulse-TDM with IGC, etc.) should be performed, with an emphasis on how instruments should best be designed to maximize performance in both research and clinical settings. Once the major engineering design trades are thoroughly understood, then DOT instrument designs will become more standardized. This will encourage financial investment, with the goal of high-volume manufacturing and distribution of DOT systems at relatively low cost.

Future bioinstrumentation design directions

Commercial small animal anesthesia and biomonitors cannot provide precise control over physiologic parameters. Anesthesia delivery and ventilator equipment for small animals is designed simply to provide surgical anesthesia, and the design emphasis has never been on precision control over metabolic, hemodynamic, or ventilatory parameters. Nor has any emphasis been placed on compactness, so the combination of a veterinary anesthesia delivery system and rodent ventilator can consume two square feet of bench space.

Equipment designed for human use will serve well, but is often overdesigned for animal use, and the extra expense is rarely justified. For example, a non-dispersive IR anesthetic vapor concentration monitor (like the Datex Capnomac) is very accurate and precise, however it is also a large, complex, and very expensive piece of equipment. Simpler technologies, such as acoustic velocity detection or UV absorption, may provide adequate performance for animal use at a fraction of the cost, volume, and weight of non-dispersive IR. So, in summary:

- The physiologic stability requirements for hemodynamic experimentation should be examined in further detail. This knowledge should then be used to develop effective, modular, and inexpensive anesthesia, ventilation, and biomonitors equipment designed specifically for research use.

Future research directions

The temporal hemodynamic measurements specifically addressed the cerebral response to electrical forepaw stimulation under eucapnic conditions. Another avenue which can be explored is the effect of superimposing the forepaw response on a non-eucapnic resting state. Hypercapnia globally increases the static levels of both CBF (through arteriolar vasodilation) and CBV (through blood volume changes in the capillary and venous compartments), and hypocapnia has the inverse effect. Yet neither state significantly alters $CMRO_2$ or CMR_{glu} . So performing a similar forepaw stimulation experiment under various baseline pCO_2 levels may provide valuable insight into the effects of baseline cerebral blood flow and blood volume in the capillary and venous compartments.

In addition to the knowledge gained from temporal hemodynamic measurements discussed above, the effects of varying stimulus current, frequency, and duration on the hemodynamic signal yielded interesting results. However these measurements represented the results from only two rats, so a more

detailed examination of these effects should be performed with a sufficient number of rats to ensure that the results will be statistically significant. If the same preparation is used for these new measurements, then the results of the prior work can be combined with the new measurements under eucapnic conditions, reducing the additional number of rats required to achieve statistical significance.

Although the work discussed in this dissertation compares the results of both fMRI and DOT measurements, the data were collected at different times and with different rats. Despite the fact that both DOT and fMRI preparations were nearly identical, there will always be some uncertainty as to both the temporal and physiological accuracy of the comparison. One means of addressing this uncertainty would be to repeat these measurements with simultaneous DOT and fMRI. A nonmagnetic optode assembly could be used, and the data recordings of both measurements could be synchronized with common timing pulses – even the stimulus pulses would do. For blood volume measurements, both a MION bolus and a continuous ICG infusion could be administered, however the optical effects of combining MION and ICG in-vivo should be quantified to ensure that the optical properties of MION are properly accounted for. So, in summary:

- Repeat the forepaw stimulation measurements under both hypercapnic and hypocapnic conditions.
- Explore the effects of stimulus pulse frequency, pulse current, and duration on the cerebral hemodynamic response to forepaw stimulation, using the same forepaw stimulation preparation.
- Perform forepaw stimulation with simultaneous DOT and fMRI measurements. Evaluate the use of MION in combination with ICG for CBV studies to differentiate the erythrocyte cytosolic volume (using Hb-based CBV measurements) from the blood plasma volume (using ICG absorption measurements).