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Introduction

Making light work: illuminating the future of biomedical optics

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In 1996, the Royal Society held a Discussion Meeting entitled 'Near-infrared spectroscopy and imaging of living systems'. In 2010, this topic was revisited in a Theo Murphy Royal Society Scientific Discussion Meeting entitled 'Making light work: illuminating the future of biomedical optics'. The second meeting provided the opportunity for leading researchers to reflect on how the technology, methods and applications have evolved over the past 14 years and assess where they have made a major impact. Particular emphasis was placed on discussions of future prospects and associated challenges. This Introduction provides an overview of the state of the art of near-infrared spectroscopy (NIRS) and biomedical optics, with specific reference to the contributed papers from the invited speakers included in this issue. Importantly, we also reflect on the contributions from all of the attendees by highlighting the issues raised during oral presentations, facilitated panel sessions and discussions, and use these to summarize the current opinion on the development and application of optical systems for use in the clinical and life sciences. A notable outcome from the meeting was a plan to establish a biennial international conference for developers and users of NIRS technologies.

Keywords: near-infrared spectroscopy; biomedical optics; optical imaging; molecular imaging; tissue oximetry

1. Introduction

In 1996, a Royal Society Scientific Discussion Meeting on 'Near-infrared spectroscopy and imaging of living systems' was held in London, UK, with the intention of bringing together leaders in the field so that 'basic scientists could

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inform themselves about the most pressing biological questions ... and biologists could acquire a better understanding of the interaction of near infrared light with tissue' [1]. Using the articles contributed by the invited speakers of that meeting [2] the state of the art of near-infrared spectroscopy (NIRS) and imaging in 1996 could be summarized as follows:

— continuous wave (CW), time and frequency domain NIRS systems were in use on brain, breast and muscle;
— investigations were taking place into possible methodologies to provide an absolute measure of tissue oxygenation;
— optical tomography imaging had been performed on breast and neonatal brain;
— multi-channel functional activation and resting state studies had been performed in adult subjects; and
— the use of intrinsic and extrinsic contrast agents was being investigated for macro- and microscopic applications.

The future of NIRS technologies was considered to be an 'easy' non-invasive monitor of tissue haemoglobin oxygenation and concentration changes. However, an absolute measure of haemoglobin concentration and high-resolution images was regarded as 'most useful' [3]. It was predicted that NIRS would find its major initial clinical use in large-scale diagnostics (e.g. breast imaging) and in an environment where high technology is already well established (e.g. intensive care or surgery) and where the patient is easy to monitor.

Biomedical optics as a field has expanded dramatically; in this paper, we focus our attention on the evolving research and clinical landscape in NIRS, optical topography and optical tomography. In 2010 many of the original 1996 participants, accompanied by new research leaders in the field, met at the Kavli International Centre to participate in a Royal Society Theo Murphy Discussion Meeting entitled 'Making light work: illuminating the future of biomedical optics'. We summarize contributions from speakers, panellists and invited participants to ask the question: What has really changed in the 14 years since 1996? What new instrumentation is being developed and what does this offer for the future of biology and medicine? We refer to other articles in this issue that provide detailed reviews and full referencing on certain topics.

2. Near-infrared spectroscopy instrumentation

The range of NIRS instrumentation currently available encompasses a huge array of CW, frequency domain, time domain and diffuse correlation spectroscopy systems employing any number of wavelengths and measurement channels. Figure 1 summarizes the physiological parameters routinely measured using these systems. While many research groups are actively developing systems which use a range of novel methodologies, the commercialization of NIRS technologies has converged on two areas: tissue oximetry, which provides a single absolute measure of tissue oxygenation, and multi-channel optical topography, which has found widespread use in mapping cortical haemodynamics during functional activation.

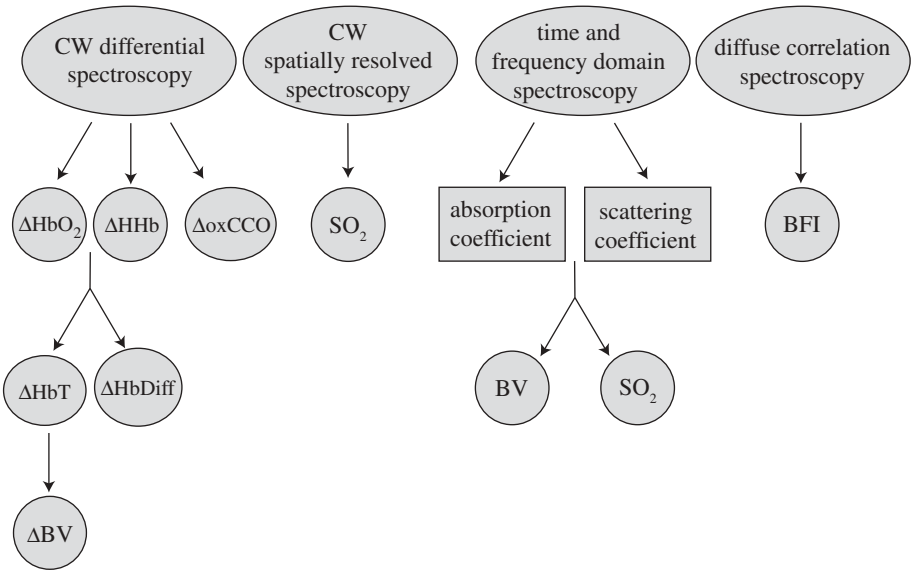

Figure 1. Schematic of the physiological measures available from a range of NIRS systems. HbO_2 = oxyhaemoglobin concentration; HHb = deoxyhaemoglobin concentration; HbT = total haemoglobin concentration = (HbO_2 + HHb); Hbdiff = (HbO_2 − HHb); BV, blood volume; oxCCO, oxidized cytochrome *c* oxidase concentration; SO_2, absolute tissue oxygen saturation; BFI, blood flow index.

Table 1. Currently available commercial tissue oximeters.

instrument	technique	no. of channels	company	website
EQUANOX[a]	multi-distance	2	Nonin, USA	www.nonin.com
FORE-SIGHT[a]	multi-distance	2	Casmed, USA	www.casmed.com
OXYMON-II A	multi-distance	2+	Artinis, The Netherlands	www.artinis.com
INVOS 5100C[a]	multi-distance	2 or 4	Somanetics, USA	www.somanetics.com
NIRO-200 NX	multi-distance	2	Hamamatsu, Japan	www.hamamatsu.com
ODISsey	multi-distance	2	Vioptix, Inc., USA	www.vioptix.com
OXIPLES[TS]	multi-distance FDS	2	ISS, USA	www.iss.com
TRS-20	multi-distance TRS	2	Hamamatsu, Japan	www.hamamatsu.com

[a]USA Food and Drug Administration's approval; FDS, frequency domain spectroscopy; TRS, time-resolved spectroscopy.

(*a*) *Commercial oximeters*

Table 1 summarizes the currently available commercial tissue oximeters (for those specially adapted for muscle studies, see [4]). Most of these oximeters use a spatially resolved (multi-distance) spectroscopy approach to deliver an absolute percentage measure of tissue haemoglobin oxygen saturation (variously termed

Table 2. Currently available commercial imagers.

instrument	technique	no. of channels	company	website
Dynot	CW	up to 8000	NIRx, USA	www.nirx.net
ETG-4000[a]	CW	52	Hitachi, Japan	www.hitachimed.com
ETG-7100	CW	72	Hitachi, Japan	www.hitachimed.com
fNIR1000	CW	16	FNIR Devices, USA	www.fnirdevices.com
FOIRE-3000[a]	CW	52	Shimadzu, Japan	www.med.shimadzu.co.jp
NIRO-200	CW	10	Hamamatsu, Japan	www.hamamatsu.com
NIRS2 CE, CW6	CW	up to 1024	TechEn, Inc., USA	www.nirsoptix.com
OXYMONMkll	CW	up to 96	Artinis, The Netherlands	www.artinis.com
Imagent	FDS	up to 128	ISS, USA	www.iss.com
HD-NI	CW	over 200	Cephalogics	www.alliedminds.com
CTLM	CW	over 100 (ring configuration)	Imaging Diagnostic Systems, Inc., USA	www.imds.com
SoftScan	TRS	raster scan	ART Inc., Canada	www.art.ca

[a]USA Food and Drug Administration's approval. CW, continuous wave; FDS, frequency domain spectroscopy; TRS, time-resolved spectroscopy.

tissue oxygenation index (TOI), tissue saturation index (TSI), regional oxygen saturation (rSO_2) and oxygen saturation (SO_2)). Most employ two wavelengths and, with only two exceptions, no attempt is made to measure optical scattering. Prices vary but are typically in the region of $30 000. This would suggest that, to date, the market has been driven by the (supposed) consumer requirement for an inexpensive 'plug and play' system which delivers a single physiological measure. Although the exact physiological relevance—and clinical utility—of this single measure of tissue oxygenation (in either brain or muscle) is a topic of much debate (see §3*b*), it has remained an attractive deliverable for the manufacturers of NIRS instrumentation.

(*b*) *Commercial near-infrared spectroscopy imaging systems*

Table 2 summarizes the currently available commercial NIRS imaging systems. The term imaging is used loosely here since not all of these systems provide an image but rather a collection of multi-channel time-course plots which can be used to generate topographic maps of haemodynamics and oxygenation. For the majority of systems the temporal and spatial resolution are specified for brain measurements (i.e. mapping the cortical haemodynamic response to functional activation), although commercial breast imaging systems are also available. Most employ CW methods and rely upon measurements at only two wavelengths. Prices vary quite widely but are mostly dictated by the number of measurement channels.

(c) Challenges

Discussions throughout the meeting, but particularly during the facilitated panel session 'Hardware innovations', suggested that there remains a healthy market for both tissue oximetry and optical imaging but that certain issues have yet to be resolved.

(i) *Providing tissue-specific information*

There appears to be a challenge in balancing the commercial requirement for generic, inexpensive, easy to use systems which can be employed on a range of tissue types and in a range of subject/patient groups, with a requirement to ensure that the systems deliver tissue-specific information or are optimized for particular applications. For example, although a range of commercial tissue oximeters is available, the resolution and sensitivity of systems being used to look for gross changes in cerebral oxygenation in brain injury may be very different from those required to monitor the performance of elite athletes where tiny fractions of a per cent may separate individuals. It was generally agreed that the 'one size fits all' approach may be flawed (quite literally in the case of a probe design as discussed below), and that there is considerable scope for continued work in determining the essential design features of systems optimized for specific applications, e.g. brain injury, neurodevelopment, sports science.

The prospect of optical methods providing tools for large-scale diagnostics was discussed at the 1996 meeting, with particular focus on applications in breast imaging. In this field, the sensitivity and specificity of absolute images for diagnostics purposes is still being investigated, especially with the use of implicit and explicit prior information (e.g. biomarkers, molecular spectra and morphology [5]). Longitudinal measurements of optical indices have been used as markers for breast tumour response during treatment, and may play a role in minimizing side effects as well as optimizing therapeutic outcome [6]. As with other applications, multiple centre clinical trials are required to establish the efficacy of optical technologies to inform diagnostics and therapy.

(ii) *Probe development*

Although significant advances have been made in single-site and multi-site probe design by commercial companies and research groups (figure 2; [7]), this clearly remains one of the most important considerations for widespread application of NIRS instrumentation. The issue of disposable versus reusable probes appears to divide the current market; some companies provide measurement systems free of charge and recoup costs from single-use disposable probes, while others incorporate reusable probes in the cost of the entire system. For measurements on brain, hair remains a confounding factor, and some manufacturers have limited the use of their systems to measurements on the hairless frontal cortex. High-density arrays are now in use for cortical mapping and are delivering images with unprecedented spatial resolution (figure 3; [8]). Prospects for further developments in this area include fibre-free head gear which could provide a viable low-weight solution, possibly even incorporating a measurement of head shape which may be used to inform image reconstruction.

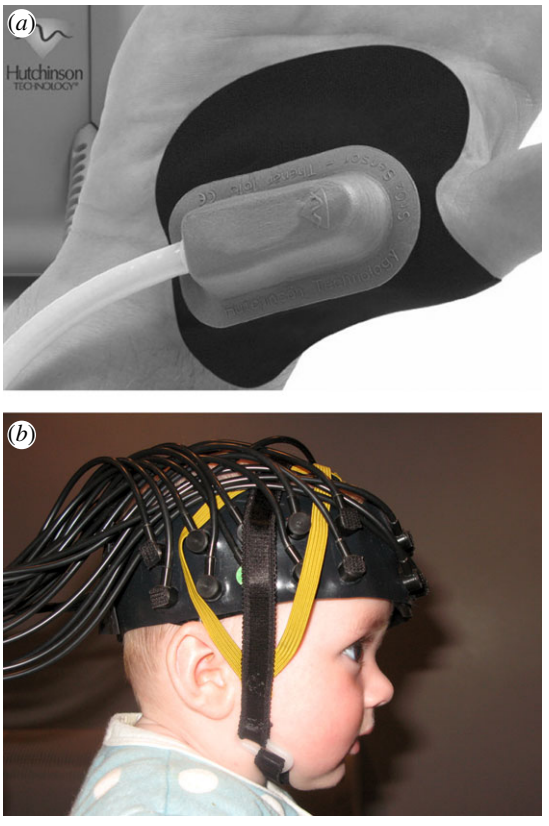

Figure 2. (*a*) Example of a commercial probe specified for the measurement of SO_2 on the thenar eminence (InSpectra, Hutchinson Technology) and (*b*) multi-channel optical topography array designed for measurements of cortical haemodynamic in young infants [7].

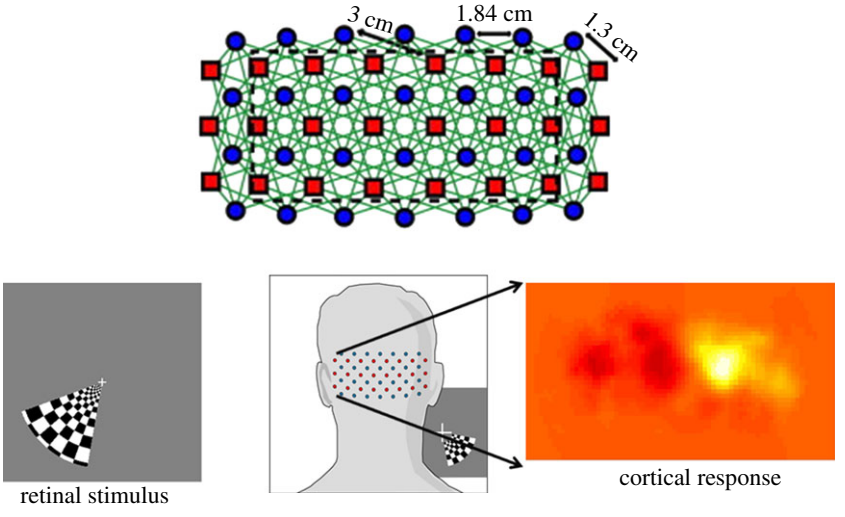

Figure 3. High-density diffuse optical tomography providing functional haemodynamic maps of the adult human visual cortex [8].

(iii) *Data analysis/image reconstruction*

Significant advances have been made in the methods used to model light transport in complex tissue structures and to reconstruct images from diffuse optical data [9]. However, it was noted that currently there exists no standardized approach to the analysis of data from multi-channel systems either for creating absolute tomographic images or topographic maps, or for determining the statistical significance of changes in haemodynamics and oxygenation. A range of methods have been developed for co-registering surface-measured optical signals with underlying anatomical structures [10,11], which will become increasingly important with the rapidly expanding application of multi-channel systems for investigating functional activation in the adult and developing brain.

(iv) *Depth sensitivity*

The issue of depth sensitivity is relevant to measurements in both brain [12] and muscle, where the extracerebral and fat layers, respectively, can have a significant influence. This is especially true in muscle where optical studies have frequently focused on the physiology of lean individuals, ignoring the complications of the enhanced adipose contamination of the optical signal in the general population [4]. One solution is to design probes to target sites where depth sensitivity is less crucial, e.g. the application of clinical muscle oximetry to a site where there is limited fat content even in obese individuals, such as the thenar eminence (see figure 2*a*).

The effects of superficial layers may be even more pertinent in pathology where changes in tissue geometry and light transport may be highly localized, dynamic and unpredictable (e.g. the variable contamination of cerebrospinal fluid by blood in vascular brain injury). Although there are known depth sensitivity benefits in using time and frequency resolved systems, it was noted that the more favourable signal-to-noise (SNR) characteristics and temporal resolution of CW systems continue to make them the instrument of choice for most users. However, whenever CW systems are used, the possible measurement confounds (e.g. the contamination of signals by changes in systemic physiology) must be accounted for. One approach is to use channels with short source–detector separations to selectively measure, and correct for, chromophore concentration changes in superficial layers. The advent of single-photon avalanche diodes may improve the technological options available for high-density multi-channel systems of this kind.

Time-resolved systems can clearly address the issue of depth selectively without the necessary complication of high-density arrays, and there is significant interest in exploiting new developments in source and detector technologies to provide effective systems at a reasonable cost. Although time domain instrumentation is inevitably more expensive, higher cost could be justified where significant benefits can be demonstrated, particularly in a clinical scenario where pathophysiological state-dependent studies are being performed (e.g. detecting deeper infarctions in stroke patients).

(v) *Wavelength selection and chromphore estimation*

The majority of commercial NIRS systems employ only two wavelengths, limiting their use to measurements of two chromophores, namely oxy- and

deoxyhaemoglobin. However, it is noted that many publications fail to report fully on even this rather limited dataset. For example, brain function activation studies frequently discuss only oxyhaemoglobin changes, whereas muscle studies typically report only deoxyhaemoglobin changes. It was felt that as a community we should ensure that analysis of both signals is presented to provide a more complete picture of the measured haemodynamic response.

Differential sensitivity of algorithms to different wavelengths of light can also result in confusion and bias. For example, some algorithms developed for muscle studies separate the small differences in light absorption by the haemoglobin and myoglobin chromophores [4]. Yet the optical differences between oxyhaemoglobin and oxymyoglobin are in the visible region while those between deoxyhaemoglobin and deoxymyoglobin are in the near-infrared region. Such algorithms may perform differently as different depths of tissue are probed.

The possibility of multiplexed laser wavelengths from a single time detection device and the utility of supercontinuum sources for improved wavelength selection were discussed, and may be more widely adopted where data acquisition rates and cost are acceptable.

(vi) *Ambulatory monitoring*

One of the major strengths of NIRS technology is its adaptability to portable, wearable devices suitable for monitoring ambulatory subjects, and was considered to be a very significant area for future development. Ambulatory devices for muscle monitoring were first developed in the 1990s by Hamaoka *et al.* [13], but only recently have robust, commercial systems become widely available [14]. Aside from the obvious application in monitoring (both the brain and the muscle) during exercise, it was expected that wireless systems would find application in a range of other subject groups, e.g. neurodevelopment studies in young infants and children, brain computer interfacing, simultaneous brain activation studies on multiple subjects, rehabilitation via gait analysis in stroke, biofeedback, at home therapy, etc. The possibility of an NIRS-based smart phone application was also discussed.

(vii) *Combined monitoring systems*

NIRS techniques lend themselves well to combination with other imaging modalities, e.g. functional magnetic resonance imaging and electroencephalography (EEG). Some commercial systems can be supplied with magnet-compatible fibres, and a co-localized NIRS and EEG research system has been developed [15]. This was viewed as an exciting prospect particularly in the field of functional brain imaging where combined functional NIRS and EEG may elucidate further the mechanisms of neurovascular coupling, particularly in the developing brain.

(viii) *Cost implications*

The commercial NIRS market continues to be driven predominantly by cerebral oximetry and mapping of cortical haemodynamics. Portable systems provide excellent prospects for expanding the application of the technology, but it was felt that a balance must be struck between simple, inexpensive measurement systems and those which deliver tissue-specific information which have real value

in informing physiological and pathophysiological processes. The challenge was identified in translating highly specified research instruments into commercially viable systems for general use, particularly where there are financial barriers for small start-up companies. There was a strong sense that the market could support technically complex and necessarily more costly technology (e.g. multi-wavelength and/or multi-channel time or frequency domain hybrid systems) if they were shown to deliver reliable, useful and relevant measures, especially for applications where high cost systems are already routinely used for monitoring or diagnosis (e.g. neurocritical care).

3. The human subject

(*a*) *Are we measuring the important?*

The opening sentence of the Preface of the proceedings of the 1996 meeting stated that 'near-infrared spectroscopy enables non-invasive observations to be made of *important* indices of tissue oxygenation and haemodynamics' [1].

The physiological parameters currently measured using NIRS systems include: absolute measures of tissue oxygen saturation and blood volume and trend measurements of the concentration of oxyhaemoglobin (HbO_2), deoxyhaemoglobin (HHb) and oxidized cytochrome *c* oxidase (oxCCO), and blood flow index (BFI) (figure 1). Much of the discussion at this 2010 meeting focused on assessing whether these really are '*important*' indices, and how they can be used effectively to provide useful information for clinical and life science applications. The dangers of making the measurable important rather than measuring the important were discussed. For example, since 1996 techniques have been perfected to deliver a percentage measure of absolute tissue haemoglobin oxygen saturation (SO_2) but, as discussed below, it remains unclear exactly what *important* information this measure provides. Conversely, a commercial system has yet to be developed which provides a robust measurement of the oxidation status of cytochrome *c* oxidase even though this parameter has the potential to inform directly on cellular oxygen metabolism. Clearly, we need to ensure that systems are developed to address targeted questions in the clinical and life sciences, and for clinical applications, address an *unmet* clinical need. There was significant interest in identifying relevant clinical studies which can test whether the parameters delivered by optical measures can inform or guide clinical management. Currently, there is a paucity of outcome study data for optical measures, although it was noted that this has not prevented other monitors from entering routine clinical use.

Discussions surrounding the clinical applications of NIRS measures highlighted the complexity of these issues and may have led to more questions than answers. What is the value of a single index? Can threshold values be related to outcome, and can the same threshold be applied across a range of pathologies in the same organ? Can useful clinical information be extracted from continuous bedside measurements of multiple NIRS parameters? Does NIRS have more value as a continuous monitor or as a diagnostic tool? Are NIRS measures only of value when used as an adjunct to other physiological measures and do they add value to these measures? There was, however, a consensus that many of these issues can only be addressed with large-scale, multi-centre trials.

(*b*) *Absolute tissue oxygen saturation*

In 1996, some absolute measure of tissue haemodynamics was highlighted as an important future prospect in NIRS [3]. As previously mentioned, this has been realized in the form of an absolute haemoglobin oxygen saturation measure which has found a commercial market in cerebral oximetry (see table 1). Discussions from the 1996 meeting indicated that, although this was a measure that clinicians wanted, it was unclear how this measure would be used to inform patient management. In its simplest form SO_2 is a percentage measure of the proportion of haemoglobin, within the sampled volume, which is oxygenated,

$$SO_2 = \frac{HbO_2}{HbO_2 + HHb} \times 100\%.$$

This description belies the complexity of this parameter, which can be influenced, for example in measurements in the brain, by arterial oxyhaemoglobin saturation (SaO_2), cerebral blood flow (CBF), venous blood volume (V_v), arterial blood volume (V_a), cerebral metabolic rate for oxygen ($CMRO_2$) and the oxygen-carrying ability of haemoglobin (k),

$$SO_2 = SaO_2 - \left(\frac{V_v}{V_a + V_v}\right)\left(\frac{CMRO_2}{k \cdot CBF \cdot [Hb]}\right) \times 100\%.$$

The NIRS measurement of SO_2 may be confounded by optical geometry which may vary between tissue types, age of subjects and in health and disease. It was noted that the complexities of this parameter are not fully appreciated by all users; the common assumption of an 'intelligent' or 'inquisitive' clinician familiar with the nuisances of this measure may need to be re-evaluated.

The situation in muscle is even more complex, given the difficulty of separating haemoglobin and myoglobin optical signals [4]. One measure (haemoglobin) integrates information from blood flow and oxygen consumption; the other (myoglobin) is a pure measure of intracellular partial pressure of oxygen (pO_2). The optimistic view of this dilemma is that—were the optical complexities to be satisfactorily resolved—a much deeper understanding of tissue oxygen metabolism is possible in organs that contain myoglobin. The difficulty of interpreting cytochrome *c* oxidase changes solely as a surrogate marker of cellular oxygen content is not present with myoglobin, whose oxygenation state is in rapid equilibrium with the pO_2. In fact, given the robustness to cellular conditions of the myoglobin p50, an absolute myoglobin oxygenation state is a direct readout of intracellular pO_2.

The use of a single measure of absolute SO_2 as a standard of care marker or robust predictor of outcome in a clinical environment is still not conclusive despite multiple studies in carotid endarterectomy, cardiac surgery and septic shock [16,17]. Concerns remain about the validity of the absolute number, particularly when taking into account the variety of different instruments which may be used for its measurement (see table 1). These issues are further complicated when the algorithms describing the derivation of this measure are not in the public domain. Concerns were also raised about test/retest variability and the

influences of probe position. Multiple studies have shown interesting changes relative to baseline but it is fair to say that the sensitivity and specificity of this measure has not yet been proved. Nevertheless, it was accepted that cerebral oximetry has already entered clinical general use. This is particularly true in adult and paediatric cardiothoracic medicine where widespread availability of systems has led, in some centres, to its routine use during surgical procedures and management of critically ill patients, even though opinion is divided as to its efficacy [18,19]. The question remains whether the use of cerebral oximetry is being driven by *faith* in the technology and the benefit it brings to patients, or *fear* of litigation.

Muscle oximetry is not currently in general clinical use. However, peripheral muscle oxygen saturation does predict the development of organ dysfunction in shock [20] and recent prospective observational studies suggest that altering management based on optical measurements may have the potential to play a useful role in patient care in both shock [21] and cardiac surgery [22].

Much of the discussion in this area reinforced the requirement for large multi-centre randomized controlled clinical trials in a range of patient groups. It was noted that, although these trials may be viewed as timely in some patient groups where a targeted question may be identified (e.g. intensive care management of preterm infants [23]), this may be more complicated in other clinical settings (e.g. adult neurocritical care [17]). Clearly, for any clinical trial, quality assurance of the chosen monitors and the measures they deliver is also of utmost importance.

Perhaps one of the most promising prospects for the use of an absolute measure of tissue oxygenation is in combination with other haemodynamic parameters to provide a marker of oxygen metabolism, e.g. combined SO_2 and BFI to estimate relative changes in $CMRO_2$ [16,24]. The influence of pathology on the models used to estimate these markers and the reliability of one-off measurements (rather than repeated measures from longitudinal studies) were discussed. The value of combining SO_2 with measures derived from other non-optical modalities (e.g. MRI-measured CBF and cerebral blood volume) was also considered.

(*c*) *Trend measurements*

Since its earliest description, trend measures of oxygenation and haemodynamics have been a central focus for NIRS measurements. More recently, the use of diffuse correlation spectroscopy has provided a trend measure of relative changes in blood flow in the form of a blood flow index.

(i) *Measurement of haemodynamic response to neuronal activation*

As predicted in 1996, one of the most exciting uses of trend measurements in haemodynamic variables has been in describing the haemodynamic response to neuronal activation, a field widely known as functional NIRS. The most significant explosion of interest in this field has been in studies of neurodevelopment. Despite the difficulties of making measurements in (semi-mobile) young infants, this remains one of the most promising applications of NIRS technology because it satisfies an unmet need; neuroimaging in infants is difficult with

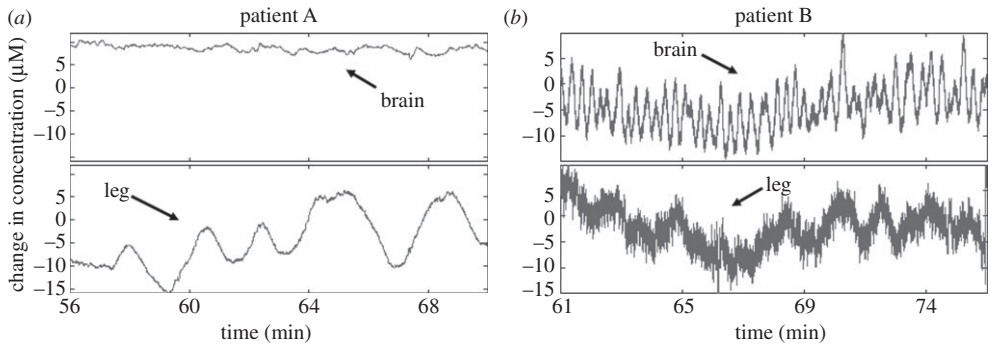

Figure 4. (*a*,*b*) Changes in HbO_2 concentration in the brain and leg from two patients undergoing extracorporeal membrane oxygenation. Systemic oscillations at varying frequencies can be seen in both patients; however, these appear to only be transmitted through to the cerebral circulation in patient B.

other modalities, and optical techniques which can provide data on functional activation, connectivity and resting state can be used to literally watch the brain develop [25]. The challenges of reducing attrition rates particularly in studies of awake infants were discussed. Typically the degree of data drop out can be associated with the type of stimulus used (i.e. worse if infant attention is poor) and the number of measurement channels used (bulky head gear is less well tolerated). In some cases, very large numbers of infants must be studied to provide statistically significant group data. Prospects for the future include studies which inform on individual rather than group behaviours, e.g. providing sufficiently robust measures of individual difference to predict outcome measures later in development. This debate is also relevant to applications in adults. The Japanese health ministry has approved NIRS as an advanced medical technology to assist the diagnosis of psychiatric disorders in adults [26]. However, there is concern that in the absence of multiple blind trials in large patient groups it is difficult to justify the current use of NIRS as a *diagnostic* neuroimaging technique, particularly in mental illness where misdiagnosis can have serious implications [27]. The practical advantages of the technology (portable, inexpensive, non-invasive, easy to use) must not outweigh the requirement for robust science-led studies with well-controlled paradigms to determine its efficacy in clinical diagnosis.

(ii) *Cerebral autoregulation and vasoreactivity*

The assessment of cerebral autoregulation and vasoreactivity was identified as another important use of trend measures. Figures 4 and 5 provide examples of how continuous, high temporal resolution trend measures are being used to assess the dynamics of the cerebral circulation in critically ill children and adults. Figure 4 shows data collected from two patients undergoing extracorporeal membrane oxygenation (ECMO) (M. Papademetriou, unpublished data). Spontaneous oscillations, at varying frequencies, can be seen in the systemic haemodynamics in both patients. The transmission of these oscillations to the cerebral circulation in one of the patients may indicate impaired cerebral autoregulation. Figure 5 shows the use of an NIRS measure of cerebral haemoglobin volume to provide

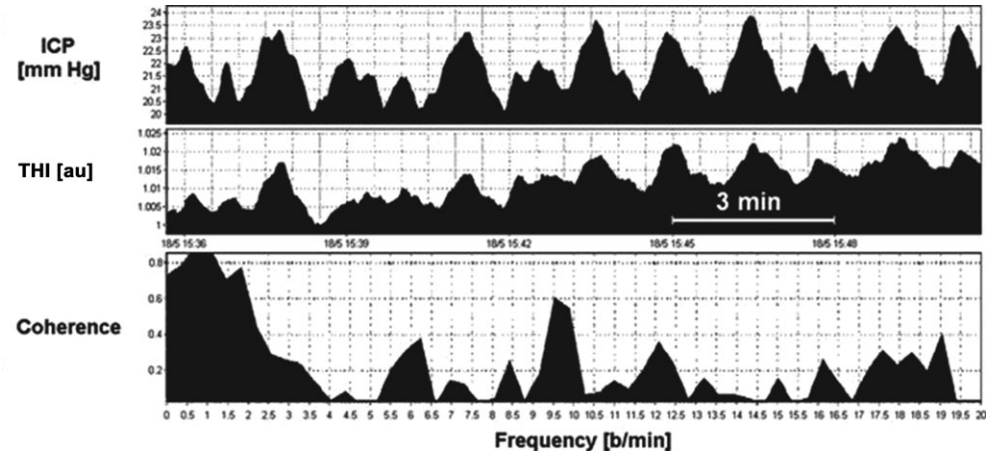

Figure 5. Slow wave oscillations in intracranial pressure (ICP) and cerebral total haemoglobin index (THI) measured in a brain-injured adult patient. The coherence analysis (calculated using a frequency band from 0.0055 to 3 cycles per min) demonstrates a good level of association between the two signals and can be used as a continuous and dynamic marker of cerebrovascular reactivity [28].

a dynamic monitor of cerebrovascular reactivity in a head-injured adult patient [28]. Studies of this kind demonstrate a good use of the technology (delivering continuous, non-invasive measures at the bedside with high temporal resolution) to address an unmet clinical need (i.e. the continuous and non-invasive assessment of cerebrovascular reactivity and autoregulation). Studies in a range of patient groups including those with stroke [29] are being performed to determine the role of NIRS techniques in elucidating important and clinically relevant pathophysiological processes related to vascular reactivity. These and other studies have been enhanced with the advent of combined diffuse correlation and absorption spectroscopy techniques to provide a measure of relative changes in blood flow as well as the usual oxy- and deoxyhaemoglobin trend measures [24], and there was significant interest in the future applications and potential commercialization of diffuse correlation spectroscopy methods.

(iii) *Cytochrome* c *oxidase*

In his 1977 *Science* paper, Jöbsis makes clear his target for the initial application of *in vivo* NIRS [30]: 'Because of the physiological importance of this enzyme (*cytochrome c oxidase*) and the favourable IR transmission characteristics in this range, it appeared useful to attempt to observe the oxygen dependent absorption peak in intact organs *in vivo*'. Since 1977, and as discussed at the 1996 meeting, there has been much debate about the validity of oxCCO measurements and in particular the choice of algorithms used for its derivation [31]. In 2010, this marker of cellular oxygen metabolism is clearly still viewed as having 'physiological importance', although the signal is not incorporated into any clinical monitors and has in fact been removed from the latest three generations of the NIRO systems produced by Hamamatsu Photonics. The signal continues to be investigated in neurocritical care [32] and during cardiac

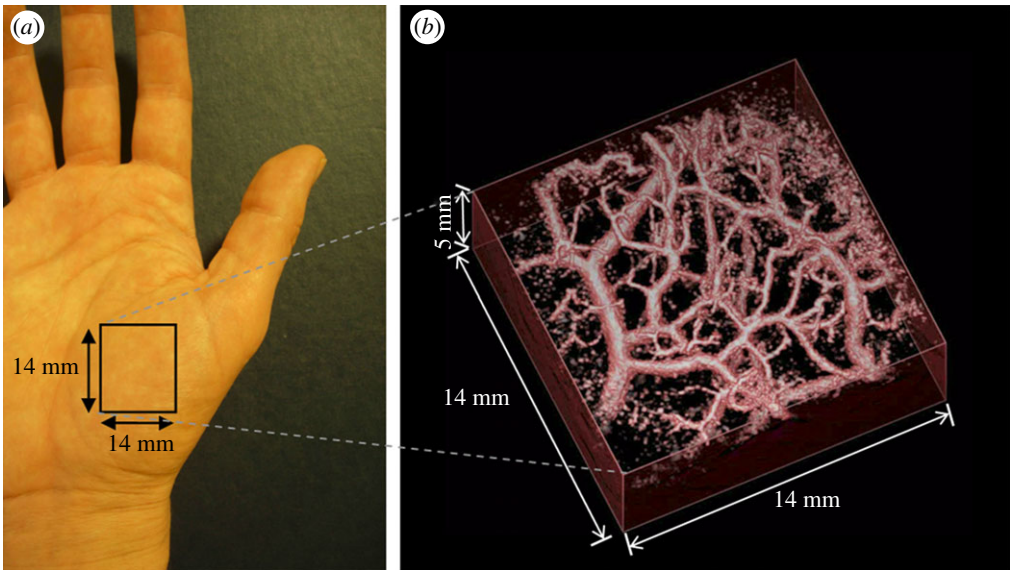

Figure 6. *In vivo* photoacoustic image of the vasculature in the palm using an excitation wavelength of 670 nm. (*a*) Photograph of the imaged region and (*b*) volume rendered image. Image courtesy of Paul Beard, University College London [42] (see the electronic supplementary material).

surgery where a relationship with post-operative neurological dysfunction has been demonstrated [33]. More recently, the use of a hybrid system which has been optimized for oxCCO measurement and incorporates broadband, frequency and spatially resolved technology is being investigated for studies in healthy volunteers [34] and brain-injured patients [35]. It was generally felt that pursuit of this metabolic marker using NIRS techniques was still worthwhile and there was some hope that ongoing developments in source and detector technology could exploit more pronounced visible spectra (e.g. at 605 and 655 nm) in studies of brain and muscle.

(iv) *Other endogenous markers*

The vast majority of NIRS devices aim to convert attenuation changes to chromophore concentration changes using *in vitro* red blood cell, haemoglobin or cytochrome *c* oxidase spectra. Occasionally, algorithms are proposed that use chemometric techniques accompanied by an *in vivo* validation [36]. Although in principle these could allow novel changes in tissue parameters to be estimated (e.g. pH), this approach has not been adopted generally. At the 1996 meeting there was interest in using scattering as a useful NIRS parameter in its own right, to report on functional changes following neuronal activation or cell swelling following brain damage [37]. However, the sensitivity of these signals has not proved susceptible to robust investigation of functional changes in deep tissues.

(*d*) *Data interpretation*

Data interpretation of NIRS measures was highlighted as one of the major challenges for the future, with emphasis on the target of extracting clinically

relevant information which can be used to guide patient management. It has already been noted that those signals which may be easiest to measure (e.g. SO_2) may be the most difficult to interpret. The lack of direct comparative measures for experimental validation of most NIRS signals also presents a significant challenge.

One approach is the development and application of physiology-informed mathematical models [38]. These may be useful for several reasons: (i) NIRS signals are rarely measured in isolation and the use of a model able to simulate, for example, vascular, regulatory and metabolic processes may aid the interpretation of multi-modal datasets; (ii) models can be used to simulate the behaviour of difficulty to validate experimentally measured signals and hence allow useful comparison between simulated and experimentally measured datasets; (iii) models can be used to provide estimates of parameters which are more difficult to measure experimentally (e.g. $CMRO_2$); and (iv) models may be used to build capacity for delivering individualized patient-specific clinical information.

It was noted that alternative approaches include signal-processing software [39] which provides a real-time assessment of specific processes, e.g. CBF regulation [40] or 'track and trigger' systems which employ statistical techniques to characterize (track) normality in multi-parameter patient datasets and subsequently identify deviations which may require medical intervention (trigger) [41]. These approaches may not fully incorporate *a priori* knowledge of physiological and pathophysiological processes but may still be used to extract clinically relevant information from measured signals.

4. Pre-clinical and biological systems

(*a*) *Why study pre-clinical systems?*

Pre-clinical measurements in animals have several advantages over clinical studies. In some cases the size of the animal enables imaging modalities to be employed that are not readily available in the human; even if they do become available the ultimate resolution is likely to be worse. A relatively new modality that is particularly amenable to pre-clinical imaging is photoacoustic imaging. This is a consequence of the small size of the animal which enables high SNR and spatial resolution to be achieved, the latter being a consequence of the much lower scattering of acoustic waves compared with light propagation in tissue. Photoacoustic techniques also offer the genuine prospect of multi-scale imaging from the organelle to the cell, tissue and organ (figure 6; [42]) [43–45].

Pre-clinical studies also allow light to be directly targeted to the areas where monitoring is required. For example, removing the skull allows for direct measurements of cortical tissue; a range of molecular and spectroscopic tools—impossible in human studies—can then be used to study the mechanism of functional activation. The fine details of the temporal and spatial dynamics of neurovascular coupling can then be dissected by combining haemodynamic markers with fluorescent probes of neuronal activation. These studies are not only interesting in their own right [46], but can be translated to less well-characterized systems. For example, the ability to image the different

layers of the haemodynamic response to functional activation can inform interpretation of the necessarily less specific information which can be obtained from studies in humans.

Although optical studies in animals are increasingly focusing on addressing basic biomedical science and drug development, there are still many cases where they are used as an intermediate step towards development of new clinical methods. Examples discussed at the meeting included dynamic contrast-enhanced small optical imaging (DYCE; [46]), Cerenkov luminescence imaging [47] and normalized epi-illumination imaging [45].

(*b*) *Optical molecular imaging*

A view that more use could be made of the developing optical technologies by combining them with more invasive clinical instrumentation such as endoscopes or fibre-based catheter systems [45] was expressed at the meeting. More direct access to the clinical target enables more sensitivity and precision. A good example is optical coherence tomography (OCT), where the incoherent scattered light can be filtered, enabling an image based purely on reflected light. The low intensity of this reflected light limits OCT to tissues—such as the eye—where there is ready access for both transmitted and reflected light. However, accessing the clinical target more directly also enables a wider range of endogenous molecular markers to be used. Rather than being restricted to the NIR spectra of haemoglobin and cytochrome *c* oxidase, there is considerable potential for measuring endogenous molecules that fluoresce outside the near-infrared region. For example, the molecule lipofuscin fluoresces in the fundus of the retina; changes in this autofluorescence can diagnose macular dysfunction in the eye [48]. Such targeted optics can in principle access other endogenous chromophores that can report on the mitochondrial energy state (NADH fluorescence [49]) or the mitochondrial pO_2 (porphyrin fluorescence [50]).

However, there is a limit to the number of endogenous chromophores and fluorophores. There has therefore been great interest in the development of a wide variety of new exogenous probes that can report on tissue function; these extrinsic probes can either be added exogenously or be attached to genes of interest in animal models. At the 1996 meeting this new field was represented by just a single paper [51]. Since 1996, however, growth has been dramatic [52]; over 2000 people recently attended the World Molecular Imaging Conference, with optics being strongly represented. Optical molecular imaging is a highly fruitful merger of advances in the field of cellular microscopy, molecular genetics and biomedical optics, the last playing the key role of translating and validating cellular studies in the whole organism.

Initial studies used the fluorescent properties of firefly luciferase, but now the most common fluorophores used to tag genes of interest are the derivatives of green fluorescent proteins. Natural and artificial mutations allow for a wide range of fluorescence proteins with the increasingly longer wavelengths enabling tissue penetration (figure 7) even in large animals such as monkeys. It is possible to add fluorophores to specific genes allowing for real-time studies of gene expression and protein–protein interactions [53]. An alternative approach is to synthesize chemical probes that are sensitive to enzymatic activity in the body. Very sensitive assays become possible if the molecule only fluoresces in the presence of the relevant substrate or enzyme [52].

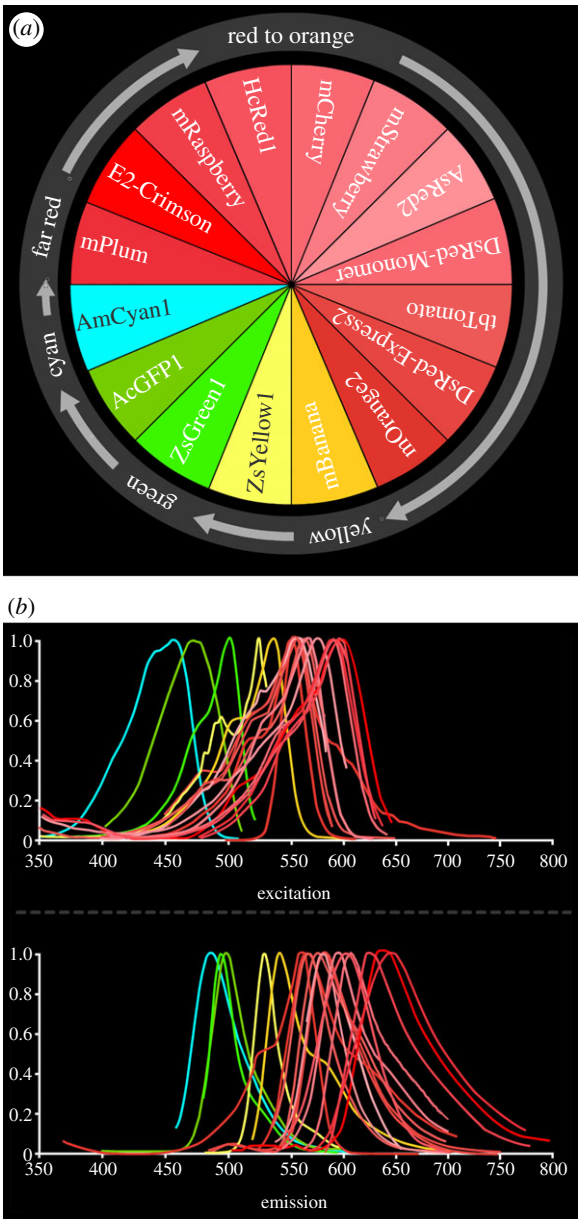

Figure 7. (*a*) Colour wheel indicating the range of colours of fluorescent proteins. (*b*) Excitation and emission spectra for proteins in (*a*); the *x*-axis is the wavelength (nm) and the *y*-axis is the normalized absorbance (or fluorescence). For molecular imaging *in vivo* excitation and emission spectra in the red and far red are favoured (such as mCherry and tdTomato). Images © 2011 Clontech Laboratories, Inc.

The commercialization of detection systems for these chromophores by companies such as Li-Cor, Carestream, Perkin Elmer, ART and Caliper has resulted in a revolution in the methods available to *in vivo* biological science; interactions that are postulated to occur in the test tube, and their resultant

biological hypotheses, can now readily be tested in the intact organism. However, for human studies chemical probes have safety concerns and genetic probes have obvious ethical constraints.

Nevertheless, molecular imaging is already having a direct impact on human health [53]. Pharmaceutical companies are keen to switch away from a policy of chasing a small number of very high risk 'blockbuster' drugs to a variety of more tailored targeted therapies. Molecular imaging is a major enabling tool in this strategy. The imaging modality is not restricted to optics. If higher resolution is required the same probe can be interrogated by photoacoustics, taking advantage for example of spectroscopic photoacoustic imaging [45]. Alternatively, it is a relatively simple task to modify an exogenous optical probe to one suitable for positron emission tomography (PET)/single-photon emission computed tomography (SPECT) studies. Once specific drug targets and enzyme activities can be visualized in whole animals, it is possible to do real-time high-throughput *in vivo* enzyme kinetics. Determining quickly whether a drug interacts with a cellular target *in vivo* (e.g. does it cross the blood–brain barrier) can take months off drug development time and, equally crucially, result in ineffectual drugs being discarded prior to human clinical trials. The outcome is cheaper, and hopefully more effective, drug discovery programmes [53]. Consequently, molecular probes that can accelerate the drug discovery programme are big business; witness Eli Lilly and Company's upfront payment in November 2010 of $300 million to acquire Avid Radiopharmaceuticals, Inc., whose lead product is a PET-sensitive molecular imaging agent for the detection of the presence of amyloid plaques in the brain.

(*c*) *Clinical studies*

It is theoretically possible to trial a drug's safety in combination with the fluorescent probe that can determine its activity *in vivo*. This would have the potential to optimize and individualize therapy. However, this is not a route that has been followed to date. We are therefore left with very few optical probes that are approved for clinical research and diagnostic use. The advantages of a small number of general probes versus a larger number of specific probes were debated at the meeting. However, at present the argument is academic; for measuring deep tissue, human studies are restricted to the clinically approved near-infrared chromophore indocyanine green (ICG). Nevertheless, ICG has proved to be incredibly versatile. It has the capability to measure brain perfusion in stroke, yield enhanced contrast in optical mammography and improve assessment of rheumatoid arthritis in finger joints. It was noted at the meeting that the ability of ICG to visualize tumour vascularization could revolutionize optical procedures in medicine, effectively allowing for real-time interactions between optical biopsy and surgical procedures [54].

5. Future near-infrared spectroscopy meetings

During discussions at the 2010 meeting it was noted that—apart from simple tissue oximeters—the information provided by many of the current advanced optical techniques is not trivial for the clinician to interpret. It is unclear whether future generations of clinicians would receive specific training in optical

technologies (as they currently do for other imaging modalities) or whether there should be a separate training programme for a class of optical technicians. In either case a regular forum to enable discussions of the latest advances in NIRS technology would be of benefit to this growing scientific community. However, in part because the field of NIRS and imaging is so interdisciplinary, there has been no single meeting which has brought together the critical mass of researchers necessary to discuss the basic physics and engineering underpinning technological development and the applications of that technology in the full range of clinical and life sciences. During discussions at this 2010 meeting, it was decided to formulate plans for a biennial international NIRS conference attracting attendees from a wide range of backgrounds, all sharing a common interest in mapping the long-term development and application of optical technologies in advancing biological and medical science. An initial meeting was held at Harvard College, MA, USA, in October 2010 and a second meeting is being planned for October 2012 at University College London, UK. Further information can be found at www.fnirs.org.

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