



Non-invasive monitoring of blood oxygenation in human placentas via concurrent diffuse optical spectroscopy and ultrasound imaging

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Direct assessment of blood oxygenation in the human placenta can provide information about placental function. However, the monitoring of placental oxygenation involves invasive sampling or imaging techniques that are poorly suited for bedside use. Here we show that placental oxygen haemodynamics can be non-invasively probed in real time and up to 4.2 cm below the body surface via concurrent frequency-domain diffuse optical spectroscopy and ultrasound imaging. We developed a multi-modal instrument to facilitate the assessment of the properties of the anterior placenta by leveraging image-reconstruction algorithms that integrate ultrasound information about the morphology of tissue layers with optical information on haemodynamics. In a pilot investigation involving placentas with normal function (15 women) or abnormal function (9 women) from pregnancies in the third trimester, we found no significant differences in baseline haemoglobin properties, but statistically significant differences in the haemodynamic responses to maternal hyperoxia. Our findings suggest that the non-invasive monitoring of placental oxygenation may aid the early detection of placenta-related adverse pregnancy outcomes and maternal vascular malperfusion.

Abnormal placental development is widely accepted as the cause of common adverse pregnancy outcomes (APOs) such as hypertensive disorders, fetal growth restriction and stillbirth. Moreover, placental dysfunction has been associated with morbidities in offspring, including perinatal mortality and long-term neurodevelopmental and cardiovascular consequences^{1–3}. To better understand human placental dysfunction associated with these APOs, non-invasive methods that measure placental oxygen dynamics are needed. Ultrasound imaging is the primary clinical modality used for assessing pregnancy. Although ultrasound imaging can provide indirect information about flow resistance in large blood vessels, it is used primarily to derive morphological information. In fact, clinical ultrasound guidelines do not include direct functional assessment of the placenta⁴.

Current knowledge about placental biology has been gleaned largely from *ex vivo* tissue and from animal research^{5,6}. Yet these models have limitations for the understanding of dynamical changes in placental pathophysiology during pregnancy^{7,8}. Similarly, the literature on placental oxygenation is derived largely from decades-old sheep studies and from scant human data using invasive sampling techniques that have yielded varying results^{7,9}. In addition, magnetic resonance imaging (MRI) approaches for assessing placental oxygenation^{10,11} are poorly suited for bedside monitoring and generally rely on indirect signals¹².

Here we report the development of an instrument and methodology based on diffuse optical spectroscopy (DOS) and ultrasound (US) that facilitate the measurement of oxygen haemodynamics in complex organs such as the placenta, which is buried far below the

tissue surface amidst intervening layered tissues. DOS measures oxy-haemoglobin and deoxy-haemoglobin concentrations, and has been successfully employed for the assessment of tissue haemodynamics^{13–21} in clinical problems, such as breast cancer diagnosis and therapy monitoring^{19,22}, brain function²³ and injury monitoring¹⁷. In most of these applications, however, reflected light penetration is limited to less than 2 cm below the surface^{24,25}. The new instrumentation and algorithms that we report here provide improvements in methodology needed to measure the oxygen haemodynamics of the anterior placenta amidst intervening heterogeneous tissue layers. Our human clinical measurements probed placental tissue located as deep as 4.2 cm below the surface, albeit more typically in the 2.3–3.3 cm range. These improvements enable continuous data collection for functional studies of the placenta at the bedside and may create opportunities for the investigation of haemodynamics in other deep organs.

Continuous-wave diffuse optical spectroscopy (CW-DOS) has been explored for the non-invasive measurement of placental blood oxygenation^{26–28}, and some of this early research suggested that higher placental tissue oxygen at baseline can arise in patients with intrauterine growth restriction^{26,28}. Although encouraging, these early measurements had limitations. The instruments used comparatively short source-detector separations (~4 cm) on the tissue surface, which limited the penetration of light to ~2 cm. Furthermore, the analysis of CW data requires major assumptions about tissue homogeneity and tissue scattering that prevent the quantification of absolute oxy-haemoglobin and deoxy-haemoglobin concentrations²⁹ and do not account for the layered morphology of

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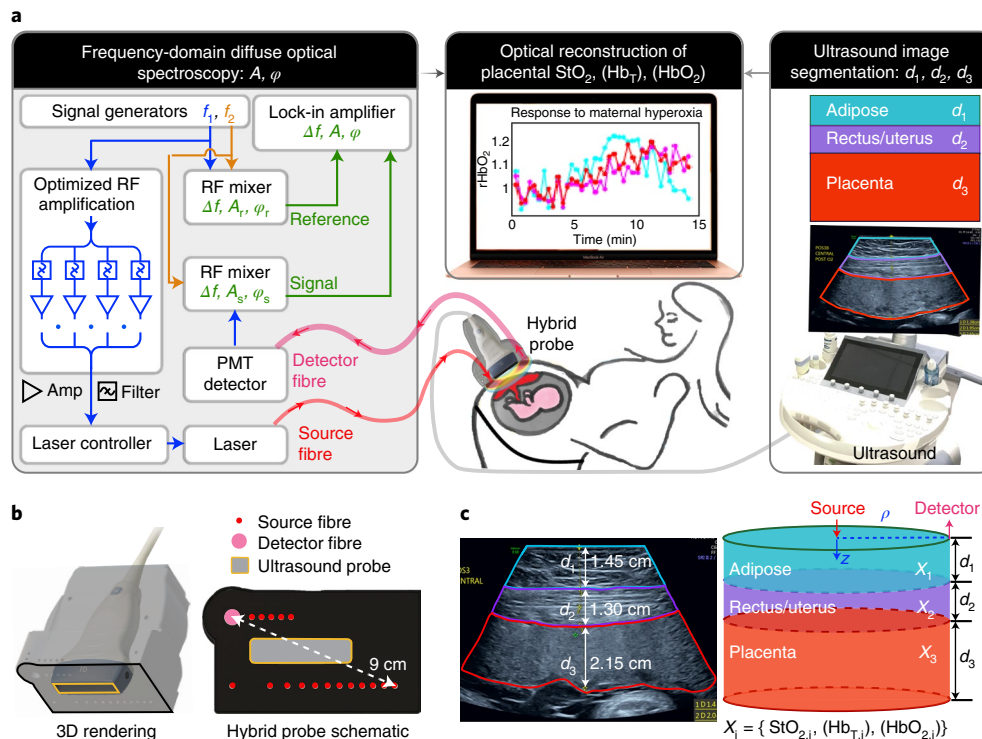


Fig. 1 | Integrated FD-DOS/US placenta instrumentation and three-layer modelling. **a**, Schematic of the hybrid FD-DOS/US instrumentation showing laser-amplitude-modulation electronics, tissue light transmission, PMT detection, heterodyne mixing for frequency down-conversion, lock-in detection of light wave amplitude/phase, simultaneous ultrasound-image segmentation and optical-image reconstruction of placental haemoglobin properties. **b**, 3D rendering and bottom-view of the probe showing FD-DOS/US integration into a single-probe head. **c**, Ultrasound image showing anatomical 'segmented' regions (left) and the corresponding three-layer model for optical-image reconstruction (right).

the abdomen. This early work also pointed to clear avenues for improvement. Our work benefits from a more accurate implementation of light-transport models^{13–15,30}, and from sophisticated frequency-domain and time-domain (FD-DOS, TD-DOS) optical instrumentation that permits the relaxation of assumptions about tissue scattering and homogeneity^{13,14,31}. Tissue-phantom experiments indicate signal-to-noise ratios (SNRs) sufficient to permit source-detector separations (SDSs) as large as 10 cm, which could enable light penetration of ~ 5 cm, thus improving on previous SDS records^{32–34}. Importantly, the optical instrument is integrated with ultrasound imaging in the same probe head²². This multi-modal FD-DOS/US combination facilitates the integration of anatomic ultrasound information about tissue-layer morphology with functional haemodynamic information about deep tissues from FD-DOS. The anatomic information enables tissue-specific and layered image reconstruction that separates the haemodynamic properties and responses of deep tissues, such as the placenta, from those of overlying layers.

We validated the methodology in layered tissue phantoms, and show its feasibility and utility by direct in vivo assessment of human placental oxygenation in 24 participants. Specifically, we measured placental oxy-haemoglobin [HbO_2] and deoxy-haemoglobin [Hb] concentrations or, equivalently, total haemoglobin [Hb_T] concentration and oxygen saturation (StO_2). We performed reproducibility and stability tests to characterize the technology. We also collected average tissue properties from each woman. We also show the detection of changes in dynamic placental oxygenation in maternal hyperoxia experiments by varying the maternal position. Notably, our pilot study shows that placental oxygen haemodynamics during maternal hyperoxia are significantly associated with placenta-related APOs and with placental maternal vascular malperfusion (MVM), a primary histopathologic pattern characteristic of placental dysfunction strongly

associated with APO and with the risk of long-term disease^{35,36}. Our findings suggest the possibility of the non-invasive detection of placental dysfunction for the generation of improved clinical understanding of placental pathophysiology in vivo.

Results

FD-DOS instrumentation. To measure placental oxygen haemodynamics in vivo, we built a low-noise heterodyne instrument for frequency-domain diffuse optical spectroscopy (FD-DOS). Tissue-phantom experiments showed that the instrument has a sufficient dynamic range and SNR to perform accurate FD-DOS measurements at SDSs of 10 cm. In the clinic, these capabilities facilitated quantitative determination of [HbO_2], [Hb] and [Hb_T] concentrations as well as StO_2 of anterior placental tissue located as deep as 4.2 cm below the skin surface. Figure 1a shows key features of the custom heterodyne FD-DOS instrument (details in Methods). It employs three lasers with wavelengths of 785, 808 and 830 nm. The output of each laser is radiofrequency (RF) amplitude-modulated at $f_1 = 100$ MHz. A new technical feature of the instrument is its nearly 100% laser-modulation depth. To achieve this improvement, we divided the source driver signal into four sub-signals, amplified each sub-signal in multiple stages with low-noise linear amplifiers, and then recombined and impedance-matched the amplified sub-signals for input to the laser drivers. Each laser's RF driver power was individually optimized to achieve $>90\%$ light-modulation depth, thereby increasing the modulated diffusive-wave amplitude and decreasing the (unmodulated) background diffuse light. As a result, measurement SNRs were better than in previous work^{32,33} (specifically, by more than 20 dB for an SDS of 8 cm), enabling longer distance SDS measurements with low laser powers (~ 40 mW).

The optoelectronic components were fibre-coupled into a custom optical probe head, within which a commercial ultrasound

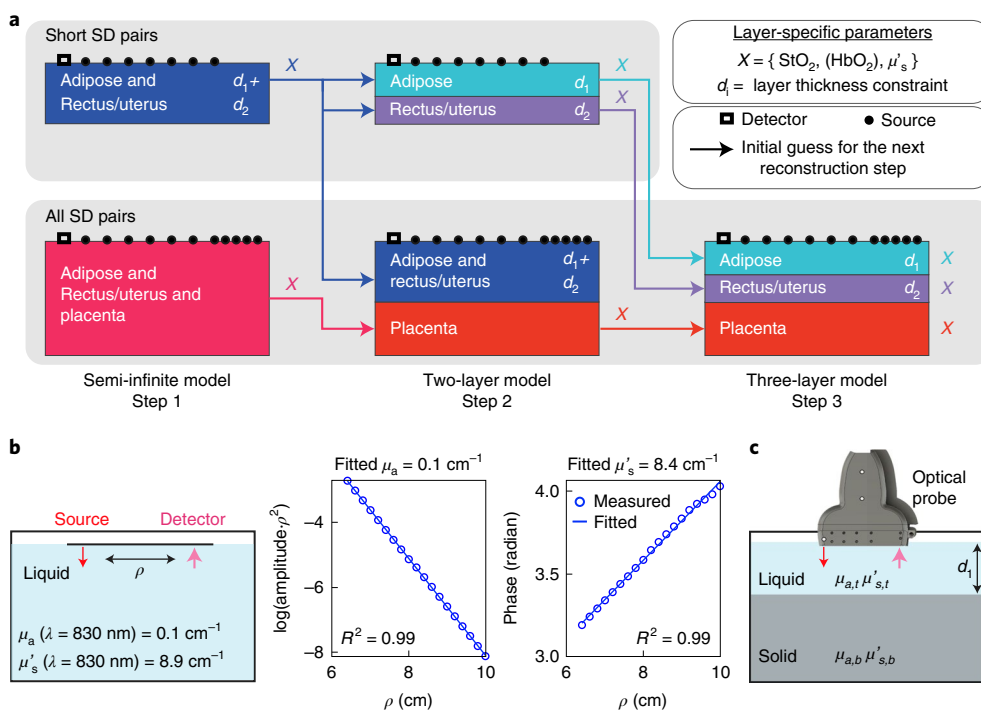


Fig. 2 | Three-layer model reconstruction algorithm and phantom-validation experiments. **a**, Three-layer model initialization and reconstruction procedures. **b**, Tissue-simulating phantom experiment for validating the accuracy and depth sensitivity of the optical properties. A translation stage moves the source fibre across the phantom for measurements at SDSs ranging from 6.2 cm to 10 cm. Nonlinear semi-infinite fitting is performed to reconstruct phantom absorption and scattering coefficients ($\lambda = 830 \text{ nm}$). **c**, Schematic of a two-layer phantom experiment.

probe was mounted. Optical source fibres in this probe offer 17 SDSs for measurements ranging between 1 and 9 cm in humans (Fig. 1b). During measurements in the participants, 10 source-fibre locations were chosen to optimize coverage over the anatomic regions of interest for each woman, and we scanned sequentially through them. At the end of each cycle, a dark count measurement is made to correct for systemic noise. A high-transmission liquid light guide (detector fibre) with 5 mm in-core diameter collects and directs light to a photomultiplier tube (PMT) detector. The PMT electrical signal is mixed with another RF wave at $f_2 = 100.2 \text{ MHz}$ to generate heterodyne down-converted signals ($\Delta f = f_1 - f_2 = 0.2 \text{ MHz}$). A high-sampling-rate lock-in amplifier captures amplitude and phase of the diffuse light waves.

Concurrent optical and ultrasound imaging. Custom integration of optics with a commercial ultrasound system (9L-D probe, Voluson E10, GE Healthcare) provides another substantial technical improvement over previous work³⁷. The custom probe facilitates concurrent measurement of tissue layer morphology and tissue physiological properties. This concept has been employed in breast cancer research²², but much deeper light penetration is required for the placenta. The ultrasound transducer at the probe's centre generates images (Fig. 1c) that we used to segment target tissue into distinct layers that constrain optical reconstruction algorithms.

Differently from previous placenta research^{26–28}, we used tissue layer morphology from ultrasound imaging to constrain the photon-diffusion tomographic inverse problem. In practice, we model the abdomen as three layers: adipose, rectus/uterus and placental tissue. We approximate each layer as homogeneous and laterally infinite, but with thickness and depth determined by ultrasound (Fig. 1c).

The measurement geometry and modelling are shown in Fig. 1c. ρ is the SDS on the tissue surface. With measured layer thicknesses and optical and physiological properties for each layer as

inputs, standard methods^{38,39} can be readily employed to generate predictions for the detected light-fluence rate on the tissue surface. Figure 2a outlines our three-step reconstruction procedure (details in Methods). Briefly, each step of the three-step reconstruction finds ‘best’ tissue properties by minimizing the differences between the measured data and the predictions of diffuse optical tissue models of increasing complexity.

Step 1 assumes that the underlying tissue is semi-infinite and homogeneous, and employs the simplest analytical model for optical-property reconstruction. Step 2 uses solutions from Step 1 as initial guesses in a two-layer diffuse optical tissue reconstruction. Step 3 uses solutions from Step 2 as initial guesses in a three-layer diffuse optical tissue reconstruction. In all steps, layer thicknesses are fixed on the basis of ultrasound imaging, but other tissue properties are permitted to vary to determine the best physiological and optical property solutions for each layer. The 3-step reconstruction approach provides accurate determination of the optical haemoglobin properties for each layer while maintaining reasonable processing time. Moreover, the 3-step algorithm helps to prevent the fit-search from becoming trapped in local solution minima.

Importantly, the image reconstructions rely on the simultaneous fitting of data from all SDSs and all wavelengths. This multispectral multiSDS approach builds global constraints directly into the inverse problem and is critical for robust fitting⁴⁰. To avoid reconstruction overfitting, Tikhonov regularization is employed to reduce ill-posedness (fitting and regularization procedures are detailed in Methods).

Validation and characterization with tissue-simulating phantoms. We first characterized the performance of the FD-DOS instrument and its SNR by using homogeneous liquid phantoms composed of ink and 20% Intralipid (Baxter) (Fig. 2b); the phantom's optical properties are known on the basis of ink concentration (for absorption) and Intralipid concentration (for scattering)⁴¹.

In the first study, fittings based on semi-infinite homogeneous solutions of the diffusion equation were employed to reconstruct the phantom's optical properties (that is, the absorption coefficient μ_a (cm^{-1}) and the reduced scattering coefficient μ'_s (cm^{-1}))¹³. The data show good SNR at SDSs up to 10 cm (Fig. 2b), wherein the mean signal intensity was 28 times greater than the standard deviation of the measured intensity. The reconstructed optical properties at each wavelength had accuracies of 3%–9%.

In two-layer phantom experiments, a solid phantom with fixed optical properties was positioned inside a liquid phantom and the optical probe (Fig. 1b) was placed on the liquid surface (Fig. 2c). The optical properties of the liquid phantom are known on the basis of ink and Intralipid concentrations. The optical properties of the solid phantom were provided by the phantom manufacturer (INO). An absorption-titration experiment evaluated the instrument's sensitivity to the absorption coefficient, holding the overlayer liquid phantom thickness (3 cm) constant while incrementally increasing the absorption coefficient of the top layer. A depth-changing experiment tested the sensitivity to superficial-layer thickness; here, the liquid phantom had fixed optical properties and the superficial-layer thickness was increased from 1.5 cm to 3.0 cm. A deep two-layer phantom experiment verified the instrument's ability to extract deep-layer optical properties for a superficial-layer thickness of 4.3 cm. Each measurement was repeated three times; the resultant means and standard deviations are reported in Supplementary Table 1. These experimental results from tissue-simulating phantoms show that the instrument and algorithms extract deep-layer optical properties accurately, with errors of <10% in absorption and <15% in scattering. Semi-infinite fitting also produces an estimate of the optical properties, but as a weighted average of both layers and with a stronger weighting of superficial layers.

Validation with finite-element simulations. We generated simulated experimental data using a finite-element simulation tool (TOAST)⁴² that facilitated the creation of a three-layer model with segmented optical properties based on the layer morphology extracted from a participant's ultrasound image (Fig. 1c). Since the participant's layer interfaces are curved, we generated test data from curved layer interfaces (Fig. 1c, left). For the inverse problem, however, we assumed each layer interface to be flat (Fig. 1c, right). We carried out computer-simulation studies with simulated experimental data (Supplementary Table 2) based on the range of participant-derived haemoglobin optical properties observed in the clinical studies (that is, we employed median and upper/lower 75th/25th percentile properties from Supplementary Table 3). The three-layer reconstructions (Supplementary Table 2) produced good estimates for mean StO_2 , $[\text{Hb}_T]$ and $[\text{HbO}_2]$ in each layer, with errors of $\lesssim 18\%$ and more typically $\lesssim 10\%$. By contrast, the semi-infinite fitting generates a weighted average of the optical properties of all three layers and is more sensitive to superficial layers; its placental layer errors are comparatively large: 30–42%. The data presented in Supplementary Table 2 compare semi-infinite fitting to three-layer reconstruction for a moderate range of variation of placental $[\text{HbO}_2]$, and a moderate range of variation of $[\text{Hb}_T]$ and StO_2 in the overlayers. The four datasets in Supplementary Table 2 may be interpreted as four different participants with different haemoglobin properties. The three-layer reconstruction predicts placental properties with increased accuracy compared with semi-infinite fitting for all participants. Specifically, by comparing the three-layer reconstructions, estimates of $[\text{Hb}_T]$ and $[\text{HbO}_2]$ based on the semi-infinite fitting are systematically worse, with ~3-fold greater percent-error. Percent-errors in semi-infinite fitting estimates of StO_2 across these participants are similar or greater than the three-layer reconstruction estimates. Additionally, it is important to appreciate that semi-infinite fittings are not typically used; they benefit from data at very large SDSs, which is made

possible by our instrumentation. The simulated datasets can also be paired to compare two different dynamic conditions within the same participant, such as would arise in response to hyperoxia versus baseline. For this comparison, the three-layer reconstruction produces good estimates of $[\text{Hb}_T]$ and $[\text{HbO}_2]$ and their changes, but the semi-infinite fittings of placental $[\text{Hb}_T]$ and $[\text{HbO}_2]$ dynamics are, by and large, underestimated.

Placental oxygen dynamics in vivo. We performed a pilot clinical study of human placental oxygen-related haemodynamic properties (study design is detailed in Methods). The study enrolled women with singleton pregnancies in their third trimester and with anterior placentas. The central region of the placenta was targeted for monitoring. Adipose, rectus/uterus and placenta layers were characterized by ultrasound and FD-DOS.

Four experiments were conducted: (1) a reproducibility experiment ($n=18$); (2) a stability experiment ($n=24$); (3) a maternal left tilt experiment ($n=3$); and (4) a maternal hyperoxia experiment ($n=24$). The women in the hyperoxia study had a median (interquartile range (IQR)) gestational age of 34.5 (32.9, 35.4) weeks and pre-gravid BMI (body mass index) of 27.7 (24.6, 30.2). The median (IQR) depth of the anterior placentas was 2.7 (2.3, 3.3) cm beneath the skin surface and the nulliparity of the participants was 29.2%. Women in the maternal tilt study had gestational age of 37.0 (35.1, 37.3) weeks and pre-gravid BMI of 30.4 (29.2, 31.7); the median (IQR) depth of these anterior placentas was 2.3 (1.9, 2.4) cm beneath the skin surface.

Reproducibility was assessed by calculating the intra-class correlation coefficient (ICC) for 3 repeated measurements at the same placental location in 18 participants. Placental StO_2 , $[\text{Hb}_T]$ and $[\text{HbO}_2]$ were highly repeatable within participants (ICC ≥ 0.9).

Stability was assessed from the standard deviation (s.d.) of data derived during a continuous 10-frame (3.5 min) baseline measurement at the same location on each participant. This single-participant s.d. was then averaged over all 24 participants to derive a mean measurement s.d., which served as our stability criterion. The resulting mean values of s.d. for placental StO_2 , $[\text{Hb}_T]$ and $[\text{HbO}_2]$ were small (2.2%, 0.9 μM and 0.8 μM , respectively). Optically derived haemodynamic properties were stable at baseline (Fig. 3a), and $\geq 88\%$ of the 24 stability measurements had s.d. for placental StO_2 , $[\text{Hb}_T]$ and $[\text{HbO}_2]$ of less than 3%, 2.0 μM and 1.5 μM , respectively (Fig. 3b).

The maternal left tilt experiment is sensitive to positional changes in cardiac output and uteroplacental perfusion, which can lead to an increase in maternal cardiac output of up to 20% (ref. 43). We measured placental haemoglobin properties of 3 participants in the supine position and then had them tilt to the left lateral position without removing the probe. The averaged relative increases in StO_2 , $[\text{Hb}_T]$ and $[\text{HbO}_2]$ were 2.4%, 8.4% and 10.9%, respectively. Mean values of StO_2 , $[\text{Hb}_T]$ and $[\text{HbO}_2]$ for each participant before and after the maternal tilt are shown in Fig. 3c. Although the number of participants is small, we observed a trend; $[\text{Hb}_T]$ and $[\text{HbO}_2]$ increased in the lateral position ($P=0.05$ and $P=0.02$, respectively, two-sided paired-sample t -test), suggestive of an accompanying increase in placental perfusion with oxygenated maternal blood.

Finally, we measured placental haemodynamic responses to maternal hyperoxia. The participants were given 100% FiO_2 via a facemask for 20 frames (~7 min). We monitored haemoglobin concentrations continuously before, during and after maternal hyperoxia. Figure 4a,b present a case example of variations in StO_2 and $[\text{HbO}_2]$. Overall ($n=24$), the method easily resolved changes in placental blood oxygenation owing to maternal hyperoxia. StO_2 and $[\text{HbO}_2]$ were found to increase by a median (IQR) of 7.1 (4.9, 9.3)% and 1.9 (1.1, 3.3) μM , respectively.

Optical biomarkers of placental dysfunction. Another goal of the maternal-hyperoxia study was to examine associations

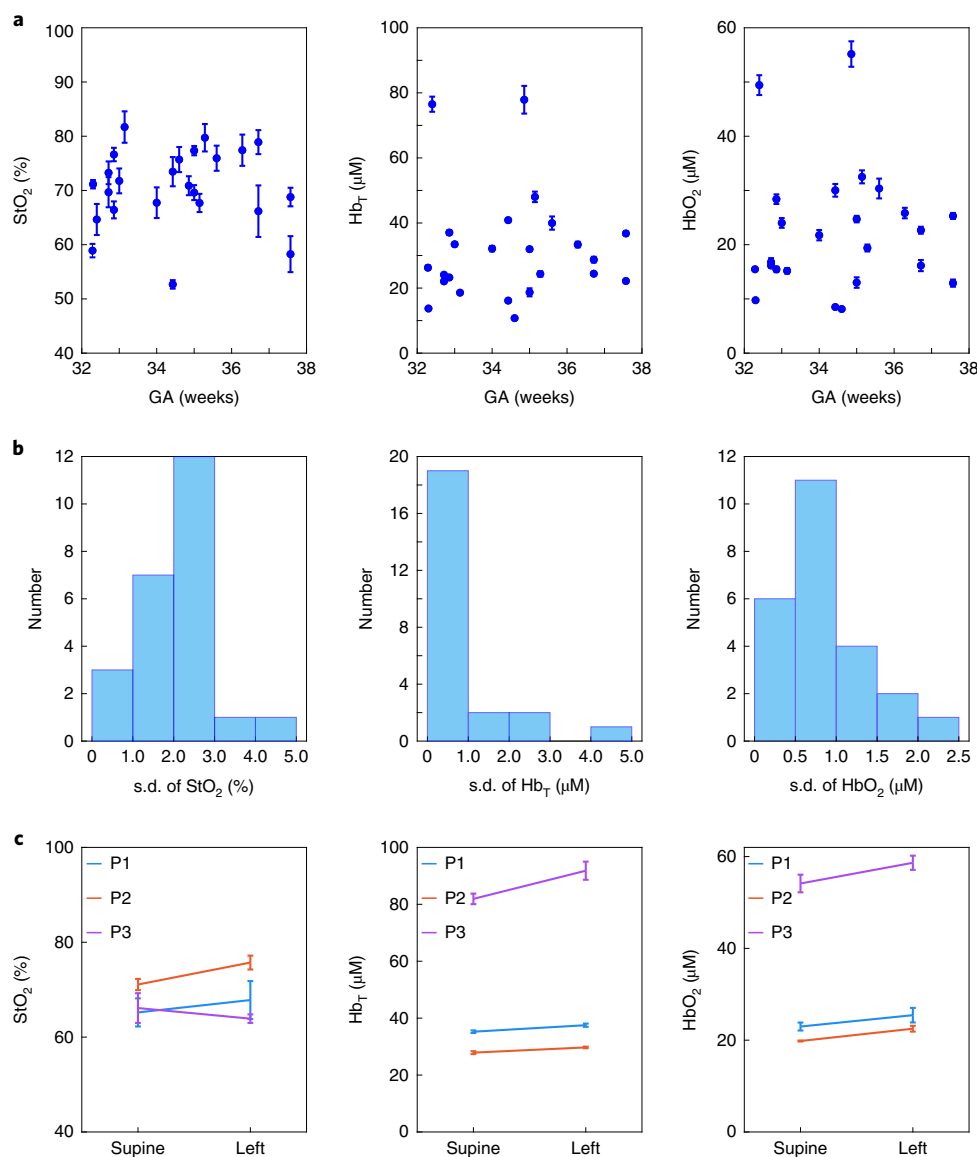


Fig. 3 | Placental haemoglobin properties during stability test measurements and the maternal left-tilt experiment. a, Mean of placental StO_2 , $[\text{Hb}_T]$ and $[\text{HbO}_2]$ versus GA derived from the stability test measurements ($n=24$; error bars indicate s.d.). **b**, Histograms showing the standard deviation of placental StO_2 , $[\text{Hb}_T]$ and $[\text{HbO}_2]$ obtained from the stability test ($n=24$). **c**, Mean of placental StO_2 , $[\text{Hb}_T]$ and $[\text{HbO}_2]$ before and after maternal left tilt ($n=3$; error bars indicate s.d.).

between placental oxygen dynamics and both APO and MVM. To this end, APO was defined as a composite of gestational hypertension (GHTN), preeclampsia or intrauterine growth restriction (IUGR), and MVM was determined from the examination of delivered placentas by a single placental pathologist (R.L.L.). None of the participants had clinical evidence of GHTN, preeclampsia or IUGR at the time of their ultrasound/FD-DOS measurement, and the mean time interval from optical measurement to delivery was 3.1 (2.1, 5.4) weeks. APO was found in 9 of the 24 participants; MVM was found in 8 of the 24 participants. Two of the 15 participants with normal pregnancy outcomes (NPOs) had MVM, and 3 of the 9 participants with APO had a normal placental pathology assessment (NPP).

We determined the absolute values of StO_2 , $[\text{Hb}_T]$ and $[\text{HbO}_2]$, as well as their variation relative to baseline during maternal hyperoxia; that is, $r\text{StO}_2$, $r\text{Hb}_T$ and $r\text{HbO}_2$. Relative data were obtained by normalizing their time-series values to the mean of the last four frames of the baseline period.

The 24 participants were categorized into two groups, according to their pregnancy outcomes: NPO or APO. Both $r\text{StO}_2$ and $r\text{HbO}_2$ increased substantially in response to maternal hyperoxia for the NPO group (Fig. 4c). However, the same parameters in participants with APO showed a more blunted response (Fig. 4d).

As for the participants' placental histopathology, the same 24 participants were categorized into two groups: NPP or MVM. We observed significant, large and positive $r\text{StO}_2$ and $r\text{HbO}_2$ in the NPP group (Fig. 4e). These same parameters, however, showed a blunted response in the MVM group (Fig. 4f). $r\text{Hb}_T$ was comparatively constant for all groups.

We next sought to quantitatively determine whether placental haemoglobin properties were significantly associated with APO. For this analysis, mean baseline StO_2 , $[\text{Hb}_T]$ and $[\text{HbO}_2]$ were calculated using the final 4 frames of the baseline period. Hyperoxia-induced changes from baseline (that is, ΔStO_2 , ΔHb_T and ΔHbO_2) were defined using mean values in the 4-frame window wherein maximum StO_2 occurred. Baseline StO_2 , $[\text{Hb}_T]$ and $[\text{HbO}_2]$ were not

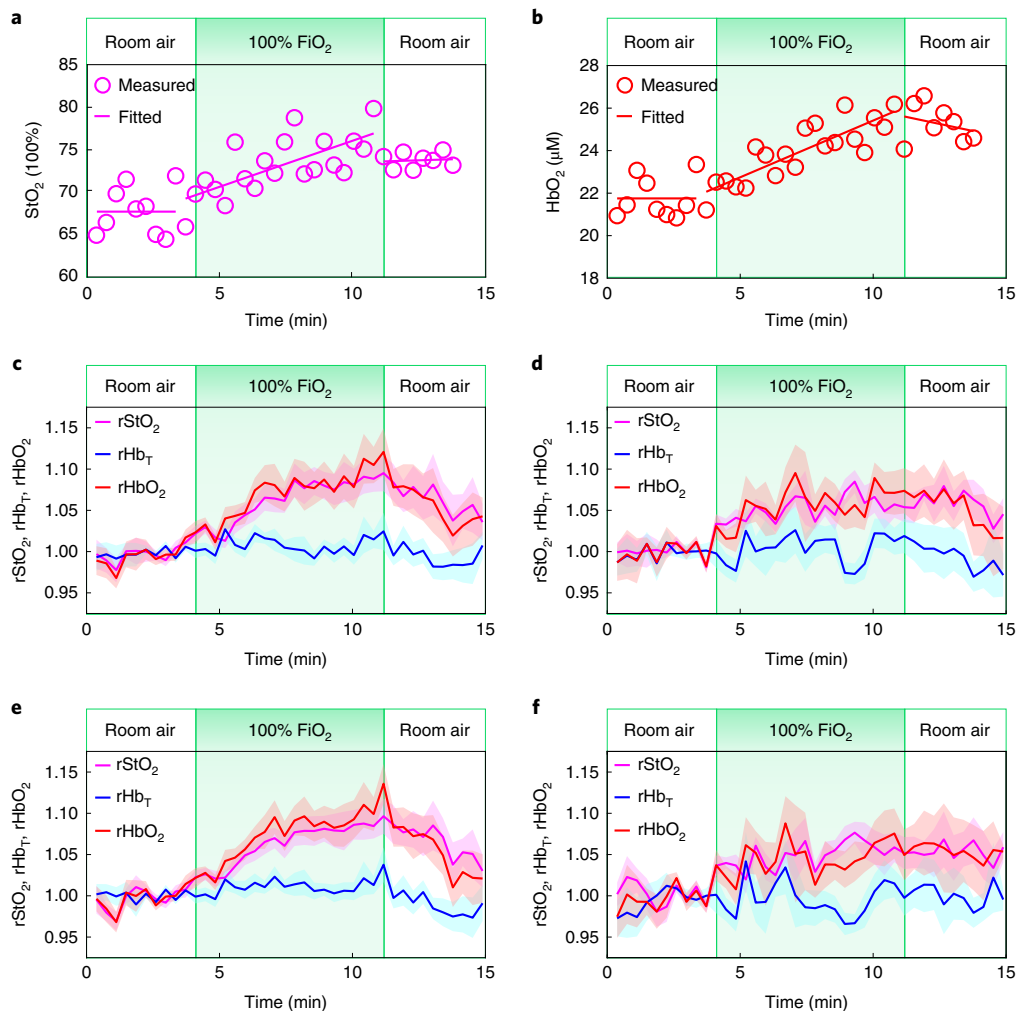


Fig. 4 | Continuous monitoring of placental haemoglobin properties during maternal hyperoxia. a,b, Example case of StO_2 and $[\text{HbO}_2]$ during maternal hyperoxia. **c,d**, Averaged placental rStO_2 (purple), rHb_T (blue) and rHbO_2 (red) for NPO participants ($n=15$) and for participants with APO ($n=9$). **e,f**, Averaged placental rStO_2 (purple), rHb_T (blue) and rHbO_2 (red) for NPP participants ($n=16$) and for participants with MVM ($n=8$). Shaded regions represent the standard error. The cohort-averaged rStO_2 and rHbO_2 exhibit a significant increase during maternal hyperoxia for the NPO and NPP groups, but a blunted response for the APO and MVM groups.

associated with APO (Fig. 5a and Table 1). On the other hand, ΔHbO_2 and ΔStO_2 were significantly reduced in cases with APO compared with NPO (Fig. 5b and Table 1).

Similarly, we determined whether placental haemoglobin properties were significantly associated with MVM.

Baseline StO_2 , $[\text{Hb}_T]$ and $[\text{HbO}_2]$ were not associated with MVM (Fig. 6a and Table 1). ΔHbO_2 and ΔStO_2 were significantly reduced in cases with MVM compared with NPP (Fig. 6b and Table 1).

As an additional check, we carried out the same univariate analysis with standard parameters such as maternal age, nulliparity, gestational age (GA) at study visit, maternal BMI, placental depth (d) and uterine-artery Doppler pulsatility index (UtA PI), the latter having been proposed as a surrogate indicator of trophoblastic invasion⁴⁴. We did not find any significant association between APO and/or MVM and either maternal age, nulliparity, GA at study visit or UtA PI (Table 2). However, we did observe a slight trend (not significant) towards larger d and BMI in participants with APO ($P=0.1$, $P=0.1$, respectively, two-sided Wilcoxon rank-sum test). Although our sample size is small and these variables are not necessarily uncorrelated, for completeness we ran binary logistic regressions with pairs of variables: d , ΔStO_2 ; d , ΔHbO_2 ; BMI, ΔStO_2 ; and BMI, ΔHbO_2 . The results confirmed that a trend towards significant association

between optically derived haemodynamic properties and outcomes of interest remained (Supplementary Table 4).

Lastly, our methodology permitted the study of adipose and rectus/uterus layers at baseline and during maternal hyperoxia (Supplementary Tables 3 and 5). The resultant adipose and rectus/uterus layer haemodynamic properties typically differed from those of the placenta. We did not find any statistically significant association of any overlayer haemodynamic property with APO or MVM (Supplementary Table 5). Collectively, these data suggest that without multilayer modelling, the computed placenta responses would have been attenuated; that is, the deep-tissue signal would have then been a weighted average of the placenta, rectus/uterus and adipose layers.

Discussion

Direct and non-invasive clinical methods to assess placental function in vivo and at the bedside would be desirable. The depth of the placenta below the skin surface and the variability in the properties of the overlying layers present substantial challenges for optical diagnostics. In this study, we report the development and performance of an instrument and methodology that substantially expand the capabilities of DOS to enable bedside dynamic monitoring of

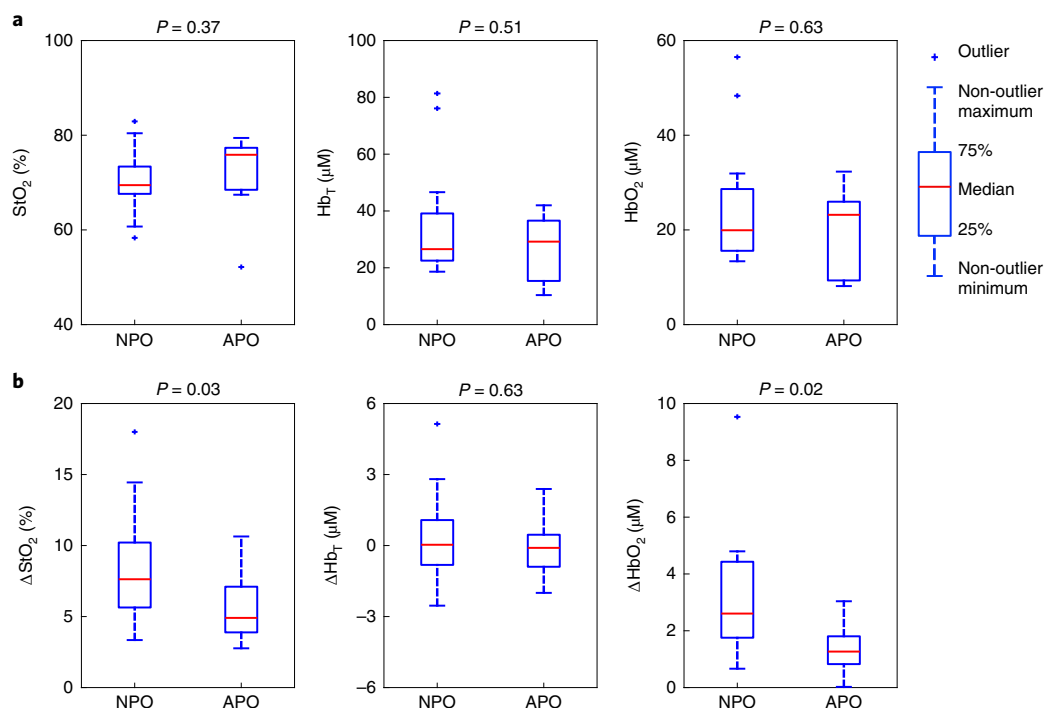


Fig. 5 | Static (baseline) and dynamic (during maternal hyperoxia) placental haemoglobin properties for participants with NPO or APO. **a**, Baseline placental StO_2 , $[Hb_T]$ and $[HbO_2]$ illustrate that they are not associated with pregnancy outcome. The data show no significant differences between NPO and APO. P values are 0.37, 0.51 and 0.63, respectively. **b**, Placental ΔStO_2 , ΔHb_T and ΔHbO_2 during maternal hyperoxia. ΔStO_2 , ΔHb_T and ΔHbO_2 exhibit trending separation, no difference and clear separation, respectively, among participants with NPO versus APO. The P values are 0.03, 0.63 and 0.02, respectively. In the boxplots, the red centre line denotes the median value and the blue box shows the IQR (25% to 75% of dataset). The blue whiskers mark the non-outlier minimum and non-outlier maximum; outliers are marked with a '+' symbol. Values of the median and IQR are presented in Table 1. P values were calculated via a two-sided Wilcoxon rank-sum test.

Table 1 | Correlations of haemodynamic variables with placental dysfunction

	$StO_2(\%)$	$[Hb_T](\mu M)$	$[HbO_2](\mu M)$	$\Delta StO_2(\%)$	$\Delta Hb_T(\mu M)$	$\Delta HbO_2(\mu M)$
Between placental static and dynamic haemoglobin properties and APO						
NPO ($n=15$)	69.5 (67.6, 73.4)	26.6 (22.5, 39.1)	19.9 (15.6, 28.6)	7.6 (5.6, 10.2)	0.0 (-0.8, 1.1)	2.6 (1.8, 4.4)
APO ($n=9$)	75.9 (68.5, 77.3)	29.2 (15.4, 36.6)	23.2 (9.3, 26.0)	4.9 (3.9, 7.1)	-0.1 (-0.9, 0.5)	1.3 (0.8, 1.8)
P value	0.37	0.51	0.63	0.03	0.63	0.02
Between placental static and dynamic haemoglobin properties and MVM						
NPP ($n=16$)	69.5 (67.9, 75.2)	27.9 (23.6, 35.0)	20.8 (15.8, 24.7)	7.6 (5.7, 10.1)	0.2 (-0.8, 1.7)	2.8 (1.5, 4.1)
MVM ($n=8$)	73.5 (65.1, 76.5)	27.7 (14.8, 39.3)	19.3 (9.0, 30.1)	5.2 (3.7, 7.1)	-0.1 (-1.7, 0.2)	1.1 (0.7, 1.8)
P value	0.83	0.69	0.69	0.05	0.15	0.01

For all correlations, the total number of participants is 24. The parameters are summarized as median (with IQR in brackets) within each group. The P values were obtained using a two-sided Wilcoxon rank-sum test.

the placenta and potentially of other organs buried far below the tissue surface.

Tissue-simulating phantom experiments showed that the instrument has sufficient dynamic range and SNR to perform measurements at long-distance SDSs (up to 10 cm), thereby suggesting that deep placental layers can be optically interrogated in the clinic. Moreover, by coupling FD-DOS instrumentation with ultrasound imaging, we directly mapped the morphology of overlying layers of the abdominal wall and uterus. This mapping permits multilayer modelling of tissue properties that effectively isolates the placenta's optical and physiological properties. We validated the methodology using tissue phantoms and finite-element simulations, and carried out a pilot study of third-trimester pregnancies.

Placental StO_2 , $[Hb_T]$ and $[HbO_2]$ were non-invasively measured at the bedside, differentiating placenta from tissue overlayers. We also showed the repeatability and stability of the optical metrics. The baseline placenta properties between the normal and abnormal pregnancies were statistically similar. This finding agrees with a previous CW study²⁷ but is different from observations of higher placental oxygenation level in IUGR participants from two early CW studies^{26,28} and from observations of lower placental oxygen level in women with pregnancy complications from a recent CW study⁴⁵. Regarding study comparisons, it should be noted that, besides important differences in technical approach (such as layer averaging, light penetration, participant BMI and other assumptions), the adverse outcomes in our study were generally not as

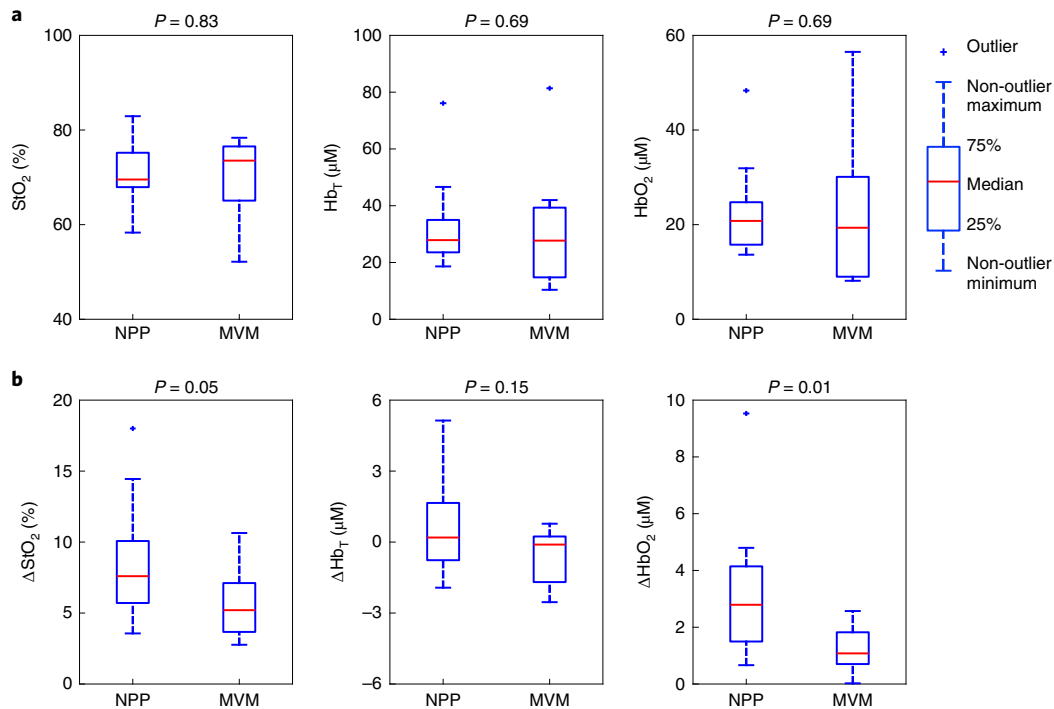


Fig. 6 | Static (baseline) and dynamic (during maternal hyperoxia) placental haemoglobin properties for participants with NPP or MVM. a, Baseline placental StO_2 , $[Hb_T]$, $[HbO_2]$ illustrate that they are not associated with placental pathology. The data show no significant differences between NPP and MVM. The P values are 0.83, 0.69 and 0.69, respectively. **b**, Placental ΔStO_2 , ΔHb_T and ΔHbO_2 during maternal hyperoxia. ΔStO_2 , ΔHb_T and ΔHbO_2 exhibit clear separation, no difference and clear separation, respectively, among participants with NPP versus MVM. The P values are 0.05, 0.15 and 0.01, respectively. In the boxplots, the red centre line denotes the median value and the blue box shows the IQR (25% to 75% of dataset). The blue whiskers mark the non-outlier minimum and non-outlier maximum; outliers are marked with a '+' symbol. Values of the median and IQR are presented in Table 1. P values were calculated via a two-sided Wilcoxon rank-sum test.

Table 2 | Correlations of demographic variables with placental dysfunction

	Age (years)	Nulliparity (%)	GA (weeks)	BMI	d (cm)	UtA PI
Between other variables and APO						
NPO ($n=15$)	27.0 (25.3, 32.3)	20.0	34.4 (32.8, 35.1)	25.7 (24.2, 28.9)	2.5 (2.3, 2.9)	0.7 (0.6, 0.8)
APO ($n=9$)	27.0 (22.8, 33.3)	44.4	34.6 (32.9, 36.4)	30.1 (24.9, 33.4)	3.2 (2.5, 3.6)	0.8 (0.6, 1.1)
P value	0.93	0.36	0.53	0.10	0.10	0.21
Between other variables and MVM						
NPP ($n=16$)	26.5 (24.0, 31.5)	18.8	34.2 (32.8, 35.2)	26.5 (24.6, 29.2)	2.5 (2.3, 3.0)	0.7 (0.6, 0.8)
MVM ($n=8$)	29.5 (24.0, 34.0)	50.0	34.7 (33.6, 35.9)	30.2 (23.4, 34.3)	3.2 (1.9, 3.5)	0.7 (0.6, 1.1)
P value	0.52	0.17	0.58	0.34	0.56	0.65

For all correlations, the total number of participants is 24. The parameters are summarized as median (with IQR in brackets) within each group except for nulliparity, which is presented as a percentage. The P values were obtained using a two-sided Wilcoxon rank-sum test, except for nulliparity which was analysed using a two-sided Fisher's exact test.

severe as in some of the others^{26,28}. Moreover, we made assessments weeks before clinical disease and delivery. Hence, although studies with larger sample size are clearly warranted, the similar baseline placenta-tissue oxygen levels that we observed are not at odds with physiological expectations, even in the presence of underlying pathology.

Since gold-standard references for placental tissue haemoglobin values do not exist, we used functional perturbations to show sensitivity to expected physiologic changes. Maternal left lateral tilt positioning relieves caval compression and augments venous return to the heart, improving cardiac output. In pregnancy, ~20% of maternal cardiac output is directed to uteroplacental perfusion.

Thus, this manoeuvre generally increases blood flow to the placenta. In fact, this position is used clinically as a fetal resuscitation manoeuvre during labour to maximize placental perfusion⁴⁶. Despite the limited sample size ($n=3$), the FD-DOS/US device detected an increase for both $[Hb_T]$ and $[HbO_2]$, consistent with the expected increased maternal blood flow into the intervillous vascular spaces of the placenta.

More importantly, the maternal-hyperoxia experiments showed that the method can detect real-time changes in placental $[HbO_2]$ and StO_2 owing to the inflow of oxygen-enriched maternal blood into the placenta, whereas $[Hb_T]$ remained comparably stable. These perturbation experiments validated our clinical expectations

and confirmed the sensitivity of the optical measures to underlying uteroplacental haemodynamics. They are also consistent with MRI findings; for example, this effect was demonstrated in normal fetuses and in those with congenital heart disease¹¹, and a similar delayed response in abnormal versus normal placentas in twin pregnancies was observed with hyperoxic BOLD (blood oxygenation level dependent) MRI¹⁷, again consistent with our observations.

We also studied how optical metrics obtained during maternal hyperoxia correlate with pregnancy outcome and placental pathology. APOs are significantly associated with both short-term and long-term morbidity and mortality^{1–3}. Similarly, MVM are associated with both APOs and long-term adverse outcomes³⁵, with prevalence estimates as high as 32% in term births and over 50% in preterm births⁴⁸. Our pilot study indicates that the non-invasive optical monitoring of placental responses to maternal hyperoxia is feasible and that it holds potential as a methodology to detect signs of poor placental perfusion weeks before delivery.

The adipose and rectus/uterus layer haemoglobin properties of healthy participants during baseline and during maternal hyperoxia were not significantly different from those of participants with APO or MVM (Supplementary Table 5). These null results for the overlayers are not necessarily surprising from a physiological perspective. For example, since MVM presents a recognizable pattern of placental injury related to altered uterine and intervillous blood flow, we would not expect a significant association between adipose haemoglobin properties and MVM. However, we noticed that a few overlayer parameters exhibited trending associations (P values as small as 0.06 for the rectus/uterus layer ΔStO_2 and APO, two-sided Wilcoxon rank-sum test); this observation should encourage further study in a larger sample size; that is, one might hypothesize that subclinical vascular dysfunction may predispose to poor placentation or be an indicator of placental dysfunction.

The need for new tools to assess placental function is well-known to clinicians. Critical knowledge gaps exist in obstetrical care as a result. For this reason, some investigators have turned to MRI to derive functional parameters related to placental oxygenation. MRI does not have the same issues of depth penetration that limit optics, and specialized MRI techniques such as $T2^*$, BOLD and magnetic resonance susceptibility are correlated with oxygen content^{10–12}. However, these correlations with oxygen have limitations, and MRI is not suitable for bedside measurements. DOS, by contrast, can directly measure deoxy-haemoglobin and oxy-haemoglobin concentrations at the bedside and non-invasively during clinical care. Here we have described a unique instrument that permits quantitative dynamic monitoring of the human placenta at the bedside, and the associations that we have found with APO and MVM support its continued development. However, our work thus far has some limitations: we have so far probed only anterior placentas within ~4 cm of the surface, and the placenta optical signals are due to a combination of maternal and fetal blood. Although the methodology holds clinical promise, a clinical study with larger sample sizes is desirable to corroborate the findings, and to enable more sophisticated statistical analyses that explore possible confounding variables and generate composite metrics with improved specificity and sensitivity.

The technology will need to be refined. With improved spatial information from ultrasound, such as 3D imaging, we may be able to improve on the uniform slab-layer tissue model and derive optical properties with greater fidelity. With improved time resolution, the temporal responses to functional activation could be explored as a test variable. Additionally, an improved time resolution would increase the sensitivity to changing conditions and would facilitate the efficient evaluation of multiple placental sites; the latter is especially important given the potential spatial heterogeneity of pathology within the placenta. Exploration of placental oxygenation at earlier gestational ages, although challenging, could lead to the identification of early signs of placental insufficiency, and

comparisons of uterine and placental responses during hyperoxia could improve our understanding of maternal and fetal oxygen consumption. Real-time optical monitoring feedback could alert clinicians to suspicious data owing to contraction or movement and thus improve data-acquisition quality. Currently, it takes ~90 min to determine the regularization hyperparameters; three-layer reconstruction of each frame data takes ~90 s on our 3.2 GHz quad-core computer. These times can be reduced to ~12 min and ~12 s, respectively, with a 3.5 GHz 32-core workstation. Looking forward, with machine learning and information from large datasets, we believe that the regularization hyperparameter could be determined in ~1 min at the beginning of the measurement and thereby facilitate real-time layer model reconstructions. Broadly, we anticipate that optical metrics of placental haemodynamics will enable scientists to better understand placental pathophysiology. Moreover, the instrumentation and methodology that we have reported here are potentially suited for in vivo studies of oxygen function in other internal organs buried deep below the tissue surface, such as the uterus and the kidney.

Methods

FD-DOS instrumentation. The details of the construction and operation of the custom heterodyne FD-DOS instrument are schematically shown in Extended Data Fig. 1. Briefly, three sinusoidal electromagnetic waves at RF ($f_1 = 100\text{MHz}$), that is, one for each laser diode and one sinusoidal wave at frequency ($f_2 = 100.2\text{MHz}$), were generated from four low-noise, fractional-N phase-locked-loop signal generators (HMC833, Hittite Microwave). The waves were synchronized by an ultra-low-jitter programmable reference clock ($f_0 = 50\text{MHz}$, LMK61E2, Texas Instruments). Each f_1 wave from the signal generator was amplified (ZX60-P103LN+, Mini-Circuits), filtered (DC to 98 MHz, SLP-100+, Mini-Circuits) and divided (2-way splitter, Z99SC-62-S+, Mini-Circuits) into two f_1 waves; one was prepared for the reference signal ('reference f_1 ') and the other was prepared for driving amplitude modulation for one laser ('signal f_1 '). Simultaneously, the f_2 wave from the signal generator was also amplified, filtered and divided (4-way splitter, ZB4PD-52-20W-S+, Mini-Circuits) into four f_2 waves; three of these were prepared for frequency-mixing with the three detected signals ('signal f_2 ') and the other was prepared for frequency-mixing with the reference signal ('reference f_2 ').

The three of 'signal f_1 ' were further amplified and input into laser controllers (CLD1011LP, Thorlabs), which drive the light amplitude modulation of the three near-infrared lasers with wavelengths of 785 nm (LP785-SF1000, Thorlabs), 808 nm (LDPC-T3-808-62.5/125-M-35-3S-3-0.5-70-150LD, OZ Optics) and 830 nm (LDPC-T3-830-62.5/125-M-35-3S-3-0.5-70-150LD, OZ Optics). The laser controllers also maintain thermal stability using digital proportional-integral-derivative control. To enhance SNR, the achievement of a modulation depth or amplitude modulation index, that is, the ratio of the modulation excursions of the RF signal to the level of unmodulated carrier, of greater than 90% for each laser is critically important. Achievement of these large modulation depths required individually optimized RF amplification according to each laser's characteristics. Each laser has a specific threshold current, maximum current and optical power efficiency (mW mA^{-1}). To minimize unmodulated background light and maximize SNR, each laser has a unique RF driver that drives its amplitude with modulation depth >90% (93% for 785 nm laser, 95% for 808 nm laser, and 98% for 830 nm laser). Specifically, each 'signal f_1 ' wave was divided into four sub-signals via a 4-way power splitter. Each sub-signal was then amplified in one or two amplification stages (in each stage, the signal was amplified by ~8.6 dB); the 830 nm channel had 2 amplification stages and the 785 nm and 805 nm channels had 1 amplification stage. The sub-signals were then combined via a 4-way power splitter. A custom-built circuit containing a low-noise amplifier (ZX60-P103LN+, Mini-Circuits) and low-pass filter (DC to 98 MHz, SLP-100+, Mini-Circuits) was used in each amplification stage.

The three amplitude-modulated laser diodes were fibre-coupled to an optical switch (MEMS 91545C4, Dicon), which was in turn connected to the 10 source fibres (400 μm core, 0.5 NA, FP400URT-Custom, Thorlabs) on the probe head (see main text Fig. 1(b)). The optical switch sequentially cycled each laser diode through each source position and also a 'dark count position' (that is, a cycle of $3 \times 11 = 33$ sequential measurements; 21 s per cycle). Of note, for the dark count measurement, no fibre was connected to the 11th position on the switch (that is, no light was delivered to the tissue).

Multiply scattered light emerging from the tissue at the detector position was collected by a high-transmission liquid light guide (5 mm core, 0.59 NA, LLG5-8H, Thorlabs) that was coupled to a high-sensitivity photomultiplier (PMT) detector (R12829, Hamamatsu). The PMT converts the diffuse light wave to a proportional electric voltage signal, which is then amplified and filtered by a high-speed current amplifier (DHPCA-100, FEMTO) and finally frequency-mixed (ZP-3-S+, Mini-Circuits) with one 'signal f_2 '. Mixing produces

a heterodyne down-converted signal, related to the diffusive light wave (that is, its amplitude and phase are proportional to those of the diffusive light wave) at frequency $\Delta f = f_1 - f_2 = 0.2\text{MHz}$. This lower-frequency signal, which can be very accurately quantified, is the heterodyne detected signal³¹. Simultaneously, a reference signal with the fixed frequency Δf , amplitude (A_r) and phase (φ_r) is generated by mixing the 'reference f_1 ' and 'reference f_2 '.

A high-sampling-rate lock-in amplifier (MFLI 500 kHz, Zurich Instruments) compares reference and detected signals to derive the amplitude (A) and phase (φ) of the diffuse light wave (that is, the lock-in output in-phase ($I = A\cos\varphi$) and quadrature ($Q = A\sin\varphi$) signals, from which A and φ are calculated). Note that a 3-to-1 RF switch (G4F-520120, Charter Engineering) was employed to pair the correct reference signal with the corresponding detected wavelength. Note also that before computing A and φ , the Q and I for each wavelength at every source position were subtracted by the corresponding Q_{noise} and I_{noise} obtained from the dark count position in the same cycle. In summary, we collected diffuse light waves from 10 source-detector pairs with SDSs ranging from ~ 1 to $\sim 9\text{cm}$ in the human probe (and up to 10cm in the tissue-phantom experiment); these data enable the depth-dependent optical determination of tissue properties.

Three-layer photon-diffusion model and Green's function. The human abdomen is multilayered. We model it as a three-layer medium, wherein each layer is assumed homogeneous and laterally infinite in extension. The experimental geometry is described using cylindrical coordinates in the main text Fig. 1c; the depth is denoted by $z(\text{cm})$ and the SDS is denoted by $\rho(\text{cm})$. Both source and detector are positioned on the surface ($z=0$). In the diffusive medium, sources on the tissue boundary are well-modelled as an isotropic point source in the medium at depth of $z_0 = l_{\text{tr},1}$ (cm), which depends on tissue optical properties and is defined below. The diffusion equations (in the frequency-domain) for the spatially dependent amplitude of the diffusive waves in each of the layers of the three-layer medium are^{38,39,49}:

$$\nabla^2 U_1(\rho, z) - \left(\frac{\mu_{a,1}}{D_1} + i \frac{2\pi f_1}{v_1 D_1} \right) U_1(\rho, z) = -\frac{MS_0}{D_1} \delta(\rho - \rho_0, z - z_0); \quad 0 \leq z < d_1; \quad (1-1)$$

$$\nabla^2 U_2(\rho, z) - \left(\frac{\mu_{a,2}}{D_2} + i \frac{2\pi f_1}{v_2 D_2} \right) U_2(\rho, z) = 0; \quad d_1 \leq z < d_1 + d_2; \quad (1-2)$$

$$\nabla^2 U_3(\rho, z) - \left(\frac{\mu_{a,3}}{D_3} + i \frac{2\pi f_1}{v_3 D_3} \right) U_3(\rho, z) = 0; \quad d_1 + d_2 \leq z. \quad (1-3)$$

Here, the diffusive wave, $\Phi_{AC,k}(\rho, z, t) = U_k(\rho, z) e^{i2\pi f_1 t}$ ($W\text{cm}^{-2}$), is a complex representation of photon fluence rate within layer $k(k=1,2,3)$. $S_0(W)$ is the time averaged power emitted by the light source, $f_1(\text{Hz})$ and M (dimensionless) are the frequency ($f_1 = 100\text{MHz}$) and modulation depth of the source, respectively. The source is point-like and located at (ρ_0, z_0) . $d_k(\text{cm})$, $\mu_{a,k}(\text{cm}^{-1})$ and $\mu'_{s,k}(\text{cm}^{-1})$ are the layer k thickness, layer k light absorption coefficient and layer k reduced scattering coefficient, respectively. $l_{\text{tr},k} \equiv 1/(\mu_{a,k} + \mu'_{s,k})$ (cm) and $D_k = (1/3) l_{\text{tr},k}$ (cm) are the photon transport mean-free path and the diffusion coefficient of layer k , respectively. $v_k = c/n_k$ (cm s^{-1}) is the light velocity in layer k , where c (cm s^{-1}) is the speed of light in vacuum and n_k is the refractive index of layer k . The boundary conditions for the photon fluence rate and its normal derivative across the interfaces are well-known and are used to derive solutions⁵⁰.

Assuming large SDS, the equations can be solved using a Fourier-transform approach and extrapolated-zero boundary conditions. The analytical Green's function for the three-layer diffusion equation in the $z=0$ plane is^{38,49}:

$$G_{3L}([\rho, z=0], [\rho_s = \rho_0, z_s = z_0]) = \frac{1}{2\pi} \int_0^\infty \tilde{G}_{3L}(s) s J_0(s\rho) ds; \quad (2-1)$$

$$\tilde{G}_{3L}(s) = \frac{Z_{3L}(s)}{Y_{3L}(s)} / \alpha_1 D_1; \quad (2-2)$$

$$\begin{aligned} Z_{3L}(s) = & N_{12} M_{23} \exp(\alpha_1 z_0 - \alpha_1 d_1 + \alpha_2 d_2 + \alpha_1 z_{b,1}) \\ & - N_{12} M_{23} \exp(\alpha_1 z_0 - \alpha_1 d_1 + \alpha_2 d_2 - \alpha_1 z_{b,1}) \\ & + N_{12} N_{23} \exp(-\alpha_1 z_0 + \alpha_1 d_1 - \alpha_2 d_2 + \alpha_1 z_{b,1}) \\ & - N_{12} N_{23} \exp(-\alpha_1 z_0 + \alpha_1 d_1 - \alpha_2 d_2 - \alpha_1 z_{b,1}) \\ & + M_{12} N_{23} \exp(\alpha_1 z_0 - \alpha_1 d_1 - \alpha_2 d_2 + \alpha_1 z_{b,1}) \\ & - M_{12} N_{23} \exp(\alpha_1 z_0 - \alpha_1 d_1 - \alpha_2 d_2 - \alpha_1 z_{b,1}) \\ & + M_{12} M_{23} \exp(-\alpha_1 z_0 + \alpha_1 d_1 + \alpha_2 d_2 + \alpha_1 z_{b,1}) \\ & - M_{12} M_{23} \exp(-\alpha_1 z_0 + \alpha_1 d_1 + \alpha_2 d_2 - \alpha_1 z_{b,1}); \end{aligned} \quad (2-3)$$

$$\begin{aligned} Y_{3L}(s) = & M_{12} M_{23} \exp(\alpha_1 d_1 + \alpha_2 d_2 + \alpha_1 z_{b,1}) \\ & + N_{12} M_{23} \exp(-\alpha_1 d_1 + \alpha_2 d_2 - \alpha_1 z_{b,1}); \\ & + N_{12} N_{23} \exp(\alpha_1 d_1 - \alpha_2 d_2 + \alpha_1 z_{b,1}) \\ & + M_{12} N_{23} \exp(-\alpha_1 d_1 - \alpha_2 d_2 - \alpha_1 z_{b,1}); \end{aligned} \quad (2-4)$$

$$M_{12} = \alpha_1 D_1 + \frac{n_2^2}{n_1^2} \alpha_2 D_2; \quad M_{23} = \alpha_2 D_2 + \frac{n_3^2}{n_2^2} \alpha_3 D_3; \quad (2-5)$$

$$N_{12} = \alpha_1 D_1 - \frac{n_2^2}{n_1^2} \alpha_2 D_2; \quad N_{23} = \alpha_2 D_2 - \frac{n_3^2}{n_2^2} \alpha_3 D_3;$$

$$\alpha_k = \frac{(D_k s^2 + \nu_k \mu_{a,k} + i 2\pi f_1)}{D_k}; \quad k = 1, 2, 3. \quad (2-6)$$

Here, J_0 is the Bessel function of the first kind and zero-order. $z_{b,1} \equiv (2/3) l_{\text{tr},1} (1 + R_{\text{eff}}/1 - R_{\text{eff}})$ (cm) is the extrapolation length, where R_{eff} is related to the indices of refraction of the media⁵⁰. In practice, we solve the integral in equation (2-1) numerically by applying Gauss-Laguerre quadrature of 5,000 points. Note that to minimize numerical errors, the hyperbolic functions are expanded and simplified.

Note that the analytical Green's function for the two-layer diffusion equation in the $z=0$ plane can be derived from a special case of the three-layer model, where $\mu_{a,1} = \mu_{a,2}$, $\mu'_{s,1} = \mu'_{s,2}$, $n_1 = n_2$, $d_1 + d_2 = d$, $D_1 = D_2$ and $\alpha_1 = \alpha_2$.

$$G_{2L}([\rho, z=0], [\rho_s = \rho_0, z_s = z_0]) = \frac{1}{2\pi} \int_0^\infty \tilde{G}_{2L}(s) s J_0(s\rho) ds; \quad (3-1)$$

$$\tilde{G}_{2L}(s) = \frac{Z_{2L}(s)}{Y_{2L}(s)} / \alpha_1 D_1; \quad (3-2)$$

$$\begin{aligned} Z_{2L}(s) = & \exp(-\alpha_1 z_0) - \exp(-\alpha_1 z_0 - 2\alpha_1 z_{b,1}) + \beta \cdot \exp(\alpha_1 z_0 - 2\alpha_1 d) \\ & - \beta \cdot \exp(\alpha_1 z_0 - 2\alpha_1 d - 2\alpha_1 z_{b,1}); \end{aligned} \quad (3-3)$$

$$Y_{2L}(s) = 1 + \beta \cdot \exp(-2\alpha_1 d - 2\alpha_1 z_{b,1}); \quad (3-4)$$

$$\beta = \frac{\alpha_1 D_1 - (n_2^2/n_1^2) \alpha_3 D_3}{\alpha_1 D_1 + (n_2^2/n_1^2) \alpha_3 D_3}. \quad (3-5)$$

The photon fluence rate measured on the tissue surface is essentially the Green's function multiplied by the constant amplitude of the source MS_0 . The detected signal intensity is directly proportional to this photon fluence rate:

$$U_1([\rho, z=0], [\rho_s = \rho_0, z_s = z_0]) = MS_0 G([\rho, z=0], [\rho_s = \rho_0, z_s = z_0]); \quad (4-1)$$

$$\begin{aligned} I_m(\rho) = & K U_1([\rho, z=0], [\rho_s = \rho_0, z_s = z_0]) \\ = & C G([\rho, z=0], [\rho_s = \rho_0, z_s = z_0]); \end{aligned} \quad (4-2)$$

$$A_m(\rho) = |I_m(\rho)|; \quad \theta_m(\rho) = \arg[I_m(\rho)]. \quad (4-3)$$

Here, $C = C_a \exp(-iC_p)$ is the complex light coupling coefficient (a proportionality constant); C_a and C_p are determined using calibration phantoms with known optical properties before/after the measurement. With equations (2) and (4), the signal at the tissue surface boundary can be calculated, given input optical properties for each layer and layer thicknesses. Such a calculation is called a solution of the forward problem. The reconstruction algorithms underlying the FD-DOS analysis solve the inverse problem by finding the tissue properties that minimize the difference between measured signal and the forward problem (theoretical) solution (with specific input properties).

Global optimization with multiple spectral/SDS channels. In practice, the two-layer or three-layer optical properties based on the photon-diffusion model were reconstructed by solving a global optimization problem. Specifically, we carried out the data inversion using all source-detector pairs and wavelengths simultaneously. This approach builds-in global constraints about chromophore absorption and layer geometry into the inverse problem and is critical for robust fitting. Note that the accuracy of our determination of [Hb] and [HbO₂] concentration (and thus StO₂, [Hb_T]) is superior to that based on independent determination of absorption and scattering coefficients as a function of wavelength. This is because the use of the known chromophore extinction coefficients, the Mie power-law model for scattering, and the full collection of

multispectral and multiSDS data to fit to all wavelengths and SDSs simultaneously, effectively constrains the reconstruction problem⁴⁰.

The multispectral fitting assumed that $\mu_{a,k}(\lambda_j)$, the absorption coefficient at layer k at wavelength λ_j ($j = 1, 2, 3, \lambda_j = 785, 808, 830\text{nm}$), is due to the absorption of $[\text{HbO}_2]_k$, $[\text{Hb}]_k$, $[\text{H}_2\text{O}]_k$ and $[\text{lipid}]_k$.

$$\mu_{a,k}(\lambda_j) = \epsilon_{\text{HbO}_2}(\lambda_j) [\text{HbO}_2]_k + \epsilon_{\text{Hb}}(\lambda_j) [\text{Hb}]_k + \epsilon_{\text{H}_2\text{O}}(\lambda_j) [\text{H}_2\text{O}]_k + \epsilon_{\text{lipid}}(\lambda_j) [\text{lipid}]_k. \quad (5)$$

In equation (5), $\epsilon_{\text{HbO}_2}(\lambda_j)$, $\epsilon_{\text{Hb}}(\lambda_j)$, $\epsilon_{\text{H}_2\text{O}}$ and $\epsilon_{\text{lipid}}(\lambda_j)$ are wavelength-dependent extinction coefficients for each chromophore, which are known⁵¹. $[\text{H}_2\text{O}]_k$ and $[\text{lipid}]_k$ are the concentration of water and lipid in layer k , which are assumed⁵¹. $[\text{HbO}_2]_k$ and $[\text{Hb}]_k$ are the concentration of oxy- and deoxy-haemoglobin in layer k ; they are determined by solving the inverse problem.

Notice that the total haemoglobin concentration $[\text{Hb}_T]_k$ and the tissue blood oxygen saturation $\text{StO}_{2,k}$ in layer k can also be readily obtained from:

$$[\text{Hb}_T]_k = [\text{HbO}_2]_k + [\text{Hb}]_k; \quad \text{StO}_{2,k} = [\text{HbO}_2]_k / [\text{Hb}_T]_k; \quad k = 1, 2, 3. \quad (6)$$

For multispectral fitting, we also assumed a Mie scattering model (equation just below) for the tissue scatterers⁵³, wherein the scattering coefficient in layer k is a power-law function with scattering amplitude γ_k and scattering power b_k . Here, $\lambda_0 = 700\text{nm}$ is a reference wavelength chosen based on the range of the three wavelengths.

$$\mu'_{s,k}(\lambda_j) = \gamma_k (\lambda_j / \lambda_0)^{-b_k}. \quad (7)$$

With equations (5), (6) and (7), $\text{StO}_{2,k}$ and $[\text{Hb}_T]_k$ can be directly determined by global optimization using all data:

$$\text{argmin} \sum_{j=1}^3 \sum_{l=1}^L |\epsilon_{l,j}(\bar{X})|^2; \quad (8-1)$$

$$\Psi(\bar{X}) = |\epsilon_{l,j}(\bar{X})|^2 = \sum_{j=1}^3 \sum_{l=1}^L \left\| \ln [A_{c,l,j} / A_{c,l_0,j}] - \ln [A_{m,l,j} / A_{m,l_0,j}] \right\|^2 + \left\| [\theta_{c,l,j} - \theta_{c,l_0,j}] - [\theta_{m,l,j} - \theta_{m,l_0,j}] \right\|^2; \quad (8-2)$$

$$\bar{X} = [\text{StO}_{2,1} [\text{Hb}_T]_1 \ Y_1 b_1 \ \text{StO}_{2,2} [\text{Hb}_T]_2 \ Y_2 b_2 \ \text{StO}_{2,3} [\text{Hb}_T]_3 \ Y_3 b_3]. \quad (8-3)$$

In this global optimization, \bar{X} is an array of all fitting variables, including haemoglobin concentrations and variants thereof, and Mie scattering model parameters for scattering. The objective function $\Psi(\bar{X})$ is a 'residual' function of \bar{X} ; it is essentially a Chi-squared function that compares calculated to measured data. $\|\cdot\|^2$ represents the L^2 norm of the vector. $A_{c,l,j}$ and $\theta_{c,l,j}$ are the calculated (predicted) amplitude and phase using the forward solver with estimated optical properties. $A_{m,l,j}$ and $\theta_{m,l,j}$ are the measured amplitude and phase. Subscripts l and j represent the l -th SDS and j -th wavelength, respectively. Notice that the normalization amplitude and phase factors are also incorporated into the objective function; these terms are denoted with the subscript l_0 .

Regularization and initialization of the optimization problem. To avoid overfitting in the reconstruction, regularization is employed to reduce the ill-posedness of the inverse problem. A Tikhonov regularization term is added to the original objective function to provide additional constraints. Thus, the new objective function $\Psi'(\bar{X})$ is the sum of the residual function in (8-2) and a weighted regularization term⁵²:

$$\Psi'(\bar{X}) = \sum_{j=1}^3 \sum_{l=1}^L |\epsilon_{l,j}(\bar{X})|^2 + \zeta_R R(\bar{X}); \quad R(\bar{X}) = \left\| \bar{X} - \bar{X}^{(0)} \right\|^2. \quad (9)$$

$R(\bar{X})$ is the Tikhonov regularization term and ζ_R is the regularization hyperparameter, which calibrates the relative weight of the residual function and regularization term. Tikhonov regularization, as used here, seeks to minimize the difference between the initial estimated value $\bar{X}^{(0)}$ and the reconstructed value \bar{X} . The value for the regularization hyperparameter is determined using an L-curve method, which is a convenient graphical tool to find the optimized regularization parameter that balances the trade-off between fluctuation size and fluctuation smoothing. Once the ζ_R is determined, the optimization problem to minimize $\Psi'(\bar{X})$ can be solved iteratively. In practice, the iterative algorithm is performed by the MATLAB 'fmincon' function, with a parallel search function 'multi-start' for global minimum⁵³.

The first and very important step in the iterative search is initialization, wherein a reasonable estimated value $\bar{X}^{(0)}$ is set. It is important that the initial guess is chosen to be reasonably close to the true value; otherwise, the iterative search may not converge to a meaningful solution. Main text Fig. 2a schematically

outlines our three-step reconstruction procedure for initialization and determination of placental haemodynamic properties.

In essence, each step of the three-step reconstruction finds 'best' tissue properties by minimizing the difference between measured data and the predictions of diffuse optical tissue models of increasing complexity. Step 1 assumes that the underlying tissue is semi-infinite and homogeneous; it utilizes short SDSs (based on the superficial-layer thickness, longest SDS $\geq 2 \times \text{Depth} + 2$) to derive an initial estimate for properties of the near-surface region and the full set of SDSs to derive an initial property estimate for the whole region. For predictions, a standard semi-infinite homogeneous medium analytic solution is used.

Step 2 utilizes estimates from step one as initial guesses in an analytic 'two-layer' diffuse optical tissue model. The two-layer (and three-layer) models fix layer thicknesses based on ultrasound data, but other tissue properties within each layer, such as scattering, StO_2 and $[\text{Hb}_T]$, are permitted to vary to minimize the difference between measurement and tissue model predictions. Short SDSs and a 'two-layer' analytical light-transport model are utilized to derive best estimates for properties of adipose and rectus/uterus layers; all SDSs and this same two-layer analytic light-transport model are utilized to derive best estimates for the overlayer (adipose plus rectus/uterus) and placental regions.

Step 3 utilizes estimates for each layer from step two as initial guesses in the final 'three-layer' diffuse optical tissue model. Again, all layer thicknesses are fixed by ultrasound-image segmentation, but the other tissue properties within each layer are permitted to vary to minimize the difference between the measurement and the predictions of the 'three-layer' analytic light-transport model.

Validation and characterization with tissue-simulating phantoms.

We characterized FD-DOS instrument performance using tissue-simulating phantoms with known optical properties ($\mu_a = 0.10\text{cm}^{-1}$, $\mu'_s = 8.9\text{cm}^{-1}$ for $\lambda_i = 830\text{nm}$). The simplest tissue phantoms were composed of water, ink for absorption and 20% Intralipid (Baxter) for scattering. During this experiment, we used a translational stage and a motion control motor to accurately change the source-detector distance with minimum change of coupling coefficient. Briefly, the detector fibre was fixed at the liquid surface and a source fibre was physically translated in the same surface plane with SDSs ranging from 6.2 cm to 10 cm using a translation stage (see main text Fig. 2b). To good approximation, the coupling coefficients in equation (4) were the same at every SDS and a nonlinear fitting based on semi-infinite homogeneous solutions of the diffusion equation was employed to reconstruct tissue phantom optical properties¹³. The measurement SNR is defined as the mean intensity of 10 distinct continuous measurement trains divided by the standard deviation of the same 10 measurements for a specified SDS; accuracies are defined as the difference of measured and expected values, divided by the expected value.

In the two-layer phantom experiments, a solid phantom ($\mu_{a,b} = 0.10\text{cm}^{-1}$, $\mu'_{s,b} = 5.0\text{cm}^{-1}$ for $\lambda_i = 785\text{nm}$) was positioned under the liquid phantom and the full ultrasound/FD-DOS optical probe was set on the liquid surface (see main text Fig. 2c). An absorption-titration experiment tested sensitivity to absorption coefficient (chromophore concentration). The overlayer liquid phantom thickness was held constant at 3 cm, the overlayer scattering coefficient was held constant ($\mu'_{s,t} = 9.10\text{cm}^{-1}$ for $\lambda_i = 785\text{nm}$) and the absorption coefficient was incrementally increased in the top layer from $\mu_{a,t} = 0.08$ to 0.13cm^{-1} . Results are given in Supplementary Table 1a. A depth-changing experiment tested sensitivity to superficial-layer thickness (see main text Fig. 2c). Here, the liquid had fixed optical properties ($\mu_{a,t} = 0.13\text{cm}^{-1}$, $\mu'_{s,t} = 9.10\text{cm}^{-1}$ for $\lambda_i = 785\text{nm}$) and the superficial-layer thickness was increased from 1.5 to 3.0 cm; note that this range encompasses the majority of our clinical placenta depths. Results are given in Supplementary Table 1b. The results are summarized as mean (with s.d. in brackets) of 3 continuous measurements. The experiment accuracy is defined as the difference of measured and expected values, divided by the expected value. In Supplementary Table 1c, we show results from a two-layer phantom experiment with superficial-layer thickness of 4.3 cm. Finally, we successfully carried out a deep-layer absorption-titration phantom experiment to test our sensitivity to deep-layer absorption coefficient. In these studies, the overlayer liquid phantom thickness was held constant at 3.25 cm, the top-layer optical properties were fixed and the bottom-layer absorption was titrated. We reconstructed bottom-layer absorption variation of $\mu_a = 0.10\text{cm}^{-1}$, $\mu_a = 0.18\text{cm}^{-1}$ and $\mu_a = 0.26\text{cm}^{-1}$ (all at $\lambda = 785\text{nm}$) with error of 8.4%, -3.6% and -14.8%, respectively. These experiments (and results) are consistent with other work in the literature³⁷. While our phantom work used larger source-detector separations to probe deep-layer optical properties, the signal-to-noise and depth-to-SDS-ratio in our measurements and those of ref. ³⁷ were comparable; importantly, our inverse problem was more robust than that in ref. ³⁷ because the overlayer thickness was known.

Ultrasound-image segmentation. The ultrasound transducer at the centre of the optical probe was critical because it enabled us to derive tissue layer morphology and geometry information needed for the optical reconstruction algorithms. At the beginning of each 'optical frame', we captured an ultrasound image. Based on this ultrasound image, the clinician determined the depth below the tissue surface of the adipose, rectus, uterus and placenta tissue layers in the left, middle and right

sections of the ultrasound image. The difference between the left, middle and right depths was thus determined to ensure effective reconstruction of tissue optical properties. If this difference was less than 0.5 cm, then we took data; on the rare occasion when the difference was larger than 0.5 cm, we repositioned the probe or adjust the probe angle to decrease the difference and then took data.

Pregnancy outcome. After delivery, medical records were reviewed and relevant data on outcomes were extracted by a reviewer blinded to the optical data. GHTN and preeclampsia were defined per American College of Obstetricians and Gynecologists criteria⁵⁴; IUGR was defined as a birth weight below the 5th percentile for gestational age⁵⁵.

Placental histopathology. The delivered placentas were evaluated using a standard procedure^{36,56} by a single placental pathologist (R.L.L.) who was blinded to the optical properties. MVM was defined as a pattern of injury including placental hypoplasia (small for gestational age), villous infarcts, retroplacental haemorrhage (abruptio placentae), distal villous hypoplasia, villous agglutination, accelerated villous maturation and decidual arteriopathy. The minimum findings required for MVM diagnosis included decidual arteriopathy or at least 2 other features including accelerated villous maturation.

In vivo monitoring of placental oxygen dynamics. We designed a pilot clinical study of human placental oxygen-related haemodynamic properties. The study enrolled women with singleton pregnancies in the third trimester, anterior placentas and pre-gravid BMI < 40. For each experiment, participants were placed in supine, semi-recumbent position and the central region of the placenta was monitored. Before proceeding with the study, approval by the Institutional Review Board (IRB) at the University of Pennsylvania was obtained. Each participant signed the resultant informed consent forms before participating in the study.

Four experiments were completed: (1) a reproducibility experiment wherein a 2-frame measurement was made both before and after lifting and placing the probe at approximately the same location three times ($n=18$); (2) a stability experiment wherein continuous data was collected for 10 frames (~3.5 min) with participant breathing room air ($n=24$); (3) a maternal left tilt experiment wherein 4 frames of data were collected, first in supine and then in left-lateral decubitus position to characterize haemodynamic changes related to increased maternal cardiac output and uterine perfusion ($n=3$); (4) a maternal hyperoxia experiment ($n=24$) wherein the placenta was monitored continuously for 10 frames (~3.5 min) of baseline at room air, 20 frames (~7 min) of maternal hyperoxia (100% FiO₂) and 10 frames (~3.5 min) of recovery at room air again. Note that a single 'optical frame' corresponds to a measurement cycle through 11 light source-detector pairs and 3 wavelengths in ~21 s.

In total, $n=24$ participants took part in this study. Detailed information about the participants is provided in Supplementary Table 6. Note that data from two other participants were excluded because their signals were either too small (tissue optical absorption coefficient was very large, $\mu_a > 0.2 \text{ cm}^{-1}$) or too unstable (due to large fluctuations during the baseline period).

Measurement reproducibility was evaluated using the ICC. We measured the haemoglobin properties multiple times at the same placental location in 18 participants.

The stability test ($n=24$) results were represented by the s.d. during the continuous 10 frames measurements (Fig. 3a). Note that occasionally during data acquisition, substantial movement artefacts can occur, causing a single frame to exhibit >10% fluctuations in StO₂, [Hb_T] or [HbO₂] compared with the values of nearby frames. We identified these motion artefacts and filtered them out from the data.

To further validate the US/FD-DOS instrumentation and methodology in a physiologic context, we performed a left tilt experiment ($n=3$). In this experiment, the impact of increased maternal cardiac output on placental oxygen haemoglobin properties were determined. Briefly, in each participant, we measured StO₂, [Hb_T] and [HbO₂] for 4 frames both before and after the maternal tilt. Figure 3c presents the mean values of StO₂, [Hb_T] and [HbO₂] for each participant before and after the maternal tilt. To calculate the mean (and s.d.) of relative increases, we first determined the 'before/after' difference in mean value of each parameter for each participant. Then we normalized this difference by the mean of the 'before' value. Finally, we averaged these fractional changes across all 3 participants (Fig. 3c). To calculate the P values, the relative haemoglobin properties were obtained by normalizing the 'after' mean values to the 'before' mean values, and paired t -test analysis was applied to the relative 'before/after' values.

The placental haemodynamic response to maternal hyperoxia was examined by monitoring the placental haemoglobin properties ([Hb], [HbO₂], [HbT], StO₂) before, during and after maternal hyperoxia. After an initial baseline period, participants were given 100% FiO₂ via facemask for ~7 min (20 frames). Concurrent ultrasound and FD-DOS data were acquired throughout the process. Overall ($n=24$), the experimental methodology easily resolved changes in placental blood oxygenation due to maternal hyperoxia, making use of all source-detector pairs. Nevertheless, in processing we identified and excluded specific SDSs from our reconstructions. For example, an SDS would occasionally saturate or become very unstable (owing to human movement), or occasionally,

the longest SDS was very noisy (owing to large absorption); such SDSs were excluded from further processing. Similarly, during the hyperoxia measurements, movement artefacts occasionally occurred, causing a single frame to exhibit unphysiologically large (negative) fluctuations compared with the nearby frames (and baseline); such a frame was excluded.

We investigated potential associations between placental oxygen dynamics during maternal hyperoxia (that is, ΔStO_2 , ΔHb_T and ΔHbO_2) and the APO/MVM outcomes. For these analyses, the mean baseline StO₂, [Hb_T] and [HbO₂] were calculated using the final 4 frames of the baseline period. ΔStO_2 , ΔHb_T and ΔHbO_2 were defined as the difference between these mean baseline values and the 'peak' values of the 4-frame window during maternal hyperoxia wherein maximum StO₂ occurred. Each participant ($N=24$) was then categorized into two groups based on pregnancy outcome: NPO or APO. We observed significantly larger ΔStO_2 and ΔHbO_2 in response to maternal hyperoxia in the NPO group compared with (the more blunted response in) the APO group (see main text Fig. 5b). Similarly, when analysing placental histopathology as the outcome of interest, we observed significant (large) ΔStO_2 and ΔHbO_2 in the NPP group compared with a blunted response in the MVM group (see main text Fig. 6b). Wilcoxon rank-sum tests were performed to calculate the P values for comparison of different variables between NPO vs APO groups and NPP vs MVM groups.

Haemodynamic properties of adipose and rectus/uterus layers. Using the three-layer model, the optical and haemodynamic properties of adipose and rectus/uterus layers can also be reconstructed. Generally, we expect the accuracy of these overlay results to be less than that of the placenta because the probe SDSs were optimized for the deeper placental tissue. Note also that due to very thin adipose or rectus/uterus layer thickness, 4 of the 24 participants were processed with two-layer rather than three-layer model reconstruction; therefore, we excluded these 4 participants in the statistical analysis of adipose or rectus/uterus layer. The StO₂ and [Hb_T] of adipose and rectus/uterus layers are reported in Supplementary Table 3; these properties are within ~10% of values reported for somewhat similar tissues; for example, breast⁵⁷ (for comparison to adipose) and muscle⁵⁸ (for comparison to rectus/uterus). For the placenta layer, we found good agreement between our reduced scattering coefficient and an ex vivo placenta study⁵⁹; the placenta layer absolute haemoglobin properties have not been previously reported (to our knowledge), and the placenta layer StO₂ values are roughly consistent with those observed by CW-NIRS (continuous-wave near-infrared spectroscopy)⁴⁴.

The adipose and rectus/uterus baseline haemoglobin properties of healthy participants were not significantly different from participants with APO or MVM (Supplementary Table 5). This finding parallels our baseline placenta results. We also analysed the adipose and rectus/uterus layer haemodynamic response to maternal hyperoxia, and we compared their differences across participant groups (Supplementary Table 5). We did not find significant association of the overlay haemodynamic properties in normal participants versus participants with APO or MVM. This finding is different from the placenta results during maternal hyperoxia. In total, these data provide in vivo evidence underscoring the importance of the multilayer modelling to separate layer responses. Without the multilayer model and associated instrumentation, quantitative estimates of placenta response are contaminated by signals from the other layers.

The null overlay results are not necessarily surprising from a physiological perspective. For example, since MVM presents a recognizable pattern of placental injury related to altered uterine and intervillous blood flow, we would not expect significant association between adipose haemoglobin properties and MVM. However, we noticed that the rectus/uterine layer blunted response to maternal hyperoxia exhibited a trending but statistically non-significant association to APO ($P=0.06$). Although it is possible that impaired uterine perfusion may be involved in the pathophysiology of placental dysfunction, in our view, further study in a larger sample size will be important to confirm and further elucidate these relationships.

Uterine-artery Doppler pulsatility index. Each uterine artery was identified using a transabdominal C1-5 ultrasound probe (GE Healthcare) via power Doppler mapping. Pulsed wave Doppler was then used to obtain three similar consecutive waveforms. PI was defined as the difference between peak systolic and end diastolic velocities divided by the mean velocity. The mean PI of the two uterine arteries was used for analysis. No association was found between UtA PI and APO or MVM (Table 2). Furthermore, when including UtA PI as a covariate, the associations between ΔStO_2 and ΔHbO_2 and our outcomes (that is APO and MVM) remained apparent (Supplementary Table 4).

Statistical analysis. Statistical analyses were performed using MATLAB 2019a. Accuracy in phantom experiments and simulations is defined as the difference between measured and expected values divided by the expected value. Depending on the data type, results from the clinical study are presented as mean (and s.d.) or median (with IQR). ICC in the reproducibility experiment was calculated by dividing the random effect variance by the total variance. Our study employed a relatively small sample size (<30 participants), and the data in the two groups were not normally distributed. In this case, a non-parametric test is appropriate (that is, a rank-sum test with continuous data). Thus, P values for studying

correlations between different variables and placental dysfunction were obtained using the two-sided Wilcoxon rank-sum test, a non-parametric test for two populations when samples are independent. *P* values for studying correlations between nulliparity and placental dysfunction were obtained by two-sided Fisher's exact test. *P* values for studying the before/after difference in the maternal left tilt experiment were calculated by two-sided paired-sample *t*-test. Binary logistic regressions were also performed to study the correlation between APO/MVM and ΔStO_2 or ΔHbO_2 but with control of other variables: UtA PI, placental depth (*d*) and pre-gravid BMI. We carried out this analysis for completeness, with caveats that the sample size is small and that different pairs of variables might be partially correlated (and if so, that future inclusion of interactions in the statistical models is desirable). Supplementary Table 4 shows the resultant *P* values of ΔHbO_2 and ΔStO_2 for prediction of APO or MVM from the binary logistic regression models. The results confirm that there remained a trend towards significant association between optically derived haemodynamic properties and our outcomes of interest. For the future, a larger sample size will permit more sophisticated statistical analyses that explore the effects of possible confounding variables and generate composite metrics with improved specificity and sensitivity.

Reporting summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability

The main data supporting the results in this study are available within the paper and its supplementary information. All optical data generated in this study, including source data and the data used to make the figures, are available from figshare with identifiers at <https://doi.org/10.6084/m9.figshare.19451882>, <https://doi.org/10.6084/m9.figshare.19451879> and <https://doi.org/10.6084/m9.figshare.19451876>. The raw clinical and ultrasound data are available from the corresponding author, subject to approval from the Institutional Review Board of the University of Pennsylvania.

Code availability

The custom code employed for processing the optical data and for performing the statistical analysis are available from figshare with identifiers at <https://doi.org/10.6084/m9.figshare.19451882>, <https://doi.org/10.6084/m9.figshare.19451879> and <https://doi.org/10.6084/m9.figshare.19451876>. The LabVIEW code and simulation code are also available from the corresponding author on request.

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References

- Burton, G. J., Fowden, A. L. & Thornburg, K. L. Placental origins of chronic disease. *Physiol. Rev.* **96**, 1509–1565 (2016).
- Thornburg, K. L., O'Tierney, P. F. & Louey, S. Review: the placenta is a programming agent for cardiovascular disease. *Placenta* **31**, S54–S59 (2010).
- Hodyl, N. A. et al. Child neurodevelopmental outcomes following preterm and term birth: what can the placenta tell us? *Placenta* **57**, 79–86 (2017).
- AIUM-ACR-ACOG-SMFM-SRU practice parameter for the performance of standard diagnostic obstetric ultrasound examinations. *J. Ultrasound Med.* **37**, E13–E24 (2018).
- Turco, M. Y. et al. Trophoblast organoids as a model for maternal–fetal interactions during human placentation. *Nature* **564**, 263–281 (2018).
- Hemberger, M., Hanna, C. W. & Dean, W. Mechanisms of early placental development in mouse and humans. *Nat. Rev. Genet.* **21**, 27–43 (2020).
- Carter, A. M. Animal models of human placentation – a review. *Placenta* **28**, S41–S47 (2007).
- Schmidt, A., Morales-Prieto, D. M., Pastuschek, J., Fröhlich, K. & Markert, U. R. Only humans have human placentas: molecular differences between mice and humans. *J. Reprod. Immunol.* **108**, 65–71 (2015).
- Nye, G. A. et al. Human placental oxygenation in late gestation: experimental and theoretical approaches. *J. Physiol.* **596**, 5523–5534 (2018).
- Horsman, M. R., Mortensen, L. S., Petersen, J. B., Busk, M. & Overgaard, J. Imaging hypoxia to improve radiotherapy outcome. *Nat. Rev. Clin. Oncol.* **9**, 674–687 (2012).
- You, W. et al. Hemodynamic responses of the placenta and brain to maternal hyperoxia in fetuses with congenital heart disease by using blood oxygen-level dependent MRI. *Radiology* **294**, 141–148 (2020).
- Logothetis, N. K. What we can do and what we cannot do with fMRI. *Nature* **453**, 869–878 (2008).
- Durduran, T., Choe, R., Yodh, A. G. & Baker, W. B. Diffuse optics for tissue monitoring and tomography. *Rep. Prog. Phys.* **73**, 076701 (2010).
- Yodh, A. G. & Boas, D. A. in *Biomedical Photonics Handbook: Biomedical Diagnostics* (ed Vo-Dinh, Tuan) Ch. 21 (2014).
- Boas, D. A., O'Leary, M. A., Chance, B. & Yodh, A. G. Scattering of diffuse photon density waves by spherical inhomogeneities within turbid media: analytic solution and applications. *Proc. Natl Acad. Sci. USA* **91**, 4887–4891 (1994).
- Waterhouse, D. J., Fitzpatrick, C. R. M., Pogue, B. W., O'Connor, J. P. B. & Bohndiek, S. E. A roadmap for the clinical implementation of optical-imaging biomarkers. *Nat. Biomed. Eng.* **3**, 339–353 (2019).
- Baker, W. B. et al. Continuous non-invasive optical monitoring of cerebral blood flow and oxidative metabolism after acute brain injury. *J. Cereb. Blood Flow Metab.* **39**, 1469–1485 (2019).
- Choe, R. et al. Transabdominal near infrared oximetry of hypoxic stress in fetal sheep brain in utero. *Proc. Natl Acad. Sci. USA* **100**, 12950–12954 (2003).
- Tromberg, B. J. et al. Predicting responses to neoadjuvant chemotherapy in breast cancer: ACRIN 6691 trial of diffuse optical spectroscopic imaging. *Cancer Res.* **76**, 5933–5944 (2016).
- Boas, D. A., Elwell, C. E., Ferrari, M. & Taga, G. Twenty years of functional near-infrared spectroscopy: introduction for the special issue. *Neuroimage* **85**, 1–5 (2014).
- Yun, S. H. & Kwok, S. J. Light in diagnosis, therapy and surgery. *Nat. Biomed. Eng.* **1**, 8 (2017).
- Zhu, Q. et al. Breast cancer: assessing response to neoadjuvant chemotherapy by using US-guided near-infrared tomography. *Radiology* **266**, 433–442 (2013).
- Eggebrecht, A. T. et al. Mapping distributed brain function and networks with diffuse optical tomography. *Nat. Photonics* **8**, 448–454 (2014).
- Konecky, S. D. et al. Imaging complex structures with diffuse light. *Opt. Express* **16**, 5048–5060 (2008).
- Ntziachristos, V. Going deeper than microscopy: the optical imaging frontier in biology. *Nat. Methods* **7**, 603–614 (2010).
- Kakogawa, J., Sumimoto, K., Kawamura, T., Minoura, S. & Kanayama, N. Noninvasive monitoring of placental oxygenation by near-infrared spectroscopy. *Am. J. Perinatol.* **27**, 463–468 (2010).
- Hasegawa, J. et al. Evaluation of placental function using near infrared spectroscopy during fetal growth restriction. *J. Perinat. Med.* **38**, 29–32 (2010).
- Kawamura, T. et al. Measurement of placental oxygenation by transabdominal near-infrared spectroscopy. *Am. J. Perinatol.* **24**, 161–166 (2007).
- Scholkmann, F. et al. A review on continuous wave functional near-infrared spectroscopy and imaging instrumentation and methodology. *NeuroImage* **85**, 6–27 (2014).
- Boas, D. A., Pitrís, C. & Ramanujam, N. (eds) *Handbook of biomedical optics* (CRC Press, 2016). <https://doi.org/10.1201/b10951>
- Fantini, S. & Sassaroli, A. in *Handbook of Optical Biomedical Diagnostics* 2nd edn (ed Valery V. Tuchin) Ch. 7. (SPIE Press, 2016). <https://doi.org/10.1117/3.2219603.ch7>
- Choi, J. et al. Noninvasive determination of the optical properties of adult brain: near-infrared spectroscopy approach. *J. Biomed. Opt.* **9**, 221–229 (2004).
- Liebert, A. et al. Assessment of inflow and washout of indocyanine green in the adult human brain by monitoring of diffuse reflectance at large source-detector separation. *J. Biomed. Opt.* **16**, 046011 (2011).
- Pifferi, A. et al. New frontiers in time-domain diffuse optics, a review. *J. Biomed. Opt.* **21**, 091310 (2016).
- Wright, E. et al. Maternal vascular malperfusion and adverse perinatal outcomes in low-risk nulliparous women. *Obstet. Gynecol.* **130**, 1112–1120 (2017).
- Ernst, L. M. Maternal vascular malperfusion of the placental bed. *APMIS* **126**, 551–560 (2018).
- Hallacoglu, B., Sassaroli, A. & Fantini, S. Optical characterization of two-layered turbid media for non-invasive, absolute oximetry in cerebral and extracerebral tissue. *PLoS ONE* **8**, E64095 (2013).
- Liemert, A. & Kienle, A. Light diffusion in N-layered turbid media: frequency and time domains. *J. Biomed. Opt.* **15**, 025002 (2010).
- Ripoll, J. et al. Recovery of optical parameters in multiple-layered diffusive media: theory and experiments. *J. Opt. Soc. Am. A* **18**, 821–830 (2001).
- Corlu, A. et al. Diffuse optical tomography with spectral constraints and wavelength optimization. *Appl. Opt.* **44**, 2082–2093 (2005).
- Pogue, B. W. & Patterson, M. S. Review of tissue simulating phantoms for optical spectroscopy, imaging and dosimetry. *J. Biomed. Opt.* **11**, 041102 (2006).
- Schweiger, M. & Arridge, S. The Toast++ software suite for forward and inverse modeling in optical tomography. *J. Biomed. Opt.* **19**, 040801 (2014).
- Lee, S. W. Y., Khaw, K. S., Kee, W. D. N., Leung, T. Y. & Critchley, L. A. H. Haemodynamic effects from aorticaval compression at different angles of lateral tilt in non-labouring term pregnant women. *Br. J. Anaesth.* **109**, 950–956 (2012).
- O'Gorman, N., Tampakoudis, G., Wright, A., Wright, D. & Nicolaides, K. H. Uterine artery pulsatility index at 12, 22, 32 and 36 weeks' gestation in screening for pre-eclampsia. *Ultrasound Obstet. Gynecol.* **47**, 565–572 (2016).

45. Nguyen, T. et al. Non-invasive transabdominal measurement of placental oxygenation: a step toward continuous monitoring. *Biomed. Opt. Express* **12**, 4119–4130 (2021).
46. American College of Obstetricians and Gynecologists. Management of intrapartum fetal heart rate tracings. *Obstet. Gynecol.* **116**, 1232–1240 (2010).
47. Luo, J. et al. In vivo quantification of placental insufficiency by BOLD MRI: a human study. *Sci. Rep.* **7**, 3713 (2017).
48. Catov, J. M. et al. Neonatal outcomes following preterm birth classified according to placental features. *Am. J. Obstet. Gynecol.* **216**, 411.e1–411.e14 (2017).
49. Liemert, A. Light diffusion in N-layered turbid media: steady-state domain. *J. Biomed. Opt.* **15**, 025003 (2010).
50. Haskell, R. C. et al. Boundary conditions for the diffusion equation in radiative transfer. *J. Opt. Soc. Am. A* **11**, 2727–2741 (1994).
51. Jacques, S. L. Optical properties of biological tissues: a review. *Phys. Med. Biol.* **58**, R37–R61 (2013).
52. Hansen, P. C. in *Computational Inverse Problems in Electrocardiology* Vol. 4 (ed P. Johnston, *Advances in Computational Bioengineering*) 119–142 (WIT Press, Southampton, 2000).
53. Ugray, Z. et al. Scatter search and local NLP solvers: a multistart framework for global optimization. *INFORMS J. Comput.* **19**, 328–340 (2007).
54. Gestational hypertension and preeclampsia: ACOG practice bulletin, number 222. *Obstet. Gynecol.* **135**, e237–e260 (2020).
55. Fenton, T. R. & Kim, J. H. A systematic review and meta-analysis to revise the Fenton growth chart for preterm infants. *BMC Pediatr.* **13**, 59 (2013).
56. Khong, T. Y. et al. Sampling and definitions of placental lesions: Amsterdam Placental Workshop Group Consensus Statement. *Arch. Pathol. Lab. Med.* **140**, 698–713 (2016).
57. Taroni, P. et al. Breast tissue composition and its dependence on demographic risk factors for breast cancer: non-invasive assessment by time domain diffuse optical spectroscopy. *PLoS ONE* **10**, e0128941 (2015).
58. Henry, B. et al. Hybrid diffuse optical techniques for continuous hemodynamic measurement in gastrocnemius during plantar flexion exercise. *J. Biomed. Opt.* **20**, 125006 (2015).
59. Khare, S. M. et al. Evaluation of the human placenta optical scattering properties using continuous wave and frequency-domain diffuse reflectance spectroscopy. *J. Biomed. Opt.* **25**, 116001 (2020).

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Author contributions

L.W., A.G.Y. and N.S. designed the study. L.W. and T.K. developed the instrument with assistance from W.B.B., K.A., L.H., D.R.B. and V.K. L.W. and J.M.C. developed the three-layer reconstruction algorithm and conducted the computer simulations. L.W. and T.K. performed phantom experiments with help from W.B.B. and L.H. K.A. designed the optical probe with input from L.W. and W.B.B. L.W. collected and analysed the optical data. S.P. and N.S. advised on human participant data interpretation. N.S. collected and analysed the ultrasound data. R.L.L. performed placental histopathologic analysis. L.W., A.G.Y. and N.S. wrote the paper with input from all authors.

Competing interests

The authors declare no competing interests.

Additional information

Extended data is available for this paper at <https://doi.org/10.1038/s41551-022-00913-2>.

Supplementary information The online version contains supplementary material available at <https://doi.org/10.1038/s41551-022-00913-2>.

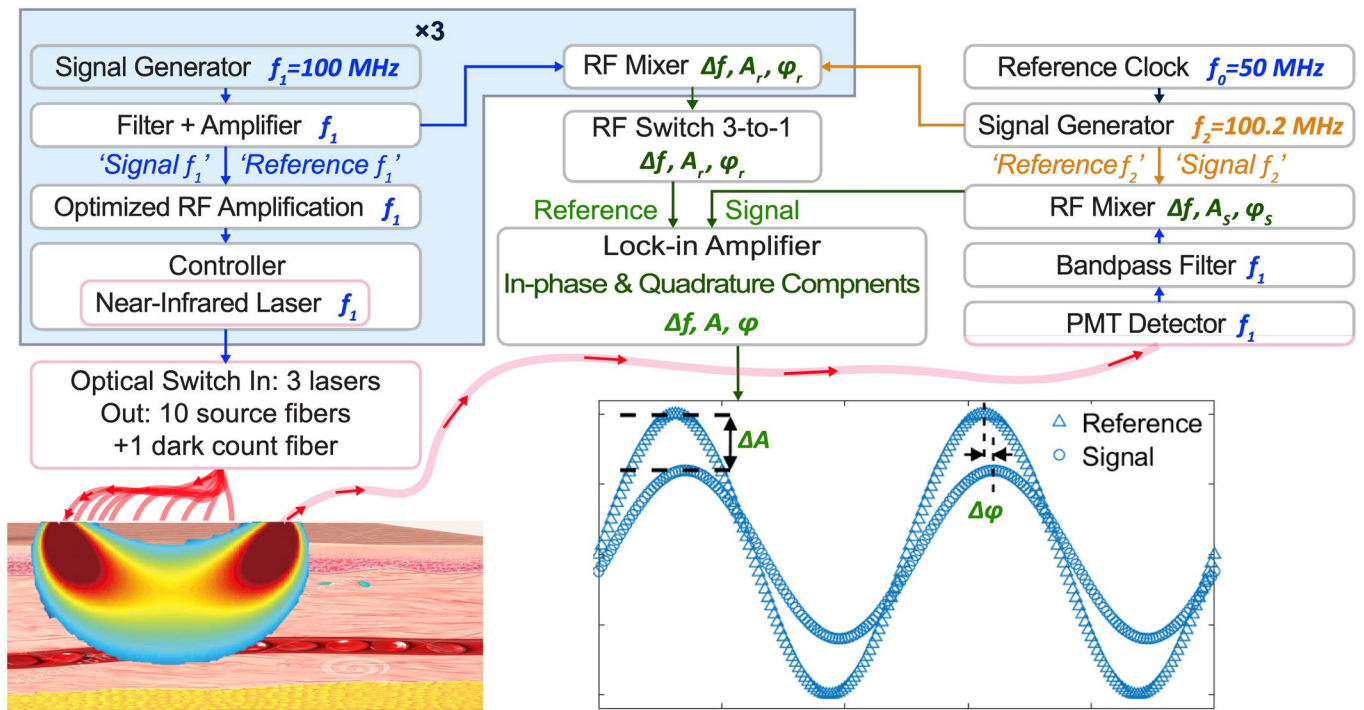
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Extended Data Fig. 1 | Detailed schematic of the custom heterodyne FD-DOS instrument.

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Software and code

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Data collection

Custom LABVIEW codes were developed under LABVIEW 2014. Custom LABVIEW codes were used to collect optical data. A commercial ultrasound system (Voluson E10, GE Healthcare) with a 9L-D transabdominal linear probe was used to collect ultrasound data during the optical measurements. A C2-5 probe was used for uterine artery Doppler measurements. Clinical data were extracted from the electronic medical record by study coordinators blinded to the optical data.

Data analysis

Optical data and statistical analyses were processed by custom MATLAB codes (in MatLab 2018b). Finite-element simulation data were generated with commercial the software TOAST. The custom code for processing the optical data and for performing the statistical analysis are available, as follows:

FD-DOS raw data and multi-layer-reconstruction processing code: <https://doi.org/10.6084/m9.figshare.19451882>.
Stability / maternal-hyperoxia statistical-analysis code: <https://doi.org/10.6084/m9.figshare.19451879>.
Left-tilt experiment statistical-analysis code: <https://doi.org/10.6084/m9.figshare.19451876>.

The LabVIEW code and simulation code are also available from the corresponding author on request.

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The main data supporting the results in this study are available within the paper and its supplementary information. All optical data generated in this study, including source data and the data used to make the figures, are available from figshare with the identifiers <https://doi.org/10.6084/m9.figshare.19451882>, <https://doi.org/10.6084/m9.figshare.19451879> and <https://doi.org/10.6084/m9.figshare.19451876>. The raw clinical and ultrasound data are available from the corresponding author, subject to approval from the Institutional Review Board of the University of Pennsylvania.

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Life sciences study design

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Sample size	For the pilot study, as it used a new device, no sample-size calculations were made. Rather, we opportunistically recruited patients with confirmed anterior placentas and who met inclusion and exclusion criteria and who consented to participation. The sample size was limited by the availability of the authors involved in obtaining the optical (L.W.) and ultrasound (N.S.) data, as well as of the study coordinators who would obtain the consent of the participants, record the data and help administer the oxygen.
Data exclusions	Data from two other participants were excluded because their signals were either too small (tissue optical absorption coefficient was very large) or too unstable (owing to large fluctuations during the baseline period).
Replication	Measurement reproducibility was evaluated by a reproducibility experiment, wherein a two-frame measurement was made both before and after lifting and placing the probe at approximately the same location for three times (n = 18). Placental oxygen saturation, total haemoglobin concentration, and oxyhaemoglobin concentration, were highly repeatable within subjects (intra-class correlation coefficient ≥ 0.9).
Randomization	This was an observational cohort study with no randomization to study arms or interventions.
Blinding	Optical-data collection and analysis was performed blinded to both the clinical pregnancy outcomes and to the placental histopathology findings. Inversely, the placental pathologist was blinded to the optical measurements when analysing the placentas.

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Human research participants

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Population characteristics The participants in the maternal-hyperoxia study had a median (interquartile range; IQR) gestational age of 34.5 (32.9, 35.4)

Population characteristics

weeks and a median (IQR) pre-gravid BMI of 27.7 (24.6, 30.2). The median (IQR) depth of the anterior placentas was 2.7 (2.3, 3.3) cm beneath the skin surface, and the nulliparity of the subjects was 29%.

37.5% of the participants had adverse pregnancy outcomes, and 33.3% had maternal vascular malperfusion. 29.2% of the participants were white, 62.5% were African American, 4% Latino, and 4% American Indian.

Recruitment

The participants were recruited as part of a larger prospective cohort exploring various placental-imaging techniques. For this study, only participants with an anterior placenta that was within 4 cm of the skin surface were approached for optical imaging. The limitations of a high body mass index and placental depth are explicitly analysed and discussed in the paper.

The sample size was limited by the availability of the authors involved in obtaining the optical (L.W.) and ultrasound (N.S.) data, as well as of the study coordinators who would obtain the consent of the participants, record the data and help administer the oxygen.

Recruitment limitations were operational and logistical, without consideration of the characteristics of the subjects, and therefore should not bias the study population.

Ethics oversight

Prior to proceeding with the study, approval by the Institutional Review Board (IRB) at the University of Pennsylvania was obtained.

Note that full information on the approval of the study protocol must also be provided in the manuscript.