Coherent hemodynamics spectroscopy in a single step

Jana M. Kainerstorfer,* Angelo Sassaroli, and Sergio Fantini
Department of Biomedical Engineering, Tufts University, 4 Colby Street, Medford, MA 02155, USA
*jana.kainerstorfer@tufts.edu

Abstract: Coherent Hemodynamics Spectroscopy (CHS) is a technique based on inducing cerebral hemodynamic oscillations at multiple frequencies, measuring them with near-infrared spectroscopy (NIRS), and analyzing them with a hemodynamic model to obtain physiological information such as blood transit times in the microvasculature and the autoregulation cutoff frequency. We have previously demonstrated that such oscillations can be induced one frequency at a time. Here we demonstrate that CHS can be performed by a single inflation of two pneumatic thigh cuffs (duration: 2 min; pressure: 200 mmHg), whose sudden release produces a step response in systemic arterial blood pressure that lasts for ~20 s and induces cerebral hemodynamics that contain all the frequency information necessary for CHS. Following a validation study on simulated data, we performed measurements on human subjects with this new method based on a single occlusion/release of the thigh cuffs and with the previous method based on sequential sets of cyclic inflation/deflation one frequency at a time, and demonstrated that the two methods yield the same CHS spectra and the same physiological parameters (within measurement errors). The advantages of the new method presented here are that CHS spectra cover the entire bandwidth of the induced hemodynamic response, they are measured over ~20 s thus better satisfying the requirement of time invariance of physiological conditions, and they can be measured every ~2.5 min thus achieving finer temporal sampling in monitoring applications.

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References and links
Near Infrared Spectroscopy (NIRS) is a non-invasive optical technique that can measure cerebral oxy- ($O$) and deoxy- ($D$) hemoglobin concentrations over time, i.e. during functional activation or physiological processes. The underlying sources of such time-dependent...
hemodynamics are changes in cerebral blood volume (CBV), cerebral blood flow (CBF), and metabolic rate of oxygen (CMRO₂). Spontaneous oscillations in hemoglobin concentrations have also been observed and correlated to spontaneous oscillations in mean arterial blood pressure (MAP) [1]. In addition to spontaneous occurrence, oscillations can be induced by paced breathing [1, 2], repeated head-up tilting [3], squat-stand maneuvers [4], and cyclic thigh cuff inflation-deflation [5]. Those maneuvers have typically been used to induce oscillations at ~0.1 Hz in MAP and cerebral blood flow that have been measured using finger plethysmography and transcranial Doppler ultrasound (TCD), respectively [6]. Using transfer function analysis, MAP and CBF oscillations have been used to assess cerebral autoregulation [7]. Understanding the interplay between MAP, CBF, and associated hemodynamic changes is the goal of hemodynamic models.

We have recently introduced one such hemodynamic model, which, in conjunction with measurements of induced hemodynamic oscillations at multiple frequencies, led to a technique we have named Coherent Hemodynamics Spectroscopy (CHS) [8]. For CHS, we have demonstrated that hemodynamic oscillations can be induced, one frequency at a time, and the phase and amplitude of those hemodynamic oscillations can be described with the novel hemodynamic model. As a result, physiological parameters such as the blood transit time in the microvasculature and the autoregulation cutoff frequency can be obtained. Being able to non-invasively measure the capillary transit times and autoregulation mechanisms in the microvasculature offers a significant promise for clinical applications. In fact, it has been found that cerebral autoregulation is altered or impaired in patients with a number of disorders such as autonomic failure [9, 10], diabetes [11–13], Parkinson’s disease [14], and stroke [15]. We have already shown that CHS is applicable in the clinical setting of the hemodialysis unit. In a study on patients undergoing dialysis, where cerebral hemodynamic oscillations were induced by periodic inflation and deflation of a pneumatic thigh cuff, we found a longer capillary transit time, indicating a reduced cerebral blood flow, in patients compared to healthy controls [16]. Understanding the chronic effects of hemodialysis on the cerebral microcirculation can contribute to assessing the origin of neurological and cognitive deficits in dialysis patients.

While CHS spectra can be obtained by inducing oscillations at multiple frequencies, the sequential measurement of oscillatory hemodynamics at each frequency results in relatively long measurement times and in CHS spectra whose individual data points at each frequency are measured at different times. Here, we introduce a new method of inducing cerebral hemodynamic perturbations from which all of the frequency information needed for CHS is obtained simultaneously. This method is based on inducing a sudden change in the systemic mean arterial blood pressure (MAP) by a fast deflation of two thigh cuff (one per each leg) after they have been kept inflated for 2 min at a pressure of 200 mmHg. The sudden, step-like, thigh cuff deflation induces a fast decrease in MAP [17], which in turn elicits a step response of the cerebral concentrations of oxy-, deoxy-, and total hemoglobin \([O(t), D(t), \text{ and } T(t)]\). This step response of the cerebral hemodynamics features a frequency information content that is suitable for CHS, and we demonstrate that CHS spectra can be obtained at once from the data collected over a period of ~20 s following the sudden release of the thigh cuffs pressure. This approach does not require sequential measurements of hemodynamic oscillations at multiple frequencies, and we show that cerebral hemodynamics measured during a single step response of MAP and from sequential MAP oscillations at multiple frequencies result in the same CHS spectra to within measurement errors.

2. Coherent hemodynamics spectroscopy (CHS)

CHS is a technique based on frequency-resolved measurements of the amplitude and phase of hemodynamic oscillations at a set of controlled frequencies. Translation of these frequency-resolved measurements into physiologically meaningful quantities is done through a hemodynamic model, which we have introduced recently [8, 18]. The model is the
quantitative framework to translate the information content of measured CHS spectra into a set of physiological and functional parameters that include the microvascular blood transit time (which is inversely related to cerebral blood flow) and local cerebral autoregulation at the microvascular level. Details about the model can be found elsewhere [8, 19], but shall be summarized briefly here.

The model describes sinusoidal hemodynamic oscillations as a function of the angular frequency $\omega$. Following the convention of Fantini’s work, oscillatory quantities are represented by phasors that are indicated in bold face. The model expressions for $\mathbf{O}(\omega)$, $\mathbf{D}(\omega)$, $\mathbf{T}(\omega)$ (i.e. the phasors that describe the oscillations of oxy-, deoxy- and total hemoglobin concentrations) as a function of $\mathbf{cbv}(\omega)$, $\mathbf{cbf}(\omega)$, and $\mathbf{cmro}_2(\omega)$ (i.e. the phasors that describe the oscillations of cerebral blood volume, blood flow, and metabolic rate of oxygen) are as follows [8, 19]:

$$
\mathbf{O}(\omega) = \mathbf{cbf}(\omega) \left[ S^{(a)} \mathbf{CBV}_0^{(a)} \mathbf{cbv}^{(a)}(\omega) + S^{(v)} \mathbf{CBV}_0^{(v)} \mathbf{cbv}^{(v)}(\omega) \right] +$$

$$+ \mathbf{ctHB} \left[ \frac{\omega^{(c)}}{\omega^{(c)} + \omega} \left( \mathbf{cbv}^{(c)}(\omega) + \mathbf{cbv}^{(v)}(\omega) \right) \right] \mathbf{cbf}(\omega) - \mathbf{cmro}_2(\omega),
$$

(1)

$$
\mathbf{D}(\omega) = \mathbf{cbf}(\omega) \left[ \left( 1 - S^{(a)} \right) \mathbf{CBV}_0^{(a)} \mathbf{cbv}^{(a)}(\omega) + \left( 1 - S^{(v)} \right) \mathbf{CBV}_0^{(v)} \mathbf{cbv}^{(v)}(\omega) \right] +$$

$$- \mathbf{ctHB} \left[ \frac{\omega^{(c)}}{\omega^{(c)} + \omega} \left( \mathbf{cbv}^{(c)}(\omega) + \mathbf{cbv}^{(v)}(\omega) \right) \right] \mathbf{cbf}(\omega) - \mathbf{cmro}_2(\omega),
$$

(2)

$$
\mathbf{T}(\omega) = \mathbf{ctHB} \left[ \mathbf{CBV}_0^{(a)} \mathbf{cbv}^{(a)}(\omega) + \mathbf{CBV}_0^{(v)} \mathbf{cbv}^{(v)}(\omega) \right],
$$

(3)

where $\mathcal{H}_{LP}^{(c)}(\omega)$ and $\mathcal{H}_{LP}^{(v)}(\omega)$ are the complex transfer functions associated with blood circulation in the capillary bed [$\mathcal{H}_{LP}^{(c)}(\omega)$, approximated by a resistor-capacitor (RC) low-pass filter, which contains as a parameter the capillary transit time $\delta^{(c)}$], and in the venous compartment [$\mathcal{H}_{LP}^{(v)}(\omega)$, approximated by a time-shifted Gaussian low-pass filter, which contains as parameters the capillary transit time $\delta^{(v)}$ and the venous transit time $\delta^{(v)}$, $\mathbf{ctHB}$ is the hemoglobin concentration in blood, $\mathcal{F}^{(c)}$ is the Fåhraeus factor (ratio of capillary-to-large vessel hematocrit)], and the superscripts $(a)$, $(c)$, and $(v)$ for CBV, cbv, and hemoglobin saturation $S$ indicate partial contributions from the arterial, capillary, and venous compartments, respectively. The total, steady state blood volume is given by $\mathbf{CBV}_0 = \mathbf{CBV}_0^{(a)} + \mathcal{F}^{(c)} \mathbf{CBV}_0^{(c)} + \mathbf{CBV}_0^{(v)}$. We have set $\mathbf{cbv}^{(c)}(\omega) = 0$ because of the negligible dynamic dilution and recruitment of capillaries in brain tissue [20–25]. Because of the high-pass nature of the cerebral autoregulation process that regulates cerebral blood flow in response to blood pressure changes [6, 9, 26], we consider the following relationship between $\mathbf{cbf}$ and $\mathbf{cbv}$ [8]:

$$
\mathbf{cbf}(\omega) = k \mathcal{H}_{LP}^{(AR)}(\omega) \mathbf{cbv}(\omega) = k \mathcal{H}_{LP}^{(AR)}(\omega) \left[ \frac{\mathbf{CBV}_0^{(a)} \mathbf{cbv}^{(a)}(\omega)}{\mathbf{CBV}_0} + \frac{\mathbf{CBV}_0^{(v)} \mathbf{cbv}^{(v)}(\omega)}{\mathbf{CBV}_0} \right],
$$

(4)

where $k$ is the inverse of the modified Grubb’s exponent, and $\mathcal{H}_{LP}^{(AR)}(\omega)$ is the RC high-pass transfer function with cutoff frequency $\omega^{(AR)}_{\text{mod}}$ that describes the effect of autoregulation. We observe that the autoregulation relationship in Eq. (4) considers blood volume as a surrogate for blood pressure because it is known that the dynamics of total hemoglobin
concentration (which is a direct measure of blood volume) closely match those of arterial blood pressure, with a relatively small time delay [1] that will be reported and discussed in this work.

For CHS, frequency dependent measurements of amplitude ratios and phase differences are considered through the following phasor ratios:

\[
\frac{D(\omega)}{O(\omega)} = \frac{D(\omega)}{O(\omega)} e^{\left[\text{Arg}[D(\omega)] - \text{Arg}[O(\omega)]\right]},
\]

\[
\frac{O(\omega)}{T(\omega)} = \frac{O(\omega)}{T(\omega)} e^{\left[\text{Arg}[O(\omega)] - \text{Arg}[T(\omega)]\right]},
\]

If the induced hemodynamic oscillations do not involve modulation of the cerebral metabolic rate of oxygen \([\text{cmro}_i(\omega) = 0]\), and if the arterial and venous blood volume dynamics are assumed to be synchronous, the model equations become [19]:

\[
\frac{D^{(\alpha / \nu)}(\omega)}{O^{(\alpha / \nu)}(\omega)} = \frac{1}{\Delta(\alpha / \nu) + 1} \left[ \frac{D^{(\mu / \nu)}(\omega)}{O^{(\mu / \nu)}(\omega)} - \frac{1}{\Delta(\mu / \nu) + 1} \right]
\]

\[
\frac{O^{(\alpha / \nu)}(\omega)}{T^{(\alpha / \nu)}(\omega)} = \frac{1}{\Delta(\alpha / \nu) + 1} \left[ \frac{O^{(\mu / \nu)}(\omega)}{T^{(\mu / \nu)}(\omega)} - \frac{1}{\Delta(\mu / \nu) + 1} \right]
\]

where we have used the shortened notation of \(V^{(c / \nu)}_0 = \frac{\text{CBV}^{(c)}_0}{\text{CBV}^{(\nu)}_0}\) for the ratio of the capillary-to-venous \((c / \nu)\) baseline blood volume, \(\Delta V^{(a / \nu)} = \left(\frac{\text{CBV}^{(a)}_0}{\text{CBV}^{(\nu)}_0}\right) / \left(\frac{\text{CBV}^{(\nu)}_0}{\text{CBV}^{(a)}_0}\right)\) for the ratio of the arterial-to-venous \((a / \nu)\) blood volume oscillation amplitudes, and \(k \nu = k \nu \nu = \text{CBV}^{(\nu)}_0 / \text{CBV}^{(\nu)}_0\) for the product of the high-frequency flow-to-volume amplitude ratio \((k)\) times the ratio of venous-to-total \((\nu / T)\) baseline blood volume. These three quantities, together with the arterial saturation \((S^{(c)}_a)\), set here to the fixed value of 0.98, the rate constant of oxygen diffusion \((\alpha)\), set here to the fixed value of 0.8 s\(^{-1}\), the autoregulation cutoff frequency \((\omega^{(\text{AutoReg})})\), the blood transit time in capillaries, \(t^{(c)}\), and the blood transit time in the venules, \(t^{(\nu)}\), are the eight model parameters used in CHS, also summarized in Table 1.

3. Methods

Near-infrared spectroscopy (NIRS) measurements were performed with a commercial tissue oximeter (OxiplexTS, ISS, Inc., Champaign, IL), operating at wavelengths of 690 and 830 nm. The optical probe was placed on the right side of the subject’s forehead and featured a source-detector separation of 3.5 cm, which is appropriate to probe the prefrontal cerebral cortex. This instrument provided relative measurements of the cerebral concentrations of oxy-hemoglobin \((O)\), deoxy-hemoglobin \((D)\), and total hemoglobin \((T = O + D)\). The arterial blood pressure was monitored continuously with a beat-to-beat finger plethysmography.
system (NIBP100D, BIOPAC Systems, Inc., Goleta, CA), which is based on an initial calibration measurement of systolic and diastolic pressures by means of an arm cuff. MAP was approximated by low-pass filtering the continuous arterial blood pressure output. While MAP is defined as a weighted contribution of systolic and diastolic blood pressures, the continuous arterial pressure was measured at a rate of 6.25 Hz, not sufficient for obtaining a reliable systolic pressure reading. Low-pass filtering proved to be a more robust estimate of MAP.

A pneumatic cuff was placed around each thigh of the subject and connected to an automated cuff inflation device (E-20 Rapid Cuff Inflation System, D. E. Hokanson, Inc., Bellevue, WA). Two inflation protocols were used in alternating order. In the first protocol, the cuffs were inflated to a pressure of 200 mmHg, kept inflated for 2 min, and then quickly deflated in ~3s (step-like change in cuff pressure) to induce a step response of the cerebral hemodynamics. In the second protocol, the cuffs were cyclically inflated (to a pressure of ~200 mmHg) and deflated for a total time of 1 min at each of four frequencies (0.050, 0.0625, 0.0714, and 0.0833 Hz) to induce oscillatory cerebral hemodynamics. The sudden deflation of the two thigh cuffs after 2 min of 200 mmHg inflation produces a quick drop in blood pressure [17], which results in cerebral hemodynamics that represent a step response before returning to equilibrium after ~20-30 s. This hemodynamic response has frequency content in the range 0-0.09 Hz (as seen in Fig. 1(a)), which is suitable for CHS. Data analysis to generate CHS spectra can be performed at any set of frequencies within the frequency band of hemodynamic response. While this response is not strictly a step response because the driving drop in arterial blood pressure is not instantaneous (see Fig. 2), for the extent of this work it will still be referred to as a step response since it results from a step decrease in the thigh cuffs pressure. The two protocols of step deflation and cyclic inflation/deflation of the thigh cuffs were alternated and repeated three times on each subject to compare variability within-subject over time and between the two methods.

Four healthy subjects participated in the study, 2 females (ages: 25 and 30 years old), and 2 males (ages: 29 and 49 years old). The experimental protocol was approved by the Tufts University Institutional Review Board (IRB) and written informed consent was obtained from all participants prior to the study.

After removing slow temporal drifts from the NIRS data by third-order polynomial detrending, intensity changes were translated into oxy- and deoxy-hemoglobin concentrations in tissue (ΔO and ΔD) using the modified Beer-Lambert law. Linear-phase, band-pass filtering with a finite impulse response filter based on the Parks–McClellan algorithm was performed for the entire time traces of ΔO and ΔD. For the analysis of the periodic inflation protocol, data were filtered around each periodic inflation/deflation frequency. For the step response, filtering was performed between 0.045 and 0.090 Hz in 0.005 Hz increments. The reason for the upper limit (0.09 Hz) can be seen in Fig. 1(a), where it is shown that the step response of cerebral total hemoglobin concentration has frequency content up to ~0.09 Hz in this study. The lower limit (0.045 Hz) was chosen to avoid slow temporal drifts in the data, which could potentially obscure this very low frequency response. The amplitude and phase of the oscillations of deoxy- and oxy-hemoglobin concentrations [respectively |D| and arg(D), |O| and arg(O)] at a given frequency were assessed by the analytic signal method, as described previously [27], resulting in time-resolved measurements of instantaneous amplitude ratios and phase differences. Averaging over the 20 s time window following cuff deflation for the step response protocol, or over the 60 s time window for each oscillatory frequency in the periodic inflation/deflation protocol yielded average amplitude ratios |D|/|O|, |O|/|T|, as well as average phase differences arg(D) - arg(O), and arg(O) - arg(T), at each frequency considered. After calculating |D|/|O|, |O|/|T|, arg(D) - arg(O), and arg(O) - arg(T) for each of the three step responses and each of the three repetitions of the periodic inflation/deflation protocol, the three measured CHS spectra per protocol were averaged for further processing using the hemodynamic model. The standard deviation of the CHS data
over the three repetitions of each protocol provides a measure of the CHS spectral variability within subjects.

Fitting for the six unknown parameters in the model (we recall that two of the eight model parameters, $S(C) = 0.98$ and $\alpha = 0.8$ s$^{-1}$, were set to fixed values) was done with a built-in fitting procedure in MATLAB (function “lsqcurvefit”) with the default reconstruction algorithm that is a trust region reflective algorithm. The fitting procedure considers the measured values of the four quantities $D/|O|$, $|O|/|T|$, $\text{arg}(D) - \text{arg}(O)$, and $\text{arg}(O) - \text{arg}(T)$ at multiple frequencies and finds the optimal set of the six unknown parameters by minimizing a cost function based on the residuals between measured CHS spectra and those computed using the hemodynamic model [Eqs. (7) and (8)]. We have used upper and lower bounds on the parameters as described previously [19]. Table 1 reports the model parameters and associated ranges (within the upper and lower bounds) considered in the fits. In order to obtain an estimate on the parameter uncertainty, fifty-four different initial guesses spread over the entire range between the upper and lower bounds for the set of parameters were used, and the stopping criterion for the fit was set to the magnitude of the variance of the data, as given by the variance over repetitions of the same protocol on the same subject.

<table>
<thead>
<tr>
<th>Table 1. Model parameters and their lower and upper bounds</th>
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<tbody>
<tr>
<td>Parameter</td>
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<td>-----------------</td>
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<tr>
<td>$t^0$ (s)</td>
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<tr>
<td>$t^1$ (s)</td>
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<tr>
<td>$\mathcal{F}(\cdot)$ CBV$^{(\cdot)}$ / CBV$^{(\cdot)}_0$</td>
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<tr>
<td>$\left(\frac{\text{CBV}^{(\cdot)}_0}{\text{CBV}^{(\cdot)}_0}\right)$ / $\left(\frac{\text{CBV}^{(\cdot)}_0}{\text{CBV}^{(\cdot)}_0}\right)$</td>
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<tr>
<td>$\frac{\omega_{(\text{Quad})}}{2\pi}$ (Hz)</td>
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<tr>
<td>$k$CBV$^{(\cdot)}$ / CBV$^{(\cdot)}_0$</td>
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A relevant question that is addressed in this work is whether the step response of cerebral $\Delta O$, $\Delta D$, and $\Delta T$ to the fast release of the thigh cuffs pressure, which occurs in the $\sim$20 s before the cerebral hemoglobin concentration ($T$) recovers to baseline, contains sufficient information to measure CHS spectra. Furthermore, it is important to validate the accuracy of data processing (detrending, digital filtering) and data analysis (model-based fitting of CHS spectra) in ideal and known conditions. To this aim, we have used simulated data to evaluate the accuracy of extracting frequency information by digital band-pass filtering of the relatively short signals of interest ($\sim$20 s). Furthermore, simulated data generated with specific values of the model parameters allowed us to test the accuracy of the fitting procedure described above. Time traces of $\Delta O$, $\Delta D$, and $\Delta T$ were simulated based on the time domain version of Eqs. (1)-(4) [18, 19]. The smoothed time trace of the measured $\Delta T(t)$ from one subject (Fig. 1(a)) was used to define $\text{cbv}(t) = \Delta T(t)/T_0$, assuming the baseline hemoglobin concentration to be $T_0 = 55$ µM. We then computed the time traces of $\Delta O(t)$, and $\Delta D(t)$ from the model equations after setting the eight model parameters to $S(C) = 0.98$, $\alpha = 0.8$ s$^{-1}$, $t^0 = 0.75$ s, $t^1 = 2$ s, $\mathcal{F}(\cdot)$ CBV$^{(\cdot)}_0$ / CBV$^{(\cdot)}_0$ = 2.4, $\left(\text{CBV}^{(\cdot)}_0/\text{cbv}^{(\cdot)}_0\right)$ / $\left(\text{CBV}^{(\cdot)}_0/\text{cbv}^{(\cdot)}_0\right)$ = 1, $\omega_{(\text{Quad})} / (2\pi) = 0.05$ Hz, and $k$CBV$^{(\cdot)}_0 / CBV_0 = 0.6$.

The results are shown in Fig. 1(a). The filtering and data processing procedures described above were applied to this simulated data set to obtain CHS spectra (by filtering and Hilbert transformation) and to recover the six model parameters of interest (by model-based fitting).

4. Results

The results of the simulated data generated by the time-domain version of the hemodynamic model can be seen in Fig. 1(a). The data were zero padded to obtain time traces that are 400 s long, with a sampling frequency of 10 Hz, with the actual changes in $\Delta O(t)$, $\Delta D(t)$, and $\Delta T(t)$ lasting for $\sim$25 s (see Fig. 1(a)). The power spectral density of $\Delta T(t)$ is also seen in Fig. 1(a), where it is clear that the step response of the cerebral hemodynamics only has a frequency
content up to ~0.09 Hz in this study. After bandpass filtering the entire time trace around frequencies between 0.030 and 0.080 Hz in 0.005 Hz steps, \(|\Delta O|/|O|\), \(|\Delta D| - \arg(O)|\), and \(\arg(O) - \arg(T)\) were averaged over the first 20 s of the step response for each filtered frequency. The spectra of \(|\Delta O|/|O|\), \(|\Delta O|/|T|\), \(|\Delta D| - \arg(O)|\), and \(\arg(O) - \arg(T)\) are shown in Fig. 1(b) (symbols). For comparison, using the frequency-domain model equations [Eqs. (1)-(4)] with the same model parameters, the CHS spectra were also calculated and they are plotted in Fig. 1(b) as solid lines. The difference between the CHS spectra directly obtained from the frequency-domain model equations and the ones obtained from processing the simulated time-domain data are, on average, ~0.3% for \(|\Delta O|/|O|\) and \(|\Delta O|/|T|\), ~1° for \(\arg(D) - \arg(O)\), and <0.2° for \(\arg(O) - \arg(T)\).

The spectral width used for digital filtering was 0.01 Hz. The discrepancy between the CHS spectra obtained from the model equations in the frequency domain and from filtered time-domain data was found to depend on the filter width, with the discrepancy increasing for greater filter width (data not shown). The CHS spectra obtained from time-domain data were fit with Eqs. (7)-(8) to solve for the six unknown model parameters. The average solutions for the fifty-four initial guesses are shown in Fig. 1(c) (symbols) and compared with the true values indicated by the horizontal lines. For all six parameters, the average ± standard deviation of the fitted parameters include the true values. For clarity of the meaning of the parameters, \(\mathcal{F}^{(c)}\)\text{CBV}\(^{(c)}\)/\text{CBV}\(^{(c)}\) is shown without the Fåhraeus factor (fixed at \(\mathcal{F}^{(c)} = 0.8\)), indicating the capillary to venous blood volume ratio.

After having demonstrated in simulation that a step response of \(\Delta O(t)\), \(\Delta D(t)\), and \(\Delta T(t)\) can be used to obtain \(|\Delta O|/|O|\), \(|\Delta O|/|T|\), \(|\Delta D| - \arg(O)|\), and \(\arg(O) - \arg(T)\) at frequencies of
interest for CHS, the approach was evaluated on experimental data on human subjects. Figure 2 shows typical time traces of measured $\Delta O(t)$ and $\Delta D(t)$ during one of the three repetitions of the experimental protocol. The time traces in Fig. 2 are low-pass filtered at 0.15 Hz for better visualization. First the thigh cuffs were inflated for two minutes as shown in Fig. 2 (top panel). After the cuff pressure was released, MAP shows a clear decrease, followed by a decrease in $\Delta T(t)$ and $\Delta O(t)$, while $\Delta D(t)$ increases (Fig. 2, middle and bottom panels). The first 20 s of the hemodynamic response after thigh cuffs release was used for extracting the CHS spectra. After the step response measurement, the cuffs were periodically inflated and deflated at four frequencies (0.050, 0.0625, 0.0714, and 0.0833 Hz), as shown in Fig. 2 (top panel). MAP, $\Delta T(t)$, $\Delta O(t)$ and $\Delta D(t)$ show oscillations that follow the cyclic cuff inflation (Fig. 2, middle and bottom panels), with a temporal offset (or relative phase) between $\Delta O(t)$ and $\Delta D(t)$, as well as between MAP and $\Delta T(t)$ that is frequency dependent. For the four investigated subjects, the time delay of $\Delta T(t)$ with respect to MAP during the step response and the cyclic occlusion protocols was measured to be between 0.5 and 2.5 s. This time delay is in agreement with a previously reported phase lag of $\sim 20^\circ$ for $\Delta T$ vs. MAP oscillations at 0.1 Hz (a phase lag of $20^\circ$ at 0.1 Hz corresponds to a time delay of 0.56 s) [1].

![Figure 2](image_url)

**Fig. 2.** Temporal trace of the thigh cuff pressure (top panel), $\Delta O$ and $\Delta D$ (middle panel), $\Delta T$ and MAP (bottom panel). The temporal hemodynamics refer to one step response and one set of periodic cuff inflations at four different frequencies. For clarity, time traces were low-pass filtered at 0.15 Hz. The bottom panel insets show a time delay of $\Delta T$ with respect to MAP during the step response as well as during the cyclic cuff inflation protocol.

After bandpass filtering the data, using a filter width of 0.01 Hz around a selected frequency, $|D/O|$, $|O/T|$, arg($D$) - arg($O$), and arg($O$) - arg($T$) were calculated. Inducing changes in MAP by either the step response or the periodic inflation causes $D$ and $O$, as well as $O$ and $T$ to be phase locked. This behavior of stable phase relationships reflects coherent hemodynamic oscillations that are the basis for CHS. To demonstrate this behavior of phase stability, Fig. 3 shows the standard deviation of arg($D$) - arg($O$) (black lines) and arg($O$) - arg($T$) (grey lines), which were calculated at each time point over a 20 s moving time window around it. Figure 3 shows that during the step response, the standard deviations of arg($D$) - arg($O$) and arg($O$) - arg($T$) are minimal at all of the four frequencies considered.
(0.05, 0.06, 0.07, 0.08 Hz), indicating coherent hemodynamics suitable for CHS over a range of frequencies. By comparison, the phase differences during the periodic cuff inflation protocol only show minimal standard deviations at the specific frequency of cyclic cuff inflation, which is the frequency of coherent hemodynamic oscillations in that case.

The CHS spectra measured on the four subjects are shown in Fig. 4 for the step response (black symbols) and periodic cuff inflation (grey symbols) protocols. The error bars in Fig. 4 correspond to the standard deviation over the three repetitions of each protocol. In the case of the periodic cuff inflation protocol, CHS is performed solely at the four frequencies considered (0.050, 0.0625, 0.0714, 0.0833 Hz). In the case of the step-response protocol, we have considered ten frequencies, namely 0.045, 0.050, 0.055 … 0.090 Hz, that are within the frequency bandwidth of the step response of cerebral hemoglobin concentration shown in Fig. 1(a). Most of the experimental CHS spectra for the step response and periodic cuff inflation protocols lie within one standard deviation from each other. The best fits of the hemodynamic model [Eqs. (7)-(8)] to the measured CHS spectra are shown by the solid lines in Fig. 4 (black line: step protocol; grey line: cyclic protocol). The fits with the hemodynamic model are usually able to closely match the experimental CHS spectra, with a few exceptions such as the \(\arg(O) - \arg(T)\) and \(|D/O|\) spectra for subject 3. We are unable to identify the reason for these exceptions, which may be related to signal-to-noise issues and potential confounding effects of biological origin. While there is a generally good agreement between the CHS spectra measured from hemodynamics induced by the step-like cuffs deflation and by the cyclic cuffs inflation-deflation, some deviations are visible. A possible reason for these deviations is the fact that the measurements were not taken simultaneous but rather separated in time by several minutes, which is also reflected in the rather large error bars on the data points.
Fig. 4. CHS spectra measured on the four subjects from cerebral hemodynamics induced by a step-like deflation (black symbols) or by a set of cyclic inflation-deflation (grey symbols) of two thigh cuffs. The lines are the best fits of the hemodynamic model to the measured CHS spectra (black lines: step protocol; grey lines: cyclic protocol).

The results of the fits for the six model parameters can be seen in Fig. 5. The symbols and error bars in Fig. 5 represent the average and standard deviation of the best-fit parameters for the fifty-four sets of initial guesses for the fitting parameters (black symbols: step response; grey symbols: cyclic cuffs inflation). For all four subjects, the step response and periodic cuff inflation protocols resulted in best-fit parameters that generally lie within one standard
deviation from each other, even though some exceptions can be noted in Fig. 5. In particular, the capillary transit time $t^{(i)}$, the autoregulation cutoff frequency $\omega_c^{(AutoReg)}$, and $k_{CBV_0}^{(v)} / CBV_0^{(v)}$ show excellent agreement between the step response and the periodic cuff inflation protocols. Again, the capillary to venous blood volume ratio is shown without the Fåhraeus factor (fixed at $\mathcal{F}^{(v)} = 0.8$) for easier understanding of the meaning of the parameter.

Based on a two-sided sign test on the results from all four subjects, none of the parameters was found to be significantly different between the step response and the periodic inflation protocols ($p > 0.6$), demonstrating the feasibility of obtaining CHS spectra from a step response in $\Delta O(t)$, $\Delta D(t)$, and $\Delta T(t)$ induced by a sudden deflation of pneumatic thigh cuffs as an alternative to a sequence of cyclic protocols that induce coherent hemodynamic oscillations at a set of specific frequencies.

5. Discussion

Coherent hemodynamics spectroscopy is a novel technique based on frequency-resolved measurements of the phase and amplitude of cerebral hemodynamic oscillations and their analysis with a quantitative mathematical model to extract physiological information such as microvascular blood transit times and the extent of local cerebral autoregulation. While
cerebral hemodynamic oscillations can be induced with a variety of protocols at select
frequencies, sequentially one at a time, this approach requires relatively long times and might
be impractical for clinical applications. Furthermore, this approach is limited to the specific
frequencies selected in the protocol. In this work, we have shown that CHS measurements can
be performed by encoding all of the relevant frequency information in a single hemodynamic
response induced by a fast release of a sustained thigh cuff occlusion. Hence, the total time
required to measure a CHS spectrum reduces to less than 2.5 min (2 min occlusion and 20 s
of hemodynamic response following the cuff release), and all of the frequency-content of the
cerebral hemodynamic response is available at once.

The reason for choosing a step function protocol, where hemodynamic signals are
measured in response to a step-like change in the externally applied thigh cuff pressure, is that
the hemodynamic response has frequency content in the desired frequency range (~0.03-
0.09 Hz) for CHS. In principle, any shape of $\Delta O(t)$, $\Delta D(t)$, and $\Delta T(t)$ can be used for
obtaining CHS spectra. It shall be pointed out that the release of cuff pressure is not a true
step function either (see Fig. 2), since it takes ~3s for the air in the cuffs to be released. A true
step function could be achieved, as demonstrated by Aaslid et al. [17], who used a modified
thigh cuff instrument, where the tube diameters to the cuffs were enlarged, guaranteeing a
faster pressure release. However, as mentioned before, a step perturbation is not strictly
necessary for CHS, as long as the associated cerebral hemodynamic response has a frequency
content in the range of interest.

For CHS, we have used an autoregulation relationship between cerebral blood flow and
cerebral blood volume (Eq. (4)), which we have considered as a surrogate for MAP. Autoregulation can be described as a high pass filter process, with MAP as the input and flow
as the output. While $\Delta T(t)$ closely follows MAP (as shown in Fig. 2), we have found a time
delay of $\Delta T$ with respect to MAP, consistent with the literature, which indicates that Eq. (4)
may underestimate the autoregulation cutoff frequency. Indeed, values of ~0.15 Hz for the
autoregulation cutoff frequency have been reported [9], which are greater than our results of
~0.03 Hz. Whether this discrepancy is due to the approximations associated with Eq. (4) or to
the fact that we are measuring local autoregulation at the microcirculation level as opposed to
organ-level autoregulation is a subject for future studies. However, we observe that one recent
study also reported a low autoregulation cutoff frequency of ~0.03 Hz [28], consistent with
our results.

Autoregulation has been previously estimated via transfer function analysis between CBF (measured with transcranial Doppler ultrasound) and MAP (measured with finger
plethysmography) [6, 26, 29]. In order to apply this method a high coherence between CBF
and MAP is required, and this is achieved, for example, by inducing MAP oscillations with
paced breathing. Here, we have demonstrated that a stable phase difference between $\arg(D) - \arg(O)$ and $\arg(O) - \arg(T)$ (Fig. 3) cannot only be achieved at a single frequency (by periodic
inflation/deflation of a pneumatic thigh cuffs) but also over a range of frequencies by the step
response of MAP to the fast release of a sustained thigh cuffs pressure. The phase stability at
all frequencies associated with the cerebral hemodynamics in response to the fast cuff release
demonstrates the feasibility of CHS according to the step protocol proposed here. In addition,
we have observed phase stability between cerebral oxy- and deoxy-hemoglobin oscillations at
some frequencies even during baseline, during the cuff occlusion, as well as during periodic
cuff inflations at a frequency different than that of the cyclic cuff inflation. This is
exemplified in Fig. 3 for the analysis at 0.05 Hz during portions of the cuff occlusion period,
where the standard deviation in $\arg(D) - \arg(O)$ and $\arg(O) - \arg(T)$ is low. However, in
comparison to the induced changes in MAP and hemodynamic signals, only the filtered
oscillations at 0.05 Hz shows a stable phase relationship, while the other frequencies do not.
This result suggests that even during baseline there might be spontaneous oscillations that
possess phase stability over an extended period of time, opening up the possibility to perform
CHS measurements without the necessity of inducing cerebral hemodynamic changes.
For the CHS technique, the hemodynamic model used to translate CHS spectra into physiological quantities operates under the assumption of time invariance, i.e. that the baseline physiological quantities do not change during hemodynamics measurements. This is another important point in favor of the step-response protocol proposed here, for which CHS spectroscopy is performed on the basis of data collected during the ~20 s hemodynamic response to the fast thigh cuffs deflation. The time invariance requirement is certainly better fulfilled during this relatively short time period of ~20 s than during the time period of a few to several minutes over which data for CHS are collected in protocols involving sequential measurements at individual frequencies.

While we have found good agreement between the CHS spectra obtained from the step response and the periodic cuff inflation protocols, two subjects (1 and 3) showed a discrepancy in the venous transit time as seen in Fig. 5. It is possible that this discrepancy may not be associated with differences between the two methods for CHS, as also supported by the simulations, but rather due to an actual difference between the venous blood transit times when the two measurements were performed.

The step response protocol for CHS introduced here requires the application of a sustained (2 min) arterial occlusion at the subject’s legs followed by a fast release. We point out that similar thigh cuff occlusion protocols are already widely used for assessing cerebral autoregulation in human subjects [17, 30, 31] and have been even applied to the vulnerable population of traumatic brain injury patients [32]. Since methods based on thigh cuff occlusions are already widely accepted for human subjects, clinical translation of CHS can be envisioned according to the methods and protocol presented here.

6. Conclusions

We have recently introduced coherent hemodynamics spectroscopy (CHS) as a method for measuring and characterizing the frequency dependence of the phase and amplitude of cerebral hemodynamic oscillations measured with NIRS. With CHS it is possible to obtain physiologically relevant information such as the microvascular blood transit times and the cerebral autoregulation cutoff frequency. Instead of inducing cerebral hemodynamic oscillations one frequency at a time, for example with paced breathing or cyclic thigh cuff occlusion protocols, we have demonstrated here that all of the frequency information needed for CHS can be encoded in a single measurement of the hemodynamic response to a step-like perturbation. For this we have introduced a paradigm that is based on two minutes of arterial occlusion by inflation of two thigh cuffs at a pressure of 200 mmHg, and measurements of the cerebral hemodynamic response following a fast release of the thigh cuffs pressure. We have demonstrated that this response to a step-like perturbation results in CHS spectra that are equivalent to those obtained by inducing hemodynamic oscillations at multiple frequencies, one at a time. As a result, we have demonstrated the feasibility of CHS “in a single step.” The advantages of the proposed method are: (1) CHS measurements can be performed over the entire frequency range over which the cerebral hemodynamic response has a significant spectral power; (2) the hemodynamics for CHS is measured over a shorter period of ~20 s, thus better satisfying the model requirement of time invariance; (3) complete CHS spectra can be measured every ~2.5 min, thus allowing for monitoring of physiological changes that occur on a time scale of ~5-10 min or slower.

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