

# The fast optical signal- robust or elusive when non-invasively measured in the human adult?

Jens Steinbrink, Florian Kempf, Arno Villringer and Hellmuth Obrig

Berlin NeuroImaging Center, Division of Optical Neuroimaging,  
Schumannstr. 20/21, 10098 Berlin, Germany

Correspondence to:

Dr. rer. nat. Jens Steinbrink, [jens.steinbrink@charite.de](mailto:jens.steinbrink@charite.de)

+49-(0)30-450-560-114; fax -952

## Abstract

Near infrared spectroscopy (NIRS) can detect vascular changes in cerebral cortical tissue elicited by functional stimulation. For some ten years another optical signal has been reported to be accessible by NIRS. This signal has been reported to correlate to the electrophysiological response rendering NIRS an exquisite non-invasive approach to investigate neurovascular coupling in the human adult. Due to their typical latency of up to hundreds of milliseconds these signals have been termed ‘fast’ optical signals and have been postulated to stem from scatter changes in neuronal tissue, as a fingerprint of the electrophysiological response. Here we utter a less optimistic view on the non-invasive detectability of these changes in the human, motivated by an upper limit signal size estimation, predicting a signal size by orders of magnitude smaller than those previously reported. Also we discuss the influence of small stimulus correlated movement artifacts potentially mimicking a fast optical signal.

Based on invasive studies, we perform an upper limit estimation for changes in intensity and mean time of flight, which can be expected assuming a scatter change in the cerebral cortex while measuring on the surface of the head of an adult subject. Since the resulting numbers are far below those previously reported, we constructed a simple system, which minimizes technical noise. The system allows us to detect rather small intensity changes ( $2 \cdot 10^{-3}$  %) when averaging over approximately 3000 Stimuli. Despite this outstandingly low noise level of the system, we find a reliable change in response to a sub-motor-threshold steady state median nerve stimulation in just one single subject (8 subjects examined, 4 subjects twice). Exceeding the motor threshold leads to large stimulus related artifacts, on a similar time scale and with comparable amplitude as previously reported signals. To check for potential modality specific problems we next performed a visual stimulation study, avoiding potential motor artifacts. For the steady state visually evoked response no subject yielded a reliable result (11 subjects examined, 4 subjects twice). The paper discusses these findings by a review of the literature on fast optical signals and their being accessible in the adult human.



## Introduction

For some ten years (Gratton et al., 1995a) a number of publications have reported on the detection of fast optical signals to be feasible in the human adult (see table 1). Such optical signals are believed to stem from changes in the optical properties of neuronal tissue which appear much faster than absorption changes related to the vascular response ('fast' versus 'slow' response). These fast optical signals share the latency of up to hundreds of milliseconds with electrophysiological event related potentials (ERPs) and are thus interpreted as a measure of the neuronal response independent of the slow, vascular response peaking some seconds after the onset of the stimulus. Such a fast optical response accessible with a method known to detect the vascular- or haemodynamic-response (Villringer et al., 1993) renders optical methods an exquisite methodological candidate to investigate the complex interdependency between neuronal excitation and the ensuing vascular response, an issue which has lately attracted great attention but is far from being resolved (Caesar et al., 2003) (Logothetis and Wandell, 2004) (Obrig et al., 2002) (Moosmann et al., 2003). Obviously, however, if optical methods are to contribute to such issues the detected signals (fast and slow) must be reliably detected through the intact skin and skull of the human adult. They should be mutually independent in that neither will the functional haemodynamic changes mimic fast changes by an aliasing of the slow response, nor in that the fast optical signal would be generated by insufficient suppression of heartbeat induced oscillations omnipresent in any optical measurement over living tissue. In addition event related movement artifacts must be shown not to interfere with or even produce the fast signals in question. Very recently Franceschini and Boas have published a set of criteria to allow for a differentiation between artifact and signal (Franceschini and Boas, 2004). This work is remarkable since for the first time the signal has been critically evaluated with respect to classical criteria for any signal attributed to focal brain activation. The criteria include basic statistical methods to assess reliability of the detection, such as odd and even comparisons, comparison between stimulation and rest as well as tests of

physiological plausibility most notably the lateralization of the cerebral response to the contralateral hemisphere when unilateral stimuli are applied.

Franceschini and Boas proceed from data on the motor and the somatosensory system and the authors find results to fulfill the criteria in 20 to 60 % of the experiments. Thus it appears a promising perspective that neurovascular coupling will be accessible by optical imaging and needs not rely on the co-registration with EEG as has been proposed by our group (Obrig et al., 2002).

In the present paper we present a complimentary although less promising result. Starting from approximations of signal size and noise level we sought to potentially find out, why our group was unable to reproduce the reported robustness of such a fast optical response in the human adult (Syre et al., 2003). This is thus a question of signal size since it should be noted here, that we by no means doubt the existence of fast optical changes in neuronal tissue. Fast optical signals have been established for more than 30 years regarding isolated single neuron (Cohen et al., 1972) (Hill and Keynes, 1945) and recently images of fast optical changes have been generated on the exposed cortex (see Table 2).

Neither do we doubt that there may be the chance to detect such a signal in the human as we have in fact been the first group to show its detectability in intensity measurements (Steinbrink et al., 2001a). Besides the discussion whether the response may be best recovered from intensity or time-of-flight data, for both parameters the issue is the signal to noise ratio and thus robustness of the signal. If this signal is to serve investigations on neurovascular coupling, *reliability* and *validity* of the very small change in optical properties induced by neuronal excitation and observed through the intact skin and skull is a prerequisite to establish this approach for non-invasive optical imaging. We hence start with an estimation of the signal size (Part I). Based on data from the exposed cortex (Rector et al., 1997b; Rector and George, 2001a) we perform an upper limit estimation for changes in intensity and in mean time of flight, which can be expected when measuring over the intact adult head. By optimizing our optical system we then report on experimental data recorded at a noise level lower than that

reported in preceding publications (Part II). The results are in contrast to the findings reported in the literature (Table 1). Though the expected signal change should be detectable by our optimized system we do not find any reliable change corresponding to the optical correlate of steady state visually evoked potentials (ssVEP). For the somatosensory system (ssSEP) we find a fast optical signal in just one single experiment and demonstrate that movement artifacts must be respected in this stimulus modality, since these are event related and can easily mimic a false positive result.

The starting point of the present publication is hence the inability to reproduce (Syre et al., 2003) the fast optical changes in non-invasive optical measurements on the adult human head, as has been multiply published in changes in mean time of flight by a single group (Gratton et al., 1995b). What we find is an expected upper limit for changes in mean time of flight and intensity when measuring over the intact skull of an adult human. Optimizing an intensity based imaging system we reproduce our previous suggestion (Steinbrink et al., 2001a) that this parameter might be superior to unravel a very small signal change, a fact also recently confirmed by two other groups (Franceschini and Boas, 2004; Wolf et al., 2002). Concerning this change in intensity our result seems less promising than previously reported, raising some doubt on being the exquisite methodological candidate to investigate issues of neurovascular coupling.

## Part I:

### **From cat-hippocampus to human cortex: estimation of the sensitivity of mean time of flight and intensity to scattering changes**

The present paper critically evaluates the potential to non-invasively detect fast optical signals in the human. While initial reports suggested a magnitude of such fast signals in the range of the slow signal changes related to the vascular response the expected magnitude has decreased in succeeding publications (Table 1). Another issue as yet unresolved is whether intensity or time-resolved measurements will be preferable for the detection of small signal changes in the human cerebral cortex. In the first part of the present publication we present a theoretical estimation of the expected signal size in intensity and mean time of flight when measuring on the head of human adults. All estimations are upper-limit estimations so that realistically the signal is expected to be smaller but is very unlikely to be larger than the numbers presented.

The estimation requires two steps. Since no data on the magnitude of the scatter change are available a value must be derived from invasive optical cerebral recordings (Rector et al., 1997b; Rector and George, 2001b; Rector et al., 2001). A second step is necessary since near infrared spectroscopy (NIRS) measurements will not sample the change on the surface of the cerebral cortex but through a ~1 cm layer of extra-cerebral tissue. This requires a second estimation on the sensitivity of the different optical signals (intensity and mean time of flight) when measured on a layered structure, i.e. the human head. Taken together these two steps will result in an upper limit estimate of the expected magnitude of changes ( $\Delta I$  and  $\Delta \langle t \rangle$ ) for non-invasive human studies.

### **Estimation of the amplitude of scattering changes**

The magnitude of the scatter changes in human cerebral tissue elicited by stimulation is not known, however, many authors have reported intensity changes in single axons or neuron ensembles. The results from single axons or brain slices corroborate the fact that there is a scatter change but it is difficult to project them onto the geometry of a bulk brain. Recently

intensity changes determined on the exposed hippocampus of the cat following collateral electrical stimulation have been reported. The largest change reported was an intensity change of 0.1 % (Rector et al., 1997b). We proceed from this value since it is the largest change reported, while the same group published smaller changes potentially due to changes in set-ups (GRIN-lens) and stimulation paradigms (see Table 2).

To derive an expected scatter change from this intensity change (Rector et al., 1997b), a Monte-Carlo simulation was performed. Due to probe geometry with probe separations of less than 2 mm, the photon-transport has to respect the scattering anisotropy  $g$  by:

$$\mu_s' = \mu_s(1-g)$$

where  $\mu_s'$  is the reduced scattering coefficient,  $\mu_s$  the scattering coefficient and  $g$  the anisotropy factor. We used a published MC-code (Wang L and Jaques S, 1992; Prahl S.A. et al., 1998) assuming tissue properties of the cat hippocampus and a refractive index of 1.4. Since these optical properties are unknown and can only be estimated, we modeled different combinations of  $g$ ,  $\mu_s$  and  $\mu_a$  (see Table 3).

Figure 1 shows the result for several assumed changes in  $\mu_s'$ . Here a linear approximation for small changes returns a translation factor of 0.95, that is, a 0.1 % change in intensity will be caused by a  $\mu_s'$ -change of 0.095 %. To check its validity the translation factor was determined for a wide range of background optical properties ( $\mu_s'$ ,  $\mu_a$  and  $g$ ) as summarized in Table 3. Runs 1-4 were performed for the semi-infinite medium and yield translation factors of  $\sim 1$ . When respecting the layered architecture of the hippocampus, a partial volume effect will increase the translation factor. This was modeled in run 5 assuming the change to be restricted to a thin superficial layer. For all runs the geometry of the image conduit and the surrounding fibers was taken into account by a spatial convolution technique. We conclude that the upper limit to estimate changes in scatter from intensity changes measured, is a factor of 4. This means the largest reported change of 0.1 % change in intensity corresponds to a scatter not larger than 0.4 %.

### Sensitivity to cortical scattering changes

The scatter change elicited by functional stimulation in the human adult will be restricted to the focus of activation on the cerebral cortex covered by about 1 cm of extra-cerebral tissue. Thus a second step is necessary to estimate expected sensitivities for scatter changes in a layered head model. Focusing on the slow changes related to the hemodynamic response, values have been published for changes in absorption yielding sensitivity profiles for intensity and mean time of flight measurements (Delpy et al., 1988) (Hiraoka et al., 1993) (Steinbrink et al., 2001b). For intensity changes the corresponding sensitivity factors are related to a mean partial photon pathlength in the tissues volume. Concerning scattering changes, however, such an intuitive sensitivity factor has not been formulated so far and the deduction of the numbers recently been published on a sophisticated anatomical model returns qualitative results only (Franceschini and Boas, 2004).

To simplify the overall analysis we regard fractional sensitivity factors for intensity changes. A fractional sensitivity factor for intensity changes

$k_I = \frac{\frac{\Delta I}{I}}{\frac{\Delta \mu_s}{\mu_s}}$  relates scatter changes in percent to fractional intensity changes.

Conversely expected changes in mean time of flight can be given in absolute numbers and the corresponding sensitivity factor  $k_{\langle t \rangle} = \frac{\Delta \langle t \rangle}{\frac{\Delta \mu_s}{\mu_s}}$ . Thus these changes are given in ps/%.

Calculated sensitivity factors strongly rely on the background optical properties chosen for the individual model. Unknown in the human, they must be estimated from invasive or in vitro measurements. This will generate an error, which can be expected to reduce accuracy to orders of magnitude rather than exact numbers. Beyond this uncertainty we were interested in the robustness of the sensitivity factors, therefore testing two models for the background optical properties of the medium (see Table 4): (i) The inhomogenous model is very similar to the one published by Okada and colleagues (Okada et al., 1995). The different compartments of the human head are modeled as adjacent layers, for whom thickness and optical properties are listed in Table 4. The optical properties are estimated for 780

nm and are a compilation of the data determined from invasive measurements (Bevilaqua et al., 1999; Doornbos et al., 1999; van der Zee and Delpy, 1993) (Sterenborg et al., 1989). (ii) The second model is characterized by homogeneous background optical properties  $\mu_s'=1/\text{mm}$  and  $\mu_a=0.01/\text{mm}$ . Both models assume a refractive index of  $n=1.4$ . To determine the sensitivity factors of interest, a model with the scattering coefficient being changed in a deep layer must be calculated. By comparing the optical signals from the baseline model with those from altered model sensitivity factors can be derived.

There is yet another uncertainty which must be respected, since the exact spatial extend of the scattering changes in the human brain is unknown. We assumed three types of model perturbation: (A) a scattering change in the gray matter, (B) a scattering change in the upper layer (1mm thickness) of the gray matter and (C) a change of  $\mu_s'$  in the gray and white matter of equal proportionality (see **Table 5**). The perturbation was induced by a change in  $\mu_s'$  of 10 %. The Monte-Carlo code was developed according to prior work from Okada and our group (Okada et al., 1997; Steinbrink et al., 2001b). For each run  $10^9$  photon bundles were launched.

For model A the resulting sensitivity factors as a function of the optode distance are given in **Figure 2**. For the more realistic inhomogeneous assumption the sensitivity factors increase with larger source detector separation. Interestingly the assumption of a homogeneous model will produce a response inversion at about 2.5 cm probe separation (i.e. around the separation commonly used in the experiments) and may thus be related to reported inversions of the response direction of the fast signal reported by (Franceschini and Boas, 2004). Relevant to the issue of magnitude, the focus of the present paper, absolute values for the sensitivity factor ( $K_I$ ) do not exceed the value of 0.1. For all three models the sensitivity factors based on a fixed source detector separation of 27 mm are presented in **Table 5**. As expected the sensitivity factors increase with the spatial extend of the scattering change. For an upper limit estimate we would thus conclude an intensity sensitivity factor of 0.1 % change in intensity per percent change in

$\mu_s'$  and a sensitivity factor of mean time of flight of about 0.1 ps per percent change in  $\mu_s'$ .

### **Overall results of the estimation**

The aim of the theoretical considerations and the above photon migration studies was to determine an upper limit estimation of expected intensity changes and changes in mean time of flight. All assumptions were based on upper limit values in order to be sure to find a number which gives the maximal change in optical parameters following a scattering change in the cerebral cortex of the human adult, when measuring on the head's surface. Proceeding from the largest intensity changes in cat hippocampus (Table 2), then applying the maximal intensity-to-scatter translation-factor of about 1 (run 1-4 Table 3) translates into a maximal scatter change of 0.1 %. Regarding the layer-sensitivity factor on the adult head model we find an upper limit change in intensity of 0.01 % and a change in mean time of flight of about 0.01 ps. Respecting the layered nature of the tissue investigated introduces a strong partial volume effect in the transformation from reported intensity changes into scatter changes. This number must then be augmented to a maximal translation factor of 4 (run 5 in Table 3).

However, so far only the upper limit of the estimation has been discussed. One other factor, not modeled here, will result in a realistic value for the expected changes much smaller than the value deduced so far:

For the invasive studies the scattering changes are given for a very focal area of the cortex, that means that the measurement perfectly 'hits' the site of activation. The non-invasive geometry and probe positioning will never reach this topographical specificity. Therefore the smearing effect of very rough topographical positioning will introduce a partial volume effect strongly reducing the magnitude of the expected change in  $\Delta I$  and  $\Delta\langle t \rangle$ . Though we cannot precisely model this effect it seems likely that such an effect might reduce the signal by an order of magnitude.

A second factor potentially reducing the signal by another order of magnitude is the stimulation modality used in different invasive measurements. Stimulus modality (collateral tract versus peripheral nerve) strongly influences signal size as can be seen in Table 2. Using vagus-nerve

instead of the Schaffer-collateral stimulation reduces the invasive- signal by more than an order of magnitude (Rector et al., 2001). We present an estimate for the larger effect (collateral stimulation) seen invasively, though stimulation paradigms performed in the human will rather correspond to peripheral nerve stimulation.

Besides the upper limit quality of our estimation these latter two aspects (partial volume effect by focal activity and peripheral nerve stimulation) realistically reduces the magnitude of signal in question to more likely be 0.0001 % for changes in intensity and  $10^{-4}$  ps for changes in mean time of flight.

## Part II

### Experimental results

In the first part of this paper we presented an estimation for the expected changes in intensity and mean time of flight ( $\Delta I/I$  and  $\Delta \langle t \rangle$ ), when measuring over the intact scalp and skull of adult human subjects. Our prediction is that the signal is detectable but will be very small. The utmost limit for such an effect would be a change of 0.04 % for intensity changes and a change of 0.04 ps in mean time of flight. Thus the question of detectability is largely a question of optimizing the signal to noise ratio. In the following sections we first describe the system we developed to maximally reduce physical noise, then we describe the analytical steps of physiological noise reduction to finally report our experimental results.

### Technical set-up and analytical noise reduction

#### NIRS-System

Though time-of-flight measurements may be more sensitive to deep layers (Okada et al., 1997) we have previously published the advantage of intensity measurements due to technical limitations when trying to increase photon flux in frequency of time-domain monitors (Steinbrink et al., 2001a). Also, in line with recent publications we increased the number of simultaneously measured probe pairs, since the signal can be expected to be highly focal (Franceschini and Boas, 2004). One should note therefore: while the focality of the changes will help to differentiate the signal in question from global

changes such as heartbeat related oxygenation changes and movement artifacts (Franceschini and Boas, 2004) the chance to detect the signal is strongly reduced since non-invasive NIRS will never reach the spatial resolution of the reported invasive reflectance changes serving as a starting point of our above estimation concerning signal magnitude.

Two different types of light sources were used. For our somatosensory study the light of a halogen bulb was band-passed between 600 and 900 nm and coupled onto a 3 mm source fiber-bundle. On the output side of the fiber-bundle 70 mW were delivered to the head. To even increase the photon flux to the tissue a high power LED (Roithner, Vienna) emitting at 810 nm with a power of 150 mW was used for the visual stimulation study. A heat sink on the back of the diode prevented undue heating of the skin. Six (somatosensory) or eight (visual) light detector fibers 3mm in diameter were concentrically placed around the source to deliver the reflected light onto eight avalanche photo diode modules (APD module C5460 Hamamatsu Photonics K.K., Japan). The output of the diodes was filtered with a hardware filter at 3 ms. Then the data was fed to an 14 BIT-AD-converter sampling each channel with 250 Hz for the somatosensory data and 550 Hz for the visual study. To emphasize the search for signals event related to the stimulation frequency (around 10 Hz) we applied a low-pass filter with a cut-off at 95 Hz.

### Heart beat filtering

As can be seen for a 12 s-period of raw data in one channel (**Figure 3A**) the signal is dominated by the pulsatile component of the blood volume changes. The magnitude of these changes ranged from three to nine percent for all the subjects and all locations. Since the expected intensity change is at least two orders of magnitude smaller, an adaptive filter is needed to attenuate the physiological noise induced by the heartbeat. **Figure 3B** shows a magnification of the raw signal. Please note the low noise-level at high frequencies.

We adapted the filter published by Gratton and colleagues (for details see (Gratton and Corballis, 1995)). Briefly, by averaging over multiple heart beat periods the filter algorithm estimates the unperturbed pulsatile effect. This pulse-average is then adapted to and subtracted from each individual

heart beat period. Special care was taken to account for differences in amplitude and length of the individual inter-pulse periods:

As is illustrated in Figure 3A the raw signal (black line) was compartmented at individual inter-pulse-intervals, easily identified by the local maxima in the intensity curve (thin lines). In a next step these individual inter-pulse-segments were compressed and stretched to one unitary length (e.g. 1s) for the duration of 10 successive inter-pulse intervals allowing for the prediction of an average pulsatile component of the raw signal. In a third step the averaged inter-pulse interval was fitted to each of the individual inter-pulse intervals by adjusting the duration, the amplitude and adding a linear trend. After this adjustment procedure the averaged 'pure pulse curve' is subtracted from the raw data. The result of this subtraction is also given in Figure 3A (bottom, gray lines).

The algorithm can be implemented in a manner that components lower than 1 Hz are maintained (upper gray line) or disregarded (lower gray line). Figure 3 shows that the variance of the signal is reduced by almost two orders of magnitude. The main effect of this adaptive filter is best seen in the frequency domain. Figure 4 shows the power spectral density for raw data (black line) and after filtering (gray line). In the raw data the pulsatile component and its higher harmonics dominate the spectrum. The filter algorithm efficiently attenuates the pulsatile components and its higher order harmonics. Figure 4 also demonstrates that this adaptive filter is superior to classical frequency filters, since it is also capable of reducing the heart beat induced artifact at the stimulation frequency of 9 and 11 Hz. In other words since the pulse is not perfectly sinusoidal its harmonics will be large enough to interfere with the stimulation frequency, expected to be smaller by orders of magnitude. Only the adaptive filter is suited to attenuate heart beat related changes while keeping the potential event related changes mostly unchanged.

### **Event related averaging**

Event related averaging is the classical way to analyze electrophysiological potentials since latencies of specific components can be determined. However singular large perturbations as may be generated by movement may interfere with the validity of the result. Variance-weighted averaging

suppresses the interference of such outliers during single inter-stimulus time courses  $y_i$ . Here the individual run (single inter-stimulus time course index  $i$ ) is weighted according to its variance:

$$\bar{y}(t) = \frac{1}{\sum_{i=1}^N w_i} \sum_{i=1}^N w_i y_i(t), \quad (1)$$

where  $N$  denotes the number of stimuli,  $\bar{y}(t)$  the averaged intensity curve and the weights can be defined by  $w_i = 1/\text{Var}(y_i(t))$  (Var indicates Variance). Note that this choice of  $w_i$  is the specialization of a covariance weighted averaging for the case of signal perturbations which are uncorrelated to the stimulus event. Determining the optimum dimension of the covariance matrix (Hoke et al., 1984; Gräbe, 1999) (Gerull, 1996) for the data given here returned an optimum dimension of 1, which justifies the use of the variance weighted averaging.

### Noise and signal reconstruction

In the first part of the present paper we deduced an absolute number for the upper limit for the changes expected from a cortical scatter change when measuring on the surface of a human adult's head. Of course the detectability of such a change critically depends on its magnitude relative to the noise, which comprises both system noise and physiological noise. Though some aspects of physiological noise can be effectively estimated and attenuated and variance-weighting is helpful for reducing the noise introduced by singular artifacts, it is impossible to model all potential sources of noise. We therefore took a practical approach to find out how large an amplitude of the signal is required to detect it with the system used.

Virtual stimuli of different magnitudes were added to unaveraged measured resting period data, to create simulated stimulation data. These data sets were then analyzed as real stimulation periods. The rationale is that by testing different amplitudes of these virtual stimuli we are able to predict the minimal amplitude, which would rise beyond the overall noise level when

all noise reduction procedures were applied. The rationale can be also illustrated by real data. Figure 5A shows ‘event related’ averaging of the intensity changes during the resting period of the visual stimulation in one of the detector channels using the set-up described in the next section. While regular averaging yields an ‘event-related’ change in the signal (gray symbols) variance weighted averaging shows no significant deviations from baseline (black symbols). Note the low noise level with a standard error of mean of about  $3 \cdot 10^{-6}$ . Thus simple averaging can result in significant deviations from the baseline even though no stimulus was presented, thus demonstrating that without variance weighting, erroneous ‘effects’ may be generated by single artifacts, irrespective of whether they stem from physiological noise or movement.

To test for the minimal amplitude which can be resolved by the given system, we next added an artificial stimulation effect to the resting period data (same duration and thus same number of stimuli as in stimulation phase). Here we used a biphasic signal with variable amplitude as indicated in Figure 5B (horizontal lines for different amplitudes). After adding the artificial signal to the unfiltered raw data, the data was re-sampled with the resolution of the analog digital converter (16bit). Figure 5B shows the result of such an analysis with different amplitudes of the artificial signal. We conclude that an amplitude of  $2 \cdot 10^{-5}$  and larger will rise over the noise level.

### **Experimental protocol and results**

Visually, somatosensory and acoustically evoked potentials are the most commonly used ERPs as is reconfirmed by every-day clinical practice. Motor potentials are less reliable especially when the execution of a finger tap is used as a trigger. Also the chance of an event related motion artifact is inherent in this stimulus modality. Since early auditory potentials arise from the brain stem and auditory cortex is located beneath the temporal muscle this ERP may also have a less favorable accessibility for non-invasive NIRS. We here present results from an electrical steady state somatosensory (ssSEP) and visual stimulation, which should be the most robust signals. For ssSEP the proximity to the brain’s surface of the known generator of the first electrophysiological component is helpful, while on the other hand the typical stimulation above the motor threshold will induce a perfectly event

related motion artifact. Please note, that in this study we used comparably high stimulation frequencies allowing us to compare our findings to steady state ERPs. The choice of this modality has the advantage to a) decrease noise by allowing for more stimuli per session b) to decrease an influence by the haemodynamic response and c) to decrease a potential movement artifact in case of the SEP, since the amplitude of the finger movement is reduced.

### **Somatosensory stimulation**

For the somatosensory study the NIRS probe consisted of a source fiber, which was concentrically surrounded by 6 detector fibers at a distance of 2.5 cm. The probe was centered on C3 (according to 10-20 system). Subjects received a median nerve stimulation of both, 9 and 11 Hz each lasting 5 minutes (contra and ipsilateral). Thus in each session 3000 events were obtained for each ipsi- and contralateral median nerve stimulation and for each stimulation frequency.

To test for the effect of potential event related movement artifacts both a sub- and a supra motor-threshold stimulation current was chosen. Since changes in electrode coupling during the five-minute stimulation period can cause changes in the motor-threshold, the experimentalist continuously controlled the stimulated hand for muscle contractions by visual and tactile inspection. Each session included a measurement with the subject at rest lasting five minutes. The study consisted in 12 sessions on 8 different subjects (4 subjects measured twice).

### **Visual stimulation**

For the visual study the emitter probe was placed over O1 (according to the 10-20 system). Here the light source (LED) was integrated in the probe and surrounded by 8 detector bundles at 2.8 cm distance. The subjects were asked to fixate on a central cross while a circular blue and yellow checkerboard was presented reversing at 7 Hz. Stimulation periods of 60 s were followed by resting periods of 60 s. Ten to fifteen cycles were performed in each of the 15 experiments. Thus at least 4200 reversals were acquired per session. 11 subjects were examined (four subjects twice).

## Results

### Somatosensory Stimulation

Figure 6 shows the event related intensity changes in the six detector channels during the stimulation period. In two of the channels a statistically significant intensity change of approximately  $2 \cdot 10^{-5}$  was observed during stimulation (black lines,  $p < 0.01$  as indicated by stars). In the resting phase (gray lines) no significant effect is seen. In line with the three applicable criteria of Franceschini and Boas Figure 7 shows the intensity change separately for odd (black line) and even (gray line) stimuli. The overall shape of the response is found in both curves. Even though the noise level for all other experiments was of similar amplitude ( $5 \cdot 10^{-6}$ ) no further deviation from the baseline and thus no signal was found in any of the experiments.

As mentioned above typical median nerve stimulation exceeding the motor threshold will potentially introduce an event related motion artifact. We therefore also included such a stimulation with a large stimulating current. For these experiments significant signals were found for all subjects in at least two channels. Figure 8 shows the intensity changes for the same subject as in Figure 7. Ipsilateral stimulation (gray lines) and contralateral stimulation (black lines) leads to a magnitude of up to  $3 \cdot 10^{-4}$ .

For the different subjects the maximal response varied between 1 to  $7 \cdot 10^{-4}$ . The signal is thus in the range of previously published findings (Steinbrink et al., 2001a) (Franceschini and Boas, 2004). The low noise level of our set-up allows us to stress two issues: 1.) The signal can be of a focal nature as can be seen in Figure 8, where the contralateral stimulation in channel 2 shows by far the largest signal amplitude. However even the channels with the lowest signals show a small but still significant intensity change rendering a global effect most likely. Thus, depending on the noise level of the set-up and the discrimination threshold chosen the here presented signal can be interpreted as a focal or a global signal, in other words 'real' or artifact. 2.) Compared to the response signal shown in Figure 6, which was obtained with a stimulation below motor threshold, the supra threshold stimulation in Figure 8 yields in a signal change, larger in amplitude by more than an order of magnitude. No electrophysiological analogue exists

for this kind of signal amplification. We hence conclude that the signal seen in response to the supra-threshold stimulation rather stems from an event related movement artifact, and might be misinterpreted as a focal change depending on the noise level of the system used.

This result demonstrates that stimulation above the motor threshold is a very unreliable argument for the validity of fast optical signals. The example shown also demonstrates that the motion artifact can well have a ‘focal’ distribution, potentially correlating to the firmness of probe to skin and skin to skull fixation. The possibility of misinterpreting a motion artifact can be reduced by increasing the number of NIRS-channels and then applying all the criteria defined in (Franceschini and Boas, 2004).

### **Visual stimulation**

For visual stimulation a motor artifact can be excluded independent of the stimulus strength chosen. Figure 9 shows the event related intensity changes determined for a subject undergoing 5600 checkerboard reversals. No significant deviations from baseline ( $p < 0.01$ ) were observed as was true for all subjects examined.

### **Potential technical improvements of the NIRS-system**

With approximately 5000 stimuli and a lowpass filter of 94 Hz we were able to achieve a noise characterized by standard error of mean of down to  $3 \cdot 10^{-6}$  per time point. Future research will have to focus on further reducing the noise level. For the system used here two sources of noise can be quantified: photon-noise and noise caused by limited resolution of the ADC. For the former quantity the number of detected photons has to be regarded. The manufacturer of the avalanche photodiodes supplies conversion-factors transforming the readout voltage in light power, which can be transformed into photon-counts. An average read-out voltage of 5 V and an internal APD-gain of 30 results in a flux of  $2.7 \cdot 10^{12}$  photons/s. Based on an effective sampling frequency of 94 Hz (the highpass filter frequency) the expected noise of  $2 \cdot 10^{-7}$  is far below the experimental noise. Of similar relevance is the dynamic range of the ADC. In the range of the voltage interval from -10 to 10 volt sampled with 16 bit and an effective sampling frequency of 94 Hz

the noise level is  $4 \cdot 10^{-7}$ . Both sources explain less than 20 % of the observed noise level, motivating further research on the origin of the observed standard error of mean.

## Conclusion and Discussion

In the past years changes in optical parameters closely resembling the latency and dynamic behavior of electrophysiological event related potentials (SEP/VEP/AEP and MP) have been reported to be easily detectable by non-invasive near-infrared spectroscopy (Table 1). Our group was not able to confirm the robustness of such ‘fast’ optical signals (Syre et al., 2003). To further enquire into the reasons for such a discrepancy we here present an upper limit estimation for the magnitude of changes to be expected in intensity ( $\Delta I/I$ ) and mean time of flight ( $\Delta \langle t \rangle$ ), when measuring on the head of human adults. Our results predict that realistically the scatter change in the human cerebral cortex will elicit a maximum intensity change not larger than 0.01 % while mean time of flight will maximally change by  $10^{-2}$  ps on the surface of the head. However the upper limit assumptions are rather dramatic and a signal change of down to 0.0001 % and  $10^{-4}$  ps is also in line with reasonable assumptions.

Since these numbers predict a signal close to or below the noise level of previously used systems we developed a system with the lowest technical noise reported in the literature. With this system and applying somatosensory stimulation below the motor threshold we detected stimulus correlated intensity changes in just one single subject. When the stimulus current was augmented above the motor-threshold large intensity changes were seen in each subject. These changes may be related to motor artifacts since minimal event-related movement will survive even sophisticated filtering. Such robust but potentially movement related changes were shown to be focal in a number of subjects and it should be noted that more than one third of these changes pass the three applicable criteria defined by Franceschini and Boas who were the first to recently critically evaluate the fast optical signals as to their validity and statistical reproducibility (Franceschini and Boas, 2004). Note, that our design does not allow to apply all 5 suggested criteria.

The largest event related electrophysiological potential commonly used even in clinical routine, the visually evoked potential (VEP), is not liable to movement related artifacts. We therefore performed another study using visual stimulation study but did not find a single significant result in any of the subjects examined. We are aware, that the poor detectability of the fast signal in the study regarding visual evoked potentials may also result from the particular experimental conditions used in this study. The visual cortex has a larger variability when relying on bony landmarks (Steinmetz et al.). Also respecting that the expected signal change may be highly focal there is a fair chance that we missed the optimal optode position. Certainly, our attempts to minimize this problem (multiple collection fibers and large number of sessions) may be addressed by an increase in source detector pairs and systematic re-location of the pad at distances of 0.5 cm or even smaller. Such procedures will necessitate multiple sessions of many hours duration, potentially overly extending the patience of the volunteer and thus limiting the versatility of the approach.

The present publication is meant to serve as a note of caution, since we find that fast optical signals are by no means robust when assessed non-invasively in the adult. The reasons which led us to thoroughly investigate signal size and detectability originate from our unsuccessful search for the signal with nearly identical experimental setups (Syre et al., 2003) ever since they were first published (Gratton et al., 1995a). Beyond this we state some issues in the literature as summarized in table 1.:

- *Phase or Intensity, which parameter is to best suit the signal detection?* In their work Gratton and co-workers claim that phase changes will reliably produce fast optical responses In the attempt to estimate the amplitude of the fast optical signal for the adult human, we found that intensity changes are superior to changes in mean time of flight due to their low noise level (Steinbrink et al., 2001a). This

view has recently been confirmed (Franceschini and Boas, 2004) (Wolf et al., 2002).

- *Optical VEPs are smaller than first reported.* Visually evoked potentials are the most commonly used ERPs. Interestingly for the first optical VEP reported (Gratton et al., 1995a) the amplitude approached the same order of magnitude as the slow response (1-25 ps) and thus gave hope for a simple determination in single subjects. In contrast to these results, studies of two groups (Syre et al., 2003) (Wolf et al., 2003) could not return a significant change in measured mean time of flight (or phase) after visual stimulation and are thus in line with the calculations in this publication
- VEPs and SEPs share the fact that their most pronounced components have stable latencies. A retardation of the P100 in the VEP by 10ms discriminates a healthy subjects from patients e.g. after optic neuritis. Even when compared within individual publications, the latencies of the most pronounced peaks vary by more than ten to one-hundred milliseconds (Steinbrink et al., 2001a) (Franceschini and Boas, 2004) (Wolf et al., 2003; Wolf et al., 2002). A direct analogy of the observed optical signal changes to ERPs thus seems questionable. Also, the timing of the previously reported changes is similar to that of the motor artifacts observed in the study described in the present paper

The present paper does not explore the physiological origin of fast optical signals. However several discussions of the authors with groups who find the fast optical signal necessitate a comment. If the scatter change elicited by the neuronal response as shown by (Stepnoski et al., 1991) or (Rector et al., 1997a) cannot be the origin of the reported changes, as shown in the present paper, a number of physiological changes beyond artifacts may potentially account for the changes reported by these groups. At low stimulation frequencies (<1 Hz) even the rather slow vascular response may elicit a true stimulus related response. Alternatively the controversially discussed early

deoxygenation (the 'dip' (Buxton R.B., 2001)) with a latency of some 100 ms may be a candidate for such changes. Even changes in the phase of the heart rate may potentially elicit changes, which are 'faster' than the full development of the typical increase in rCBF. Finally physiologically insufficiently explained, 'slow' scatter changes (Holthoff K et al., 1994) may elicit a response with a shorter latency. Here we do not find such a phenomena. If on the other hand signals not explicable by the typical fully developed vascular response are found, these candidates must be considered. They may be of great relevance but do not touch the issue of neurovascular-coupling in the sense of an electrophysiological and a vascular-metabolic side of the stimulus related response.

The findings of the present publication thus foster a less optimistic view on the detection of a non-invasive optical approach to assess electrophysiological signals in the adult human. This by no means doubts the existence of fast optical scatter changes in neuronal tissue (Rector et al., 1997b), neither do we doubt that a further investigation in the issue by means of invasive approaches during neurosurgical procedures is of high interest. For a non-invasive approach in the human adult, however the results presented here render co-registration with standard EEG or MEG technology the presently only feasible option when interested in investigating neurovascular coupling.

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publication			experimental protocol				effect		NIRS system				specials	
Citation	year	author	no. of sub.	no. of trials	stimulus	stim freq.	type	size	com	wave-length in nm	P in mW	samp freq. in Hz		
(Gratton et al., 1995b)	1995	Gratton et al.	4		mot	0.8	increase in delay	relative units	ISS	715	0.3	12.5		
(Gratton et al., 1995a)	1995	Gratton et al.	3	>300	vis quadr	2	increase in delay	0.4° (112MHz)/9ps	ISS	715	0.2	20	slow effect -7 ps	
(Gratton, 1997)	1997	Gratton	3		vis attention	1.25	increase in phase	-0.2° (112MHz)	ISS	715	<1	50		
(Gratton et al., 1997)	1997	Gratton et al.	3	456	vis quadr	2	increase in phase	-0.3° (112MHz)	ISS	715	0.2	20	comb BOLD /+VEP, difference area 17 /19	
(Gratton et al., 1998)	1998	Gratton et al.	4	600	vis mem	0.5	increase in phase	-0.06° (112MHz)	ISS	715	< 1	50		
(Rinne et al., 1999)	1999	Rinne et al.	6	2000	auditory	2.5	increase in phase	-0.2° (112MHz)	ISS	750	< 1	50		
(Gratton et al., 2000)	2000	Gratton et al.	9	1280	vis excent.	5	increase in phase	<0.2° (112MHz)	ISS	750	1.5	50	depth resolution, fMRI correlation	
(Desoto et al., 2001)	2001	DeSoto et al.	11	-	mot / spatial stroop	-0.3	increase in phase	-0.04° (112MHz)	ISS	750	-1	25	comb EEG (LRP)	
Proc Nat Acad Sci **	2001	Gratton & Fabiani	review article											
(Gratton and Fabiani, 2001)	2001	Gratton & Fabiani	review article				increase in phase	0.2-2 ps 0.01-0.1°		ISS				
(Steinbrink et al., 2001a)	2000	Steinbrink et al.	6	2000	somato (elec.)	5	decrease in intensity	0.05% ( $\Delta I/I$ )	home made	650-950	150	550	first theoret. signal estim.	
(Wolf et al., 2002)	2002	Wolf et al.	5	-1800	mot (tapping)	-3	change in intensity and phase	0.006 % ( $\Delta I/I$ ) 0.008° ( $\Delta\Phi$ )	ISS	758 & 830	-	96	only one result in phase at p<0.01	
(Syré et al., 2003)	2003	Syré et al.	12	800	vis	1.95	no change	noise: <0.02° ( $\Delta\Phi$ )	ISS	750	0.7	30	no change in phase obtained,	
(Gratton and Fabiani, 2003)	2003	Gratton et al.	8	600 per quad.	vis	2	change in phase	-0.03° (112MHz)	ISS	750			reproduction of 1995 finding, with a 10 times smaller effect size	
(Wolf et al., 2003)	2003	Wolf et al.	4	2000	vis	4-5	change in intensity	0.02-0.3% ( $\Delta I/I$ )	ISS			64-80	no phase signal, int. change in 2 subj.	
(Franceschini and Boas, 2004)	2004	Franceschini & Boas	10	700-1000	somato (elec + tactile), mot	4	change in intensity	0.04-0.1%	home made	690 & 830	< 5	40	Defintion of 5 criteria for fast optical signal	

**Table 1** Synopsis of publications on fast optical signals detected non-invasively on the human adult. The table gives the signal sizes reported, which vary by about two orders of magnitude, even in response to similar stimulation modalities. The signal changes in gray were determined by a cross correlation analysis of attenuation changes and they cannot be directly transformed into amplitude changes. In the first publication G. Gratton demonstrated a phase shift of 0.4° corresponding to a delay of the photon mean time of flight of  $\Delta\langle t \rangle \approx 10$ ps occurring about 100 ms after onset of the stimulus.

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Model	Area	stimulus	$\Delta I/I$ in %	Ref
Cat	Hippocampus	Electrical brain stim. (Schaffer collaterals)	0.1	(Rector et al., 1997b)
Rat	Brain stem	Vagus nerve aortic nerve	0.02	(Rector and George, 2001b)
Rat	Brain stem	Vagus nerve	0.005	(Rector et al., 2001)

**Table 2** Fast optical intensity changes in response to electrical stimulation, invasively measured in the animal brain.

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run	d of layer in mm	$\mu_a$ in 1/mm	$\mu_s'$ in 1/mm	g	k
1	Inf	0.01	10	0.9	0.9
2	Inf	0.01	3	0.7	1.0
3	Inf	0.03	10	0.9	0.9
4	Inf	0.01	5	0.9	0.8
5	0.2	0.01	10	0.9	4.0

**Table 3**

The background optical properties assumed for the MC-study and the related proportionality factor  $k$ . A proportionality factor 0.9 indicates that an intensity change of 0.1% can be explained by a change in  $\mu_s$  of 0.09%. Note that assuming a change in a thin layer only (run 5) returns a larger proportionality factor.

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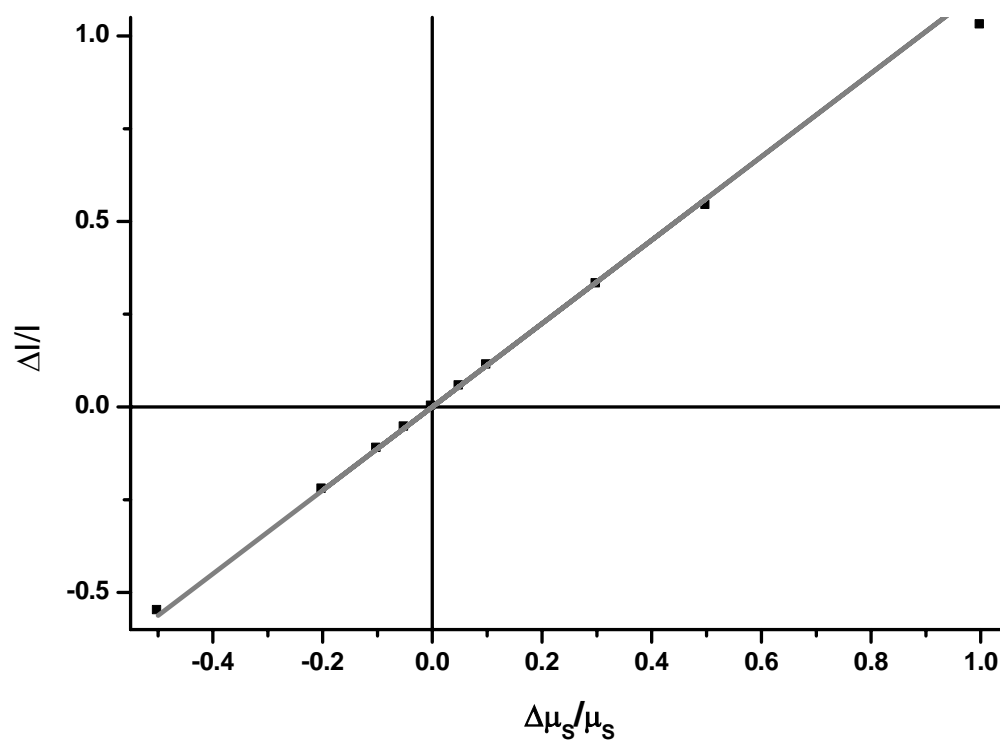
Compartment	d in mm	$\mu_a$ in 1/mm	$\mu_s'$ in 1/mm
Scalp	4	0.008	1.7
Skull	9	0.023	2.0
SHB	11	0.016	0.5
Gray matter	16	0.03	2.2
White matter	Inf.	0.007	8.5

**Table 4** Optical properties used for the compartments of the inhomogenous brain model.

Location of change in $\mu_s'$	d in mm	Inhomogenous model		Homogenous model	
		$K_I$	$K_{\Delta\langle t \rangle}$ in ps/%	$K_I$	$K_{\Delta\langle t \rangle}$ in ps
A) Total gray matter	5	0.06	0.07	0.04	-0.06
B) Layer in gray matter	1	0.05	0.04	0.02	-0.02
C) Gray and white matter	Inf.	0.04	0.03	-0.02	-0.07

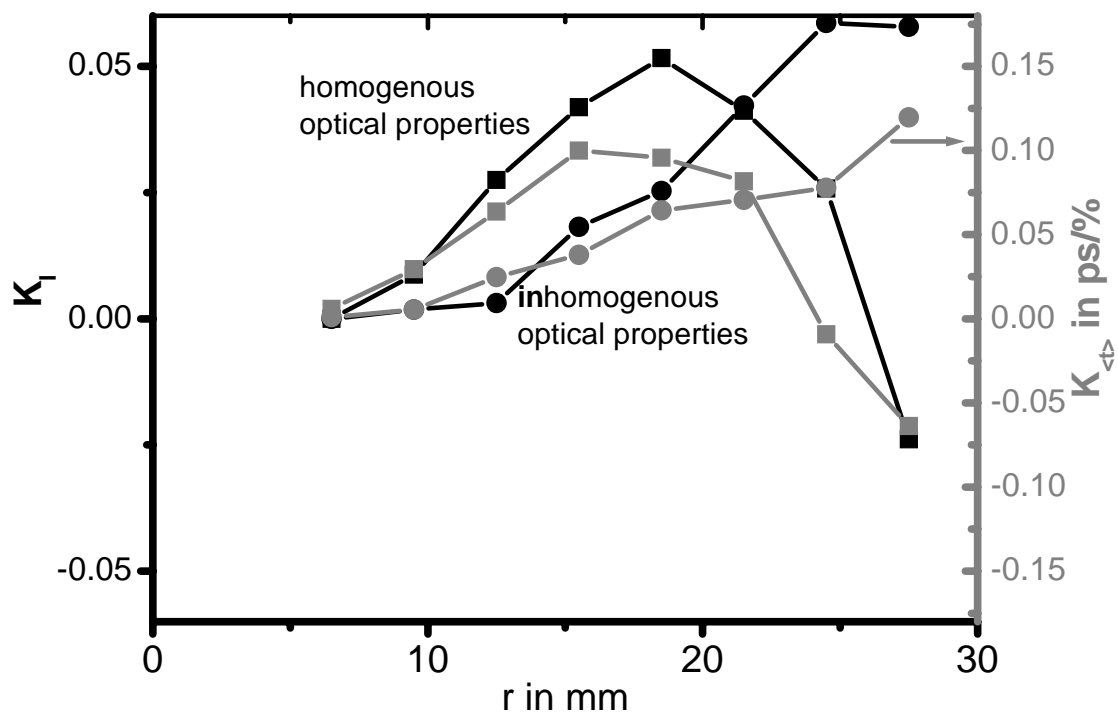
**Table 5**

The sensitivity factors of intensity  $K_I$  and mean time of flight  $K_{\Delta\langle t \rangle}$  calculated by a Monte-Carlo Simulations for a scattering change of different spatial extent. If one assumes a scattering change of 0.1% in the gray matter (case A) the resulting change in the optical signal corresponds to  $\Delta I/I=0.006\%$  and  $\Delta\langle t \rangle=0.007\text{ps}$ .

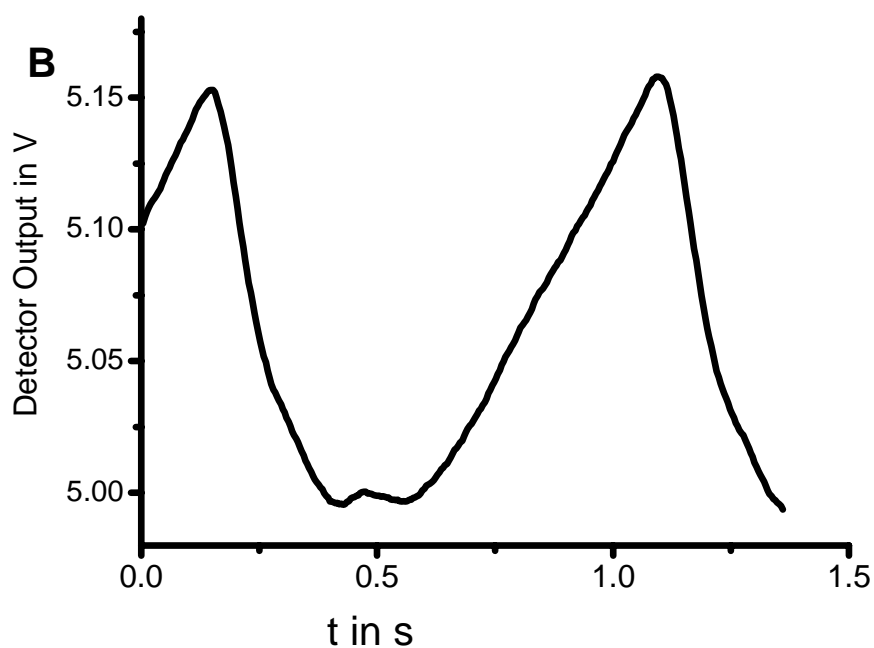
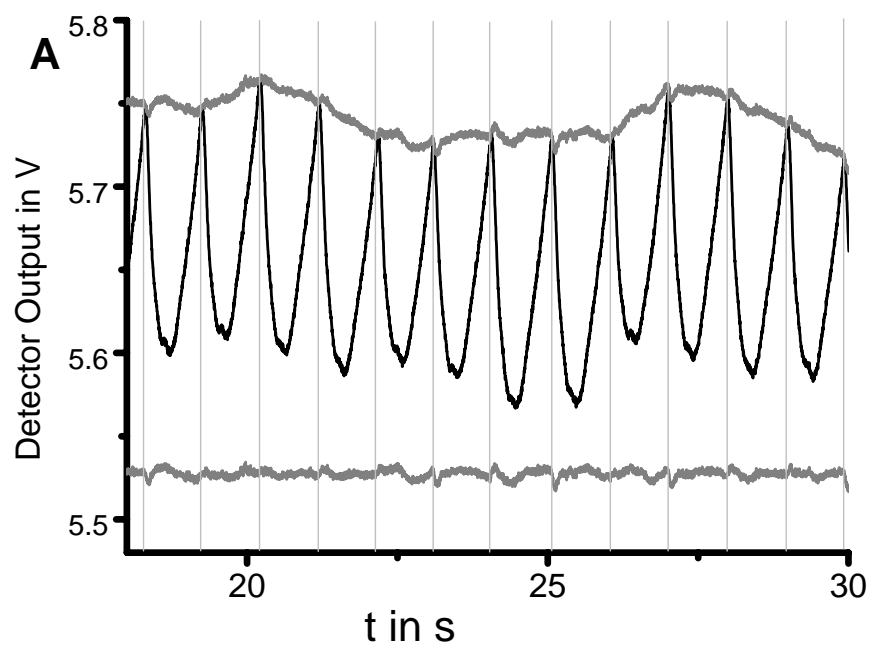


**Figure 1**

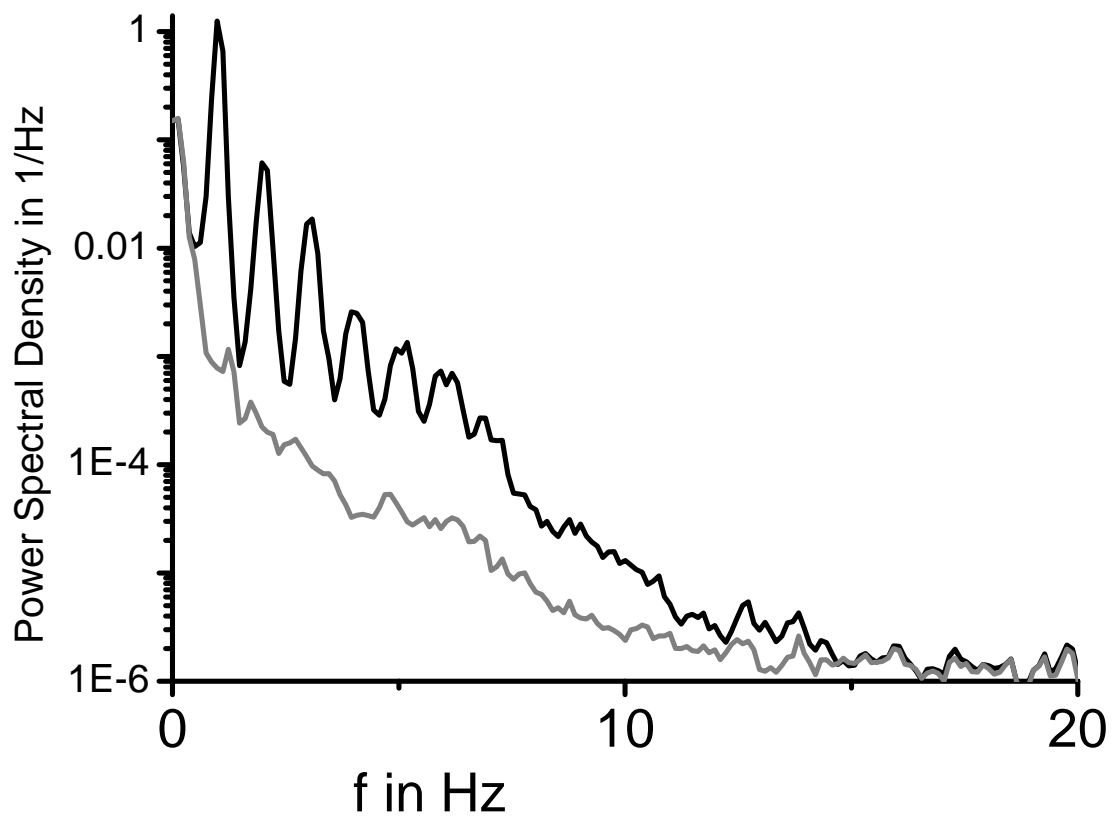
Calculated relative intensity changes (y-axis) induced by changes in  $\mu_s$  (x-axis). A linear approximation around the origin allows for an estimation of  $\Delta\mu_s$  for small intensity changes.



**Figure 2** Fractional sensitivity factors for intensity ( $K_I$ ) and for mean time of flight ( $K_{\langle \tau \rangle}$ ) as a function of the source detector separation.

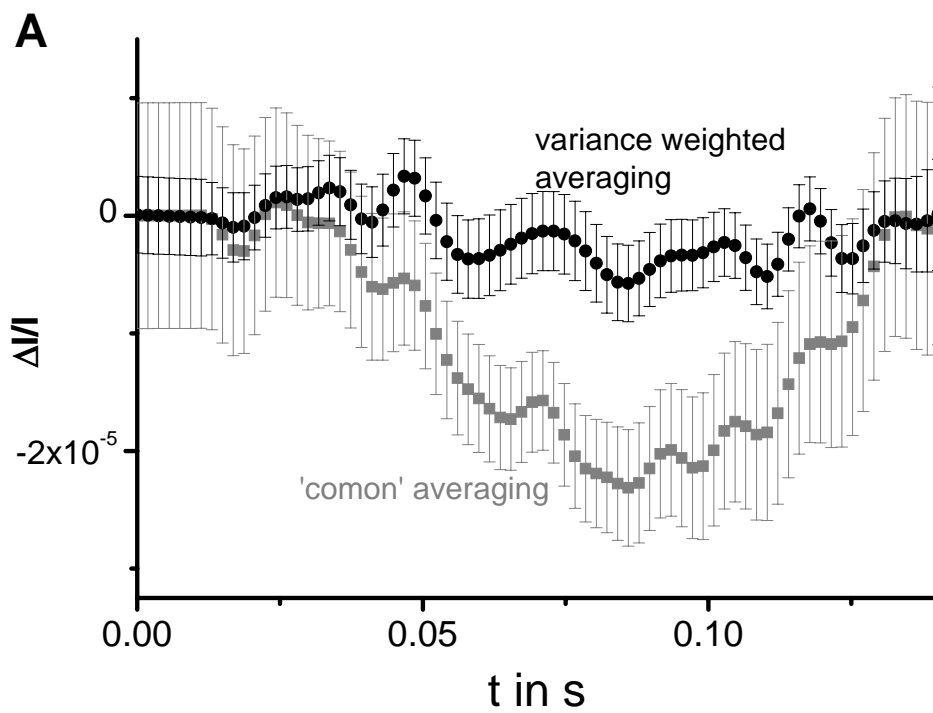


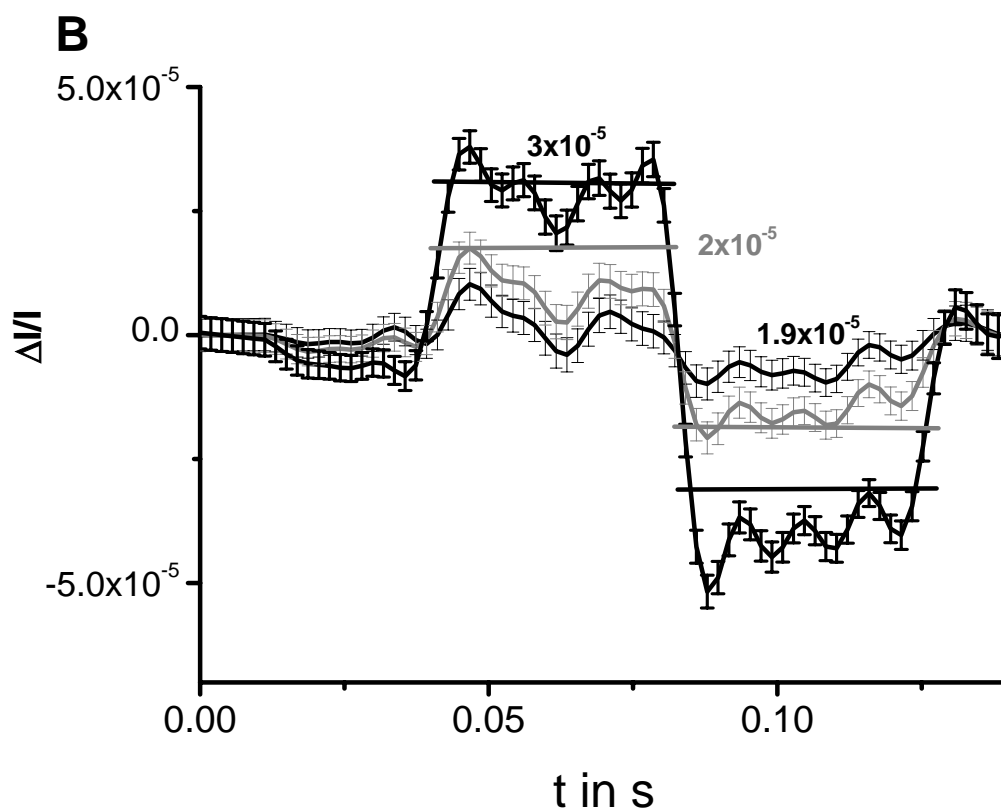
**Figure 3** A) The raw signal (output of the photodiode in Volt) of one of the channels of the imaging system. Gray lines represent the filtered signal. B) The magnification of the raw signal shows the low-level of high frequency noise of the set-up.



**Figure 4**

Power spectral density plot of the original (black) and the filtered (gray) data for one of the subjects. Note, that heartbeat related artifacts can be seen up to 14Hz.

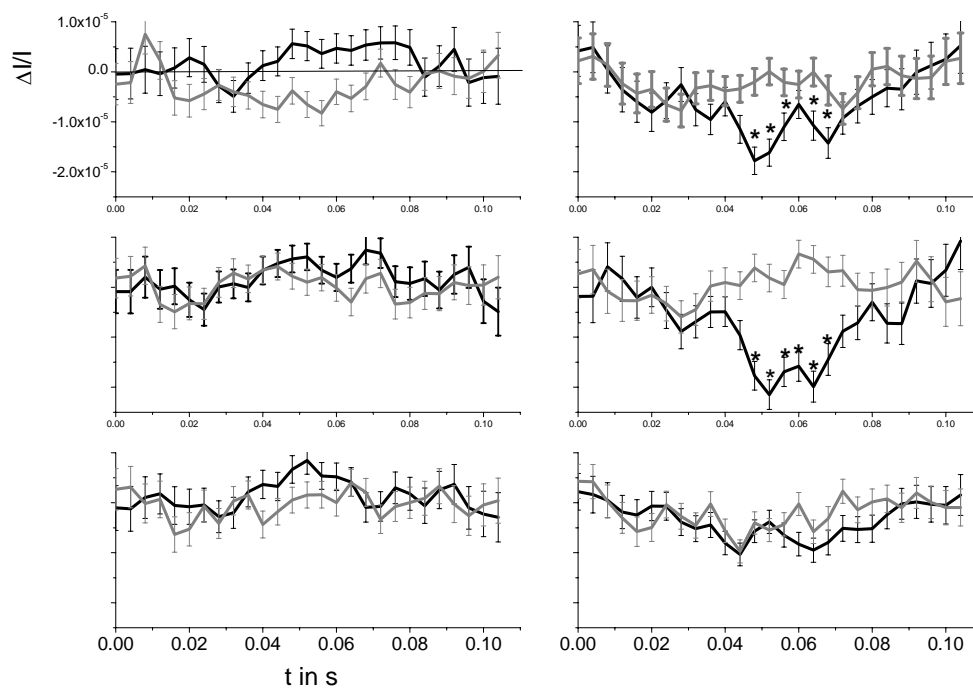




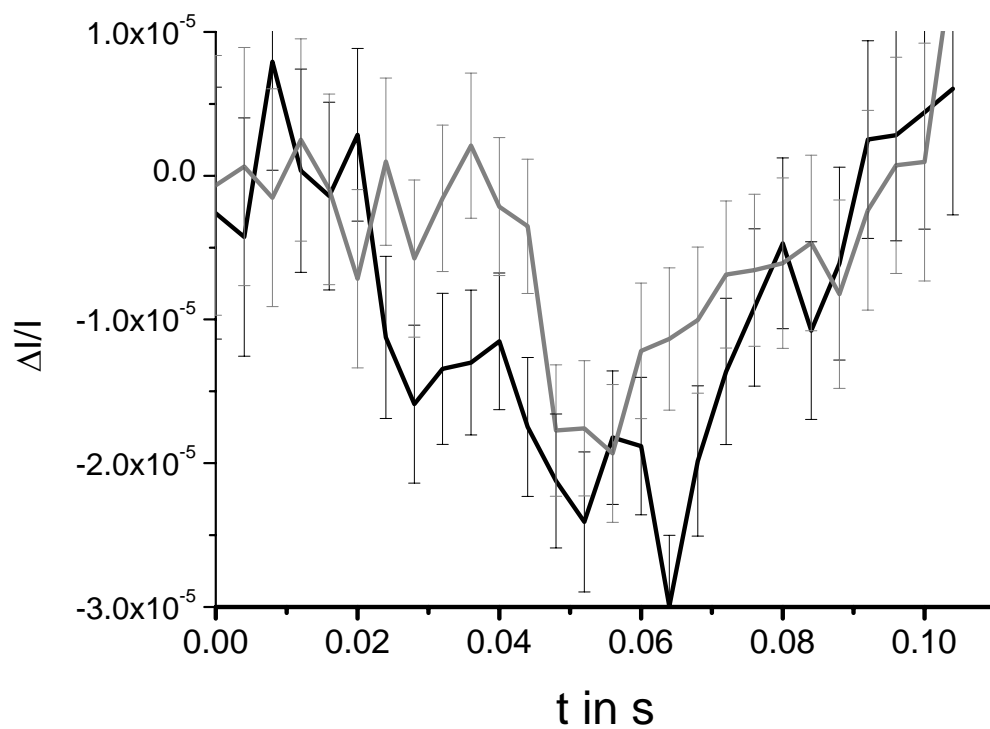
**Figure 5**

A) 'Event' related intensity change for periods during which no stimulation were applied. Black symbols represent a variance-weighted average. The same set-up as for the visual stimulation was used.

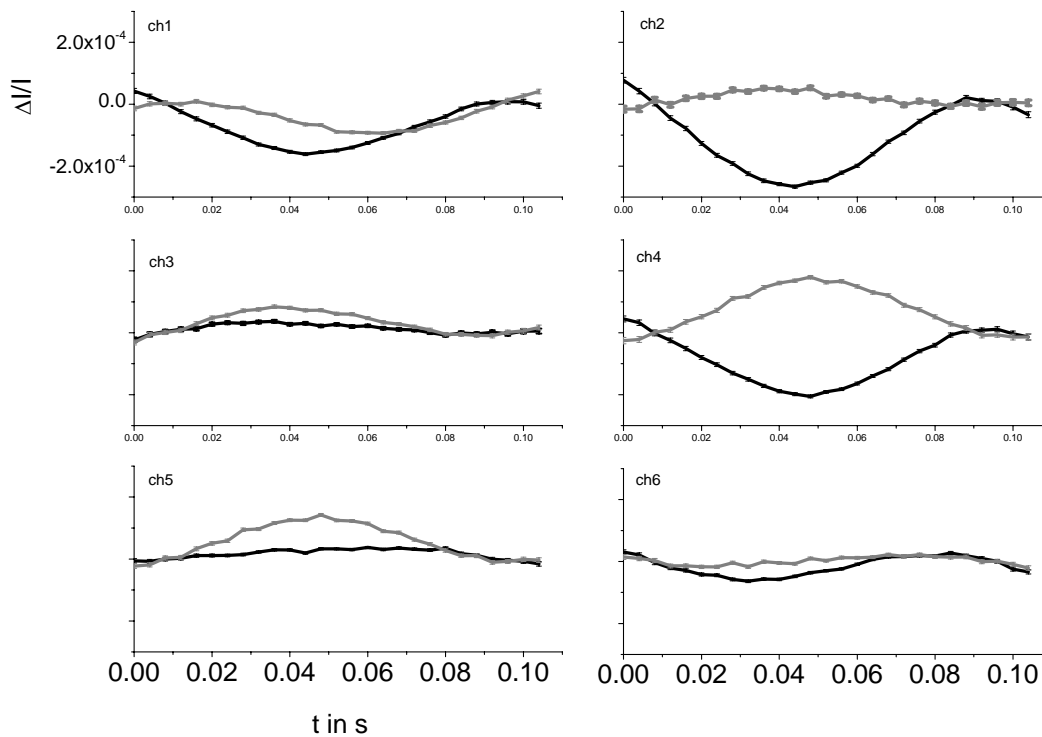
B) To test for the minimal amplitude, which can be resolved, artificial test signals of increasing magnitude (horizontal lines) were added to each stimulation period.

**Figure 6**

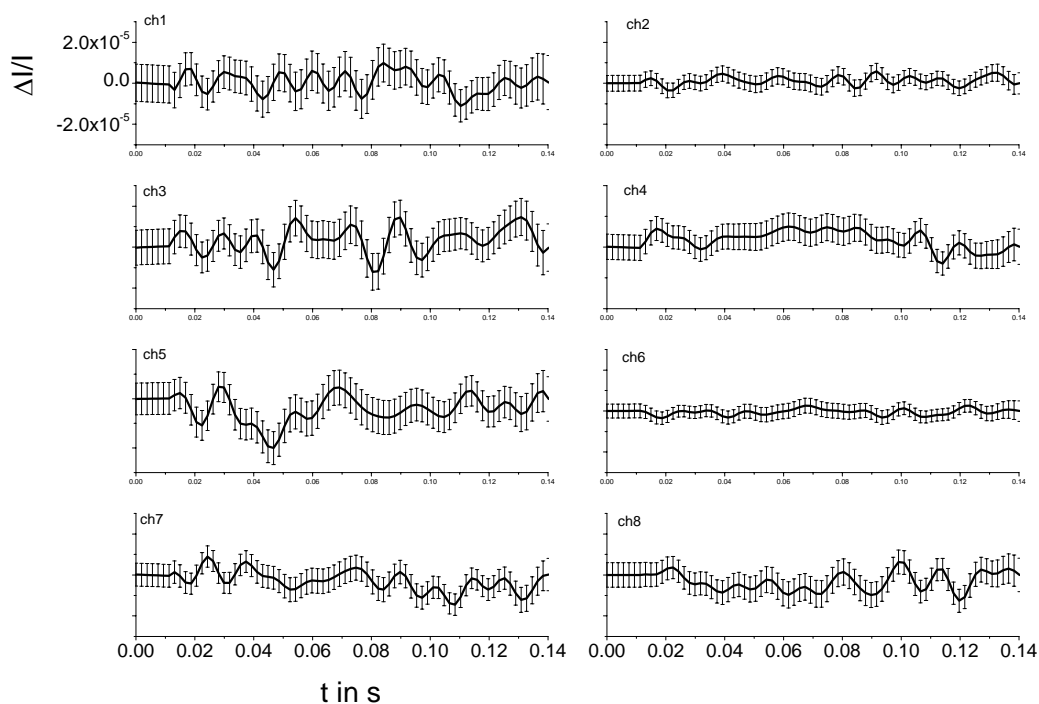
Event related intensity changes in response to sub-motor-threshold median nerve stimulation (black) at 11 Hz. Resting periods are shown in gray. Statistically significant derivations from the baseline are marked with \* ( $p < 0.01$ ).

**Figure 7**

Comparing the response to the odd (black) and even (gray) stimuli, returns two similar curves.

**Figure 8**

Intensity changes (ERIC) measured in response to supra-motor threshold median nerve stimulation (black contra- and gray ipsilateral stimulation). These are accompanied by event related jerks in the subjects stimulated thumb.

**Figure 9**

The event related optical signal following a visual stimulation did not show a significant response in any of the subjects. Note that the noise in some of the detector positions was below  $5 \times 10^{-6}$ .

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