

# Journal of Biomedical Optics

SPIEDigitalLibrary.org/jbo

## **Optical properties of neonatal skin measured *in vivo* as a function of age and skin pigmentation**

Nienke Bosschaart  
Rosaline Mentink  
Joke H. Kok  
Ton G. van Leeuwen  
Maurice C. G. Aalders

# Optical properties of neonatal skin measured *in vivo* as a function of age and skin pigmentation

Nienke Bosschaart,<sup>a</sup> Rosaline Mentink,<sup>a</sup> Joke H. Kok,<sup>b</sup> Ton G. van Leeuwen,<sup>a,c</sup> and Maurice C. G. Aalders<sup>a</sup>

<sup>a</sup>University of Amsterdam, Biomedical Engineering and Physics, Academic Medical Center, P.O. Box 22700, NL-1100 DE Amsterdam, The Netherlands

<sup>b</sup>University of Amsterdam, Neonatology Department, Academic Medical Center, P.O. Box 22700, NL-1100 DE Amsterdam, The Netherlands

<sup>c</sup>University of Twente, MIRA Institute for Biomedical Technology and Technical Medicine, Biomedical Photonic Imaging Group, P.O. Box 217, NL-7500 AE Enschede, The Netherlands

**Abstract.** Knowledge of the optical properties of neonatal skin is invaluable when developing new, or improving existing optical techniques for use at the neonatal intensive care. In this article, we present *in vivo* measurements of the absorption  $\mu_a$  and reduced scattering coefficient  $\mu_s'$  of neonatal skin between 450 and 600 nm and assess the influence of age and skin pigmentation on the optical properties. The optical properties were measured using a spatially resolved, steady state diffuse reflectance spectroscopy setup, combined with a modified spatially resolved diffusion model. The method was validated on phantoms with known values for the absorption and reduced scattering coefficient. Values of  $\mu_a$  and  $\mu_s'$  were obtained from the skin at four different body locations (forehead, sternum, hand, and foot) of 60 neonates with varying gestational age, postnatal age, and skin pigmentation. We found that  $\mu_a$  ranged from 0.02 to 1.25 mm<sup>-1</sup> and  $\mu_s'$  was in the range of 1 to 2.8 mm<sup>-1</sup> (5th to 95th percentile of the patient population), independent of body location. In contrast to previous studies, no to very weak correlation was observed between the optical properties and gestational maturity, but a strong dependency of the absorption coefficient on postnatal age was found for dark skinned patients. © 2011 Society of Photo-Optical Instrumentation Engineers (SPIE). [DOI: 10.1117/1.3622629]

Keywords: optical properties; tissues; absorption; scattering; spectroscopy; reflectance; diffusion.

Paper 11109RRR received Mar. 8, 2011; revised manuscript received Jul. 11, 2011; accepted for publication Jul. 14, 2011; published online Sep. 1, 2011.

## 1 Introduction

Light-tissue interactions in the visible wavelength range are the basis of many experimental and routinely used diagnostic and therapeutic procedures at the neonatal intensive care, e.g., in transcutaneous bilirubinometry,<sup>1</sup> pulse oximetry,<sup>2</sup> and phototherapy during jaundice.<sup>3</sup> Knowledge of the optical properties of the tissue is essential when predicting or modeling light-tissue interactions, which in turn is invaluable for the development and improvement of these procedures. Although the majority of optical techniques at the neonatal intensive care depend on the interaction of light with skin, very little information can be found in literature about the optical properties (e.g., absorption and scattering coefficients) of neonatal skin.

Earlier research on the optical properties of neonatal skin was performed by Saidu et al., who measured the absorption coefficient and the reduced scattering coefficient as a function of age with an integrating sphere setup.<sup>4,5</sup> They found that the reduced scattering coefficient increases with age, due to maturation of the skin. These measurements were performed *in vitro* on excised nonpigmented skin. However, optical properties measured *in vitro* may differ substantially from those measured *in vivo* due to the unavoidable sample preparation procedures.<sup>6</sup> Moreover, it is expected that the absorption coefficient depends on skin pigmentation, which was not investigated. We expect that

optical properties of neonatal skin, assessed by *in vivo* measurements as a function of age and skin pigmentation, can assist many optical devices such as transcutaneous bilirubinometers in improving their performance. The readings of current bilirubinometers commonly have a spread of  $\pm 40$   $\mu\text{mol/L}$  around the bilirubin concentration (ranging from 0 to 400  $\mu\text{mol/L}$ ) that is measured by the gold standard for bilirubin measurements, total serum bilirubin analysis.<sup>1</sup> This inaccuracy may be partly caused by variations in the often unknown probed volume inside the skin. Knowledge of the optical properties can be used as input to, for example, Monte Carlo simulations of photon distributions inside the skin. These simulations can provide insight into the probed tissue volume, which in turn can lead to a more optimal design for optical diagnostic probes, methods of analysis, and comparison between devices.

Steady state, spatially resolved diffuse reflectance spectroscopy has proven to be a reliable technique for measuring the optical properties of tissue and tissue simulating phantoms in the near-infrared<sup>7,8</sup> and visible<sup>8,9</sup> wavelength range. When using short source-detector distances, the investigated tissue volume is small<sup>9</sup> and can be confined to, for instance, the skin. Therefore, in this study we used a steady state, spatially resolved diffuse reflectance spectroscopy setup and a semi-empirical three-dimensional (3D) diffusion model, modified for our detection geometry, to measure the absorption and reduced scattering coefficient of neonatal skin from 450 to 600 nm.

Address all correspondence to: Nienke Bosschaart, University of Amsterdam, Biomedical Engineering and Physics Academic Medical Center, Meibergdreef 9, Amsterdam, Noord-Holland 1100DE, The Netherlands; Tel: 0031205665207; Fax: 0031206917233; E-mail: n.bosschaart@amc.uva.nl.

The optical properties were measured on the skin of 60 preterm neonates with varying gestational maturity, postnatal age, and skin pigmentation. All measurements were performed on four body locations (forehead, sternum, hand, and foot), which are common measurement sites for either transcutaneous bilirubinometry, or pulse oximetry. The method was validated on phantoms with known values for the absorption and reduced scattering coefficient.

## 2 Methods

### 2.1 Experimental Setup

The diffuse reflectance spectroscopy setup (similar to that of Doornbos et al.<sup>7</sup> and Nichols et al.),<sup>8</sup> is shown in Fig. 1(a). Tissue illumination and detection of re-emitted light are performed by a multi-fiber probe. The probe consists of 5 fibers (400  $\mu\text{m}$  core diameter, length  $\sim 2$  m), which are cast in-line in an aluminum sensing head with a core-to-core distance of 600  $\mu\text{m}$ . The first fiber acts as the illumination fiber and guides the white light from a tungsten halogen source (AvaLight-HAL, Avantes, USA) to the tissue. The other four fibers detect the re-emitted light (source-detector distances of  $r_1 = 0.6$  mm,  $r_2 = 1.2$  mm,  $r_3 = 1.8$  mm, and  $r_4 = 2.4$  mm) and guide it to an imaging spectrograph (Olympus ISS, Olympus, USA). A 16-bit  $128 \times 1024$  pixel CCD camera (DH501-18F-01, Andor Technology, USA) cooled to  $0^\circ\text{C}$  detects the four spectra originating at different distances from the illumination fiber, with a wavelength resolution of 9 nm over the detection range of 400 to 820 nm. The image tracks illuminated by the fibers are averaged

for each fiber to obtain four averaged spectra. The total exposure time for the *in vivo* measurements ranged between 15 and 35 ms. In order to minimize system noise and to correct for temporal variation, each measurement in this article consists of the average of three subsequent measurements with an interval of 1s.

### 2.2 Setup Calibration

The diffuse reflectance measurements require three types of calibration: 1. wavelength calibration of the spectrograph, 2. calibration to correct for the collection efficiency of the setup and the spectral emission of the source, and 3. correction for the (small amount of) ambient light and system noise.

The wavelength calibration of the spectrograph is performed by measuring the emission of a 543 and a 633 nm HeNe laser, and the emission of a fluorescent lamp. An additional calibration is done prior to each patient/phantom measurement by measuring the spectrum of a fluorescent light tube. The collection efficiency of the setup and the spectral emission of the source are calibrated using the method described in detail by Doornbos et al.<sup>7</sup> which is comprised of measuring the collected intensity for each detection fiber at the entrance of an integrating sphere, illuminated by the illumination fiber of the probe. This calibration results in a calibration spectrum for each fiber distance  $r_j$ , indicated by  $\mathbf{I}_0(r_j)$  (all wavelength dependent parameters in this article will be denoted by a bold-faced character).

To correct for the collection of ambient light and system noise, a dark measurement was taken prior to each patient/phantom measurement with a shielded probe. The dark measurement results in a dark spectrum for each fiber distance, indicated by  $\mathbf{I}_{\text{dark}}(r_j)$ .

### 2.3 Data Analysis

To extract the absorption and reduced scattering coefficient from the acquired spectra, we use the steady state diffusion approximation to the photon transport equation described by Farrell et al.<sup>10</sup> This diffusion model describes the 3D photon distribution inside a medium for photons injected by a point source, by modeling an isotropic source at a depth  $\mathbf{z}_0$  inside the tissue, and a virtual source at a distance of  $2\mathbf{z}_b + \mathbf{z}_0$  above the medium. The remittance  $\mathbf{R}_{\text{theor}}$  at the tissue surface as a function of source-detector distance  $r$ , and the optical properties of the tissue is given by:<sup>10</sup>

$$\mathbf{R}_{\text{theor}}(r) = (4\pi)^{-1} \cdot [\mathbf{z}_0 \cdot (\mu_{\text{eff}} + \mathbf{r}_1^{-1}) \cdot \exp(-\mu_{\text{eff}} \cdot \mathbf{r}_1) \cdot \mathbf{r}_1^{-2} + (\mathbf{z}_0 + 2\mathbf{z}_b) \cdot (\mu_{\text{eff}} + \mathbf{r}_2^{-1}) \cdot \exp(-\mu_{\text{eff}} \cdot \mathbf{r}_2) \cdot \mathbf{r}_2^{-2}], \quad (1)$$

where  $\mu_{\text{eff}} = [3\mu_a(\mu_a + \mu_s')]^{\frac{1}{2}}$ ,  $\mathbf{r}_1 = (\mathbf{z}_0^2 + r^2)^{\frac{1}{2}}$ ,  $\mathbf{r}_2 = [(\mathbf{z}_0 + 2\mathbf{z}_b)^2 + r^2]^{\frac{1}{2}}$ ,  $\mathbf{z}_0 = (\mu_a + \mu_s')^{-1}$ , and  $\mathbf{z}_b = 2/3 \cdot A(\mu_a + \mu_s')^{-1}$ . Note that all parameters, except  $r$ , are wavelength dependent. The value of the empirical parameter  $A$  in the definition of  $\mathbf{z}_b$  was set to  $A = 1$ , since this gave the most reliable results (i.e., smallest residuals) when fitting our model to the data. Fitting the diffusion model to the spatially measured reflectance of the four detection fibers all together, using  $\mathbf{R}_{\text{meas}}(r_j) = \alpha \cdot \mathbf{R}_{\text{theor}}(r_j)$  yields a single set of the parameters  $\mu_a$  and  $\mu_s'$ . The proportionality factor  $\alpha$  is adapted from Doornbos et al.<sup>7</sup> and contains factors such as the numerical aperture and the efficiency of coupling light into the sample. The value of  $\alpha$  was

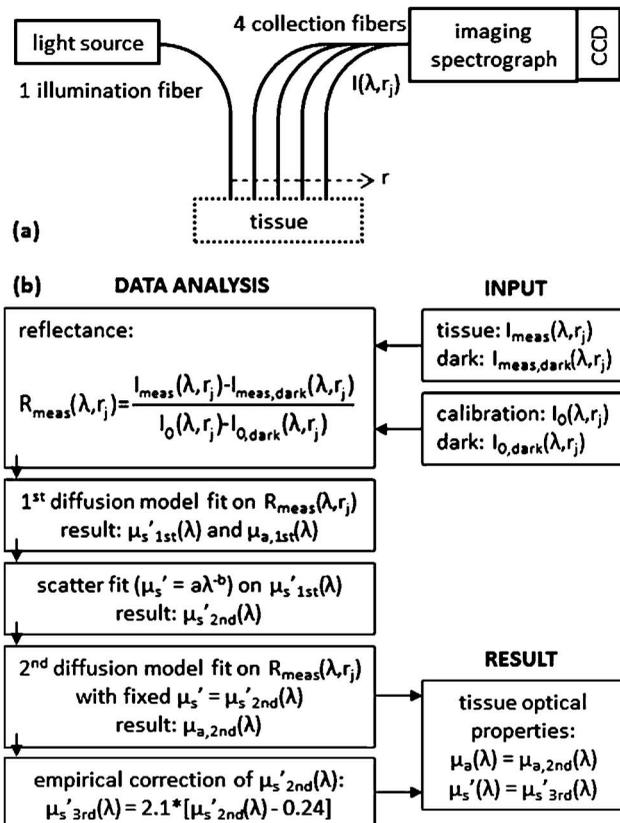


Fig. 1 (a) Illustration of the measurement setup. (b) Schematic illustration of the data analysis.

found by fitting the diffusion model to the reflectance of a phantom with known  $\mu_a$  and  $\mu_s'$  (Sec. 2.4).

Our method of analysis [schematically depicted in Fig. 1(b)] can be explained in the following steps, in which we adapted steps 1 to 4 from Doornbos et al.: 1. calculation of the measured reflectance for each fiber distance:  $\mathbf{R}_{\text{meas}}(r_j) = [\mathbf{I}_{\text{meas}}(r_j) - \mathbf{I}_{\text{meas,dark}}(r_j)] / [\mathbf{I}_0(r_j) - \mathbf{I}_{0,\text{dark}}(r_j)]$ , 2. fitting the diffusion model to  $\mathbf{R}_{\text{meas}}(r_j)$ , using a nonlinear least-square Levenberg–Marquardt algorithm, which results in a first approximation for the wavelength dependent absorption and reduced scattering coefficient  $\mu_{a,1\text{st}}$  and  $\mu_{s',1\text{st}}$ , 3. fitting the function  $\mu_{s'} = a\lambda^b$  (where  $b$  is the scatter power and both  $a$  and  $b$  are free running fit parameters) to the obtained  $\mu_{s',1\text{st}}$  using a nonlinear least-square Levenberg–Marquardt algorithm, which results in a second approximation for the reduced scattering coefficient  $\mu_{s',2\text{nd}}$ , 4. re-fitting the diffusion model to  $\mathbf{R}_{\text{meas}}(r_j)$  with  $\mu_{s',2\text{nd}}$  as a fixed parameter, which results in a second approximation for the absorption coefficient  $\mu_{a,2\text{nd}}$ , 5. correction of  $\mu_{s',2\text{nd}}$  for an initial underestimation using the empirically determined relation  $\mu_{s',3\text{rd}} = 2.1 * (\mu_{s',2\text{nd}} - 0.24)$  (see Sec. 3.1.1). The obtained values for  $\mu_{a,2\text{nd}}$  and  $\mu_{s',3\text{rd}}$  are regarded as the bulk optical properties of the tissue in the measurement volume, from now on are indicated by  $\mu_a$  and  $\mu_s'$ .

The diffusion model is valid for semi-infinite, homogeneous media where scattering dominates absorption ( $\mu_a/\mu_s' \leq \beta$ , commonly with  $\beta \ll 1$ ) and a source-detector separation that ensures a diffuse photon distribution ( $r > \gamma/(\mu_a + \mu_s')$ , commonly  $r$  is at least one mean free path,  $1/(\mu_a + \mu_s')$ , hence  $\gamma = 1$ ). Note that for our probe geometry, the value of  $\gamma$  depends entirely on the lowest value of  $(\mu_a + \mu_s')$  that can be accurately measured, since the (minimal) source-detector separation  $r$  is fixed. To determine the validity and accuracy of the model for our measurement geometry and neonatal skin, we performed measurements on phantoms with optical properties in the range of neonatal skin and found the limiting values for  $\beta$  and  $\gamma$  (Sec. 2.4). In contrast to Doornbos et al., who could only reliably measure the optical coefficients for wavelengths larger than 600 nm, we found that  $r > \gamma/(\mu_a + \mu_s')$  is in general only valid for wavelengths shorter than 600 nm. This can be explained by a difference in probe design (i.e., shorter source-detector separations) and the relatively low value of  $\mu_s'$  for neonatal skin. Therefore, we only determined the optical properties in the 450 to 600 nm wavelength region of the signal. The homogeneity of neonatal skin was investigated by optical coherence tomography (OCT) measurements (Sec. 2.5).

## 2.4 Phantom Validation Study

The method of analysis described above was validated with measurements on 14 phantoms with known values for  $\mu_a$  and  $\mu_s'$ . The optical properties of the phantoms ( $\mu_a = 0$  to  $2.7 \text{ mm}^{-1}$ ,  $\mu_s' = 0.3$  to  $3.7 \text{ mm}^{-1}$ ) were chosen to be in the same range as the expected values of  $\mu_a$  and  $\mu_s'$  of neonatal skin. The phantoms consisted of two series: series 1, with a fixed concentration of 1.5% Intralipid (Intralipid®20%, Fresenius Kabi, Germany) and varying concentrations of a nonscattering, absorbing magenta dye (0.125 to 2%, Ecoline #337, Royal Talens, The Netherlands), and series 2, with a fixed concentration of 0.25% magenta dye and varying concentrations of Intralipid (0.25 to 2%). The reduced scattering coefficient of the phantoms

was estimated using the predictions of Van Staveren et al.<sup>11</sup> for  $\mu_s'$  of Intralipid. The absorption coefficient of the dye only was determined in a separate transmission measurement by a spectrograph (USB4000, Ocean Optics, USA). During the measurements, the probe was in contact with the phantoms to minimize reflections from the phantom surface. From these phantom measurements, we determined the accuracy of our determination of  $\mu_a$  and  $\mu_s'$  and the validity limits of our analysis, i.e., the limiting values of  $\beta$  and  $\gamma$  [ $\mu_a/\mu_s' \leq \beta$  and  $r > \gamma/(\mu_a + \mu_s')$ ] for which the diffusion model can be applied in this measurement geometry.

To verify that the comparison with the predicted  $\mu_s'$  of Van Staveren is justified for these phantoms, we also measured the  $\mu_s'$  of National Institute of Standards and Technology (NIST)-certified spheres with a diameter of 409 nm, suspended in water (0.48 vol.%  $\mu_s' = 2$  to  $3 \text{ mm}^{-1}$ , Thermo Scientific, USA). The measured  $\mu_s'$  of this phantom was compared to the theoretical  $\mu_s'$ , obtained from Mie theory.

For measurements of the optical properties of neonatal skin, it is important that the measurement volume is confined to the skin only. Therefore, knowledge of the probing depth is required. To investigate the probing depth of the system, the probe was submersed at varying distances (0 to 5 mm, in steps of 100  $\mu\text{m}$ ) from the lower bottom of a phantom with  $\mu_a$  and  $\mu_s'$  similar to neonatal skin (1.5% Intralipid, 0.5% dye:  $\mu_a$  up to  $0.35 \text{ mm}^{-1}$ ,  $\mu_s' = 2$  to  $2.6 \text{ mm}^{-1}$ ). The lower bottom of the liquid phantom consisted of a stationary solid layer of diffusively scattering white rubber. The measurements were repeated with a diffusively scattering black rubber layer at the lower bottom.

## 2.5 Patient Study

Sixty neonates of the neonatal intensive care unit in the Academic Medical Center of Amsterdam were enrolled in this study. Approval of the medical ethical committee and informed consent from the patient's legally authorized representative were obtained. The patient group varied in gestational age (i.e., pregnancy duration):  $29.4 \pm 3.3$  weeks (mean  $\pm$  sd, normal distribution), postnatal age: 11 (6 to 28) days [median (25<sup>th</sup> to 75<sup>th</sup> percentile), nonnormal distribution], and skin type. The skin type of the patients was classified into three categories, based on observation and ethnicity of the patient and the parents: type 1 (Caucasian or "white": 43 patients), type 2 (Mediterranean or "intermediate": 12 patients), and type 3 (Negroid or "dark": 5 patients). The majority of the patients were subjected to more than one measurement during their admission at the hospital, with a time lapse of one day to several weeks between measurements and a median of 3 measurements per patient. An overview of the patient population and the number of measurements is listed in Table 1 (All measurements).

The optical properties were determined at four locations on the skin: the glabella of the forehead, the lower end of the sternum, the plantar side of the foot, and the dorsal side of the hand. The presence of visible large blood vessels in the measured volume was avoided. Due to medical reasons (e.g., presence of a ventilator, pulse oximeter, and/or intravenous line), not all skin areas were accessible at all times, resulting in a different number of measurements between locations (Table 1). To minimize reflections from the probe-tissue interface, the probe surface was covered with lubricating gel (Euroband Pedicat, Pollak, France).

**Table 1** Overview of the patient population and number of measurements per skin type and measurement location.

Location	All measurements				Measurements within validity limits			
	# measurements				# measurements			
	type 1	type 2	type 3	All	type 1	type 2	type 3	all
Forehead	137	47	33	217	132	45	10	187
Sternum	135	41	29	205	133	39	9	181
Hand	137	47	30	214	134	41	11	186
Foot	136	49	33	218	136	48	32	216
	# patients				# patients			
Forehead	43	12	5	60	42	12	4	58
Sternum	43	12	5	60	43	12	4	59
Hand	42	12	5	59	42	12	5	59
Foot	42	12	5	59	42	12	5	59
Total # patients	43	12	5	60	43	12	5	60
Gestational age (wks)	29.9	29.2	27.4	29.4	29.9	29.2	27.5	29.4
mean ( $\pm$ sd)	( $\pm$ 3.7)	( $\pm$ 1.8)	( $\pm$ 2.3)	( $\pm$ 3.3)	( $\pm$ 3.7)	( $\pm$ 1.8)	( $\pm$ 2.3)	( $\pm$ 3.3)
Postnatal age (days)	11	6	18	11	11	6	18	11
median (p25 to p75)	(6 to 35)	(4 to 13)	(9 to 34)	(6 to 28)	(6 to 35)	(4 to 13)	(10 to 32)	(6 to 28)

Attention was paid to keep a constant probe pressure on the skin during the measurements.

A general linear multivariate analysis model in SPSS for Windows (version 16.0, SPSS Inc.) was used to assess the influence of measurement location and skin type on the optical coefficients at three wavelengths (475, 525, and 575 nm), where we assumed a normal distribution of the data. To avoid the influence of dependent measurements in the multivariate analysis, one measurement event per patient (including all four locations) was randomly selected from our dataset.

To investigate the anatomy and homogeneity of neonatal skin compared to adult skin, OCT B-scans were made with a 1310 nm OCT system (HSL-2000, Santec, Japan) at the four investigated skin areas of several patients and adults.

### 3 Results

#### 3.1 Phantom Validation Study

##### 3.1.1 Validity and accuracy

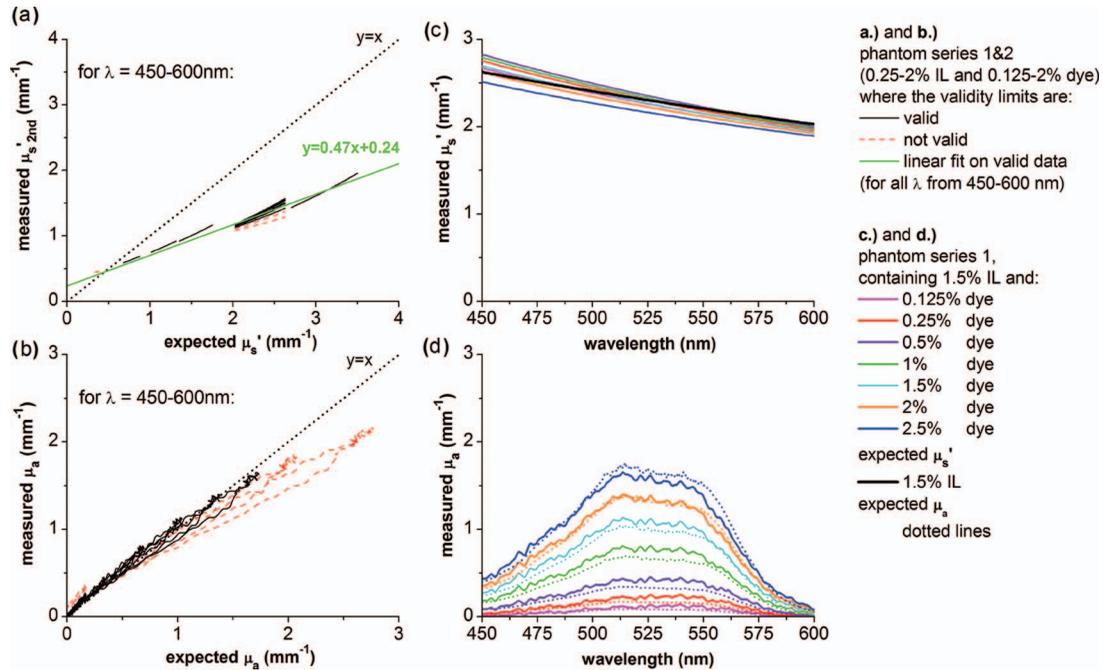
Figures 2(a) and 2(b) show the measured  $\mu_s'_{2nd}$  and  $\mu_a$  versus the expected values (from Van Staveren and transmission spectroscopy, respectively) for all phantoms of series 1 and 2. Difference is made between phantoms for which the validity limits [i.e.,  $\mu_a/\mu_s' \leq \beta$  and  $r > \gamma/(\mu_a + \mu_s')$ ] are valid for all wavelengths (solid lines) and phantoms for which the validity

limits are not valid for at least one wavelength between 450 to 600 nm (dashed lines). The obtained values for  $\beta$  and  $\gamma$  will be described below.

From Fig. 2(b), we can conclude that  $\mu_a$  is predicted with 15% accuracy by our measurements up to a value of  $1.7 \text{ mm}^{-1}$ . Since the corresponding expected  $\mu_s' = 2.3 \text{ mm}^{-1}$  for this value of  $\mu_a$ , we conclude that the limiting ratio between  $\mu_a$  and  $\mu_s'$  for which the diffusion model can be applied in this measurement geometry, is  $\beta = \frac{3}{4}$ . This value of  $\beta$  is considerably lower than the commonly used value  $\beta = 1/10$  in other diffusion model based studies.<sup>7,8</sup> For the phantoms where  $\mu_a/\mu_s' \leq \frac{3}{4}$  is not valid,  $\mu_a$  is underestimated by our measurement.

Figure 2(a) shows a consistent underestimation of  $\mu_s'_{2nd}$ , which can be described by the linear relation ( $\mu_s'_{2nd} = 0.47\mu_s'_{expected} + 0.24$ ) for the range of  $\mu_s'$ -values within the validity limits. The same exact linear relation was found between the measured  $\mu_s'_{2nd}$  of the 409 nm NIST certified spheres and the expected  $\mu_s'$  from Mie theory (no graph), indicating that the underestimation is consistent and not caused by an incorrect comparison to the prediction of Van Staveren. We therefore corrected all measured  $\mu_s'_{2nd}$  (phantom and tissue measurements) for this underestimation, using the linear relation  $\mu_s' = \mu_s'_{3rd} = 2.1 * (\mu_s'_{2nd} - 0.24)$ .

Figures 2(c) and 2(d) show the measured  $\mu_s'$  after correction and the measured  $\mu_a$  versus wavelength for the phantoms of series 1 for which the validity limits are valid. Since the



**Fig. 2** Phantom validation study. For all phantoms in series 1 & 2: (a) measured  $\mu_{s'2nd}$  versus expected  $\mu_{s'}$ , and (b) measured  $\mu_a$  versus expected  $\mu_a$ . Difference is made between phantoms for which  $\mu_a/\mu_{s'} \leq \beta$  and  $r > \gamma/(\mu_a + \mu_{s'})$  are valid (solid lines) and not valid (dashed lines). For all phantoms in series 1 for which  $\mu_a/\mu_{s'} \leq \beta$  is valid: (c) measured  $\mu_{s'}$  after correction and expected value versus wavelength and (d) measured and expected  $\mu_a$  versus wavelength.

Intralipid concentration is fixed for the phantoms of series 1,  $\mu_{s'}$  is expected to be equal for all phantoms. After correction, the measured  $\mu_{s'}$  agree within 10% with the prediction of Van Staveren and a maximum variation of 10% can be observed between the measured  $\mu_{s'}$  of all phantoms. As expected from Fig. 2(b), the measured  $\mu_a$  in Fig. 2(d) agrees within the reported accuracy with the expected  $\mu_a$  from transmission spectroscopy.

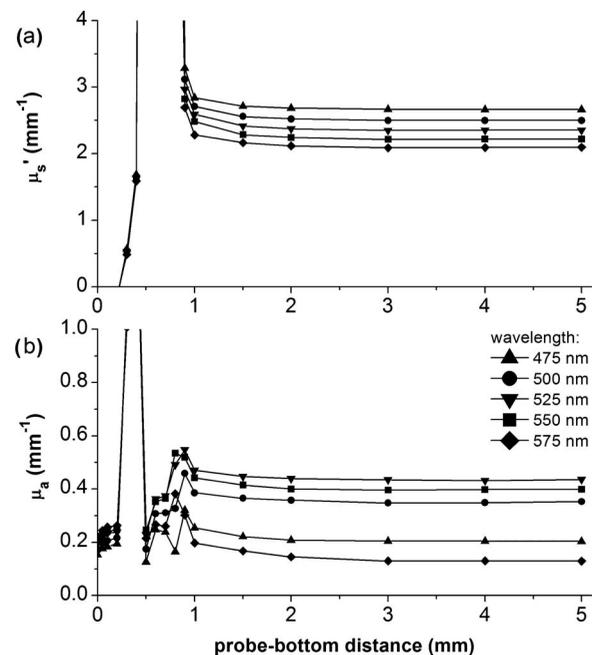
We obtained the value of  $\gamma$  from the phantom series 2 with fixed  $\mu_a$  and varying  $\mu_{s'}$ . For these series, the  $\mu_a$  and  $\mu_{s'}$  were predicted correctly for the phantoms with  $(\mu_a + \mu_{s'}) > 0.8 \text{ mm}^{-1}$ . For the phantom with  $(\mu_a + \mu_{s'}) < 0.8 \text{ mm}^{-1}$ ,  $\mu_a$  was overestimated by our measurement. Since the minimal source-detector separation is 0.6 mm in our measurement geometry, we conclude that  $\gamma = \frac{1}{2}$  in the validity limit  $r > \gamma/(\mu_a + \mu_{s'})$ .

In summary, our method is valid when measuring on semi-infinite homogeneous media with values of  $\mu_a$  and  $\mu_{s'}$  that meet the validity limits  $\mu_a/\mu_{s'} \leq \frac{3}{4}$  and  $(\mu_a + \mu_{s'}) > 0.8 \text{ mm}^{-1}$ . Our phantom study shows that these limits hold for this specific probe geometry and method of analysis. When measuring on neonatal skin, the validity of these limits needs to be investigated for every wavelength within the measured spectral range. Since we need to apply the empirically determined correction on the initial value of  $\mu_{s'2nd}$ , our method of analysis based on the diffusion model is semi-empirical.

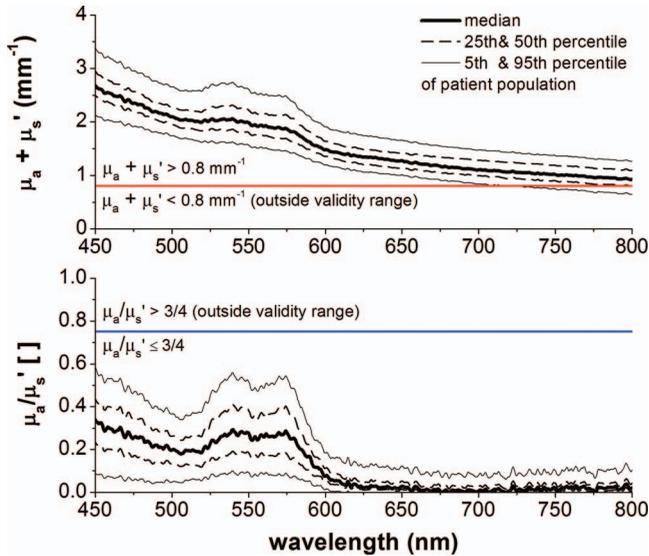
### 3.1.2 Probing depth

Figures 3(a) and 3(b) show the dependency of the measured coefficients on the probe-bottom distance for the phantom with the black bottom layer. For probe-bottom distances  $< 1$  mm,

the measured coefficients are inconsistent, due to presence of the black bottom layer in the measurement volume. For probe-bottom distances  $\geq 1$  mm, the measured  $\mu_a$  and  $\mu_{s'}$  are consistent and agree with the expected values within the reported accuracy.



**Fig. 3** Measured values of (a)  $\mu_{s'}$  and (b)  $\mu_a$  as a function of probe-bottom distance for the 1.5% Intralipid-0.5% dye phantom and the black bottom layer. Results are shown for the wavelengths 475, 500, 525, 550, and 575 nm.



**Fig. 4** Median values and 5th, 25th, 75th, and 95th percentiles for the measured  $\mu_a/\mu_s'$  and  $(\mu_a + \mu_s')$  versus wavelength for the patient measurements at the foot, after selection of the data between 450 and 600 nm by the validity limits (i.e.,  $\mu_a/\mu_s' \leq \frac{3}{4}$  and  $(\mu_a + \mu_s') > 0.8 \text{ mm}^{-1}$ ).

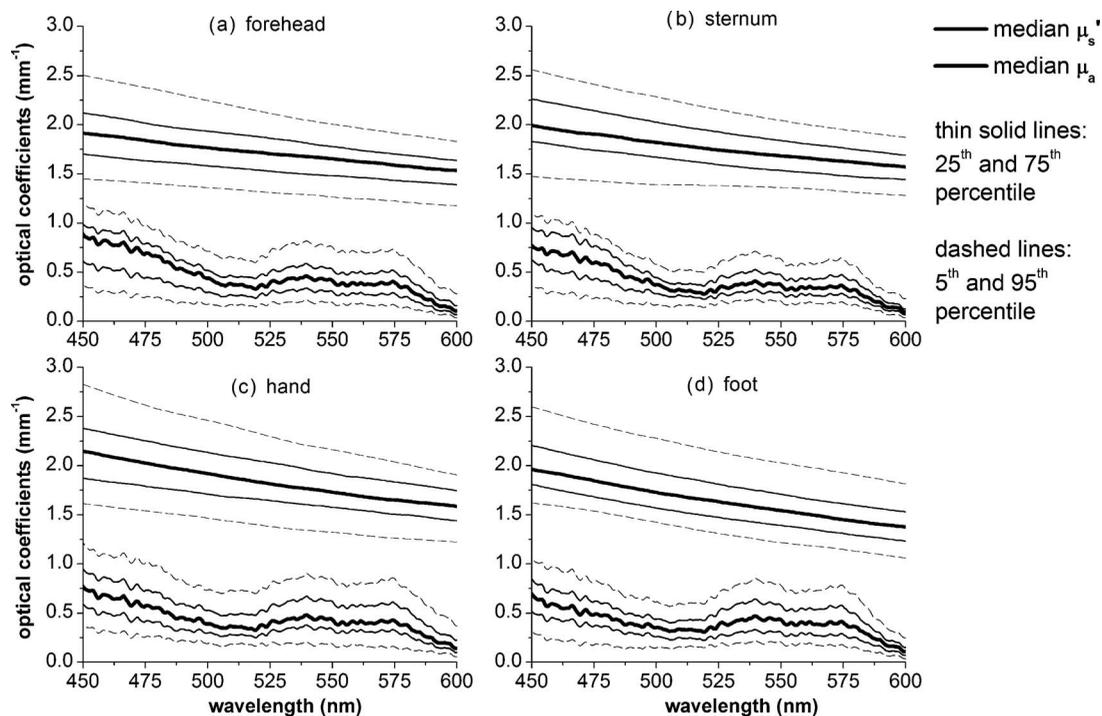
Also for the phantom with the white bottom layer, the measured  $\mu_a$  and  $\mu_s'$  are consistent for distances  $\geq 1 \text{ mm}$ . We therefore conclude that the probing depth of the system is 1 mm for homogeneous media with optical properties comparable to the 1.5% Intralipid-0.5% dye phantom (i.e.  $\mu_s' \approx 2.3 \text{ mm}^{-1}$  and  $\mu_a \approx 0.4 \text{ mm}^{-1}$ ).

## 3.2 Patient Study

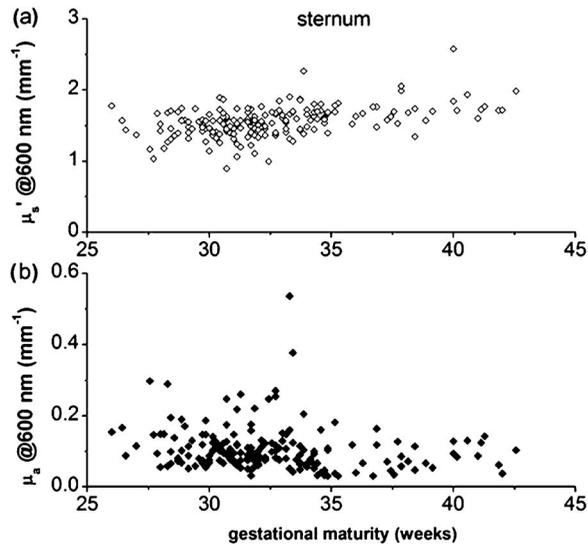
### 3.2.1 Dependency on location and age

The validity of the diffusion model [ $\mu_a/\mu_s' \leq \frac{3}{4}$  and  $(\mu_a + \mu_s') > 0.8 \text{ mm}^{-1}$ ] was checked for all patient measurements in the wavelength range of 450 to 600 nm, resulting in a reduction of the number of measurements and patients (Table 1 Measurements within validity limits). Figure 4 shows the 5th to 95th percentiles of the selected patient measurements in the wavelength range of 450 to 800 nm. The validity of the limit  $(\mu_a + \mu_s') > 0.8 \text{ mm}^{-1}$  is in general not valid for the longer wavelengths, hence our choice to only show the optical properties from 450 to 600 nm. Within the wavelength range of 450 to 600 nm, all patient measurements fall well within the validity limits of our analysis. The  $\mu_s'$  and  $\mu_a$  for all patients and measurements within the validity limits are shown in Fig. 5 for each location, regardless of skin type. The optical coefficients from this patient population were not normally distributed for all wavelengths; therefore, we present  $\mu_s'$  and  $\mu_a$  with the median, and 5th, 25th, 75th, and 95th percentile. The median value for the scatter power  $b$  in  $\mu_s'$  was  $-0.82$  (forehead),  $-0.87$  (sternum),  $-1.06$  (hand), and  $-1.34$  (foot). The multivariate analysis proved no significant differences ( $p > 0.05$ ) in the optical coefficients between locations for each patient.

The dependencies of  $\mu_s'$  and  $\mu_a$  at  $\lambda = 600 \text{ nm}$  on gestational maturity (gestational + postnatal age) are shown for the sternum in Fig. 6 for all patients and measurements within the validity limits, regardless of skin type. After random selection of one measurement event per patient, no to very little correlation can be observed between  $\mu_s'$  and gestational maturity (Spearman  $r^2 = 0.14$ ,  $p < 0.05$ ) and  $\mu_a$  and gestational maturity (Spearman  $r^2 = 0.12$ ,  $p < 0.05$ ). The same is true for the correlation



**Fig. 5** For (a) forehead, (b) sternum, (c) hand, and (d) foot: median values and 5th, 25th, 75th, and 95th percentiles for the measured  $\mu_s'$  and  $\mu_a$  versus wavelength for all patient measurements within the validity limits.

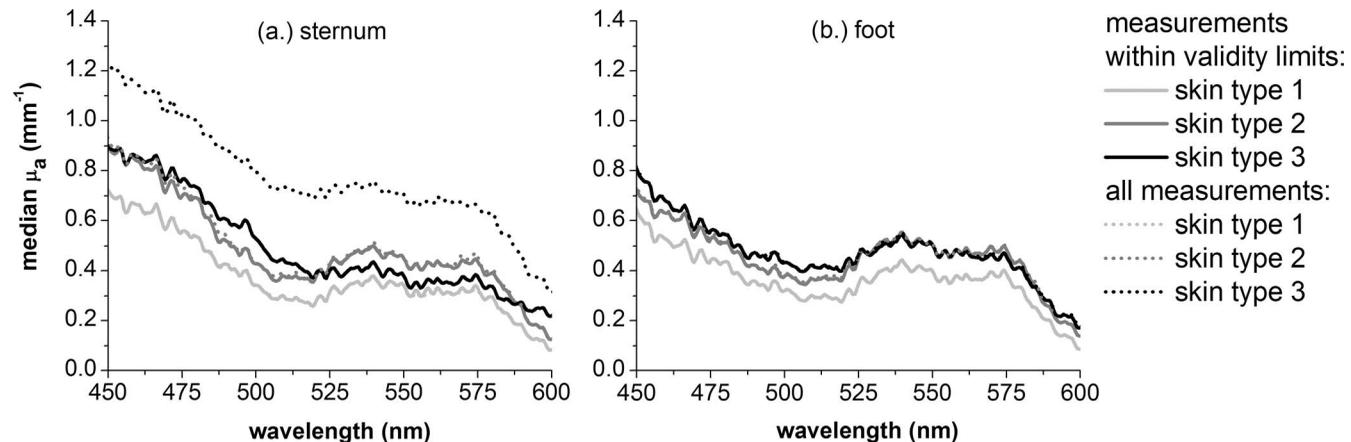


**Fig. 6** Measured values at  $\lambda = 600$  nm of (a)  $\mu_s'$  and (b)  $\mu_a$ , versus gestational maturity (gestational + postnatal age) at the sternum, for all patient measurements within the validity limits.

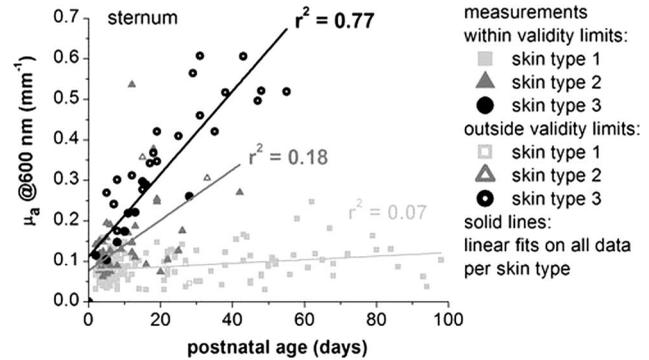
between  $\mu_s'$  and gestational maturity at the other locations [Spearman  $r^2$  of 0.22 (forehead), 0.11 (hand), and 0.29 (foot),  $p < 0.05$ ] and the correlation between  $\mu_a$  and gestational maturity [Spearman  $r^2$  of 0.06 (forehead), 0.01 (hand), and 0.10 (foot),  $p < 0.05$ ]. Similar results were obtained at other wavelengths.

### 3.2.2 Dependency on skin type

The median values of  $\mu_a$  versus wavelength are shown in Fig. 7 for each skin type at the sternum [Fig. 7(a)] and the foot [Fig. 7(b)]. Results for the forehead and the hand (no graph) were similar to those of the sternum. When only regarding the measurements that fall within the validity limits (solid lines, Table 1), the multivariate analysis proved no significant ( $p > 0.05$ ) influence of skin type on the measured optical properties, neither for  $\mu_s'$  nor for  $\mu_a$ . The latter is caused by the exclusion of the highest  $\mu_a$  values because of the  $\mu_a/\mu_s' \leq \frac{3}{4}$  validity limit, which is most abundant for patients with skin type



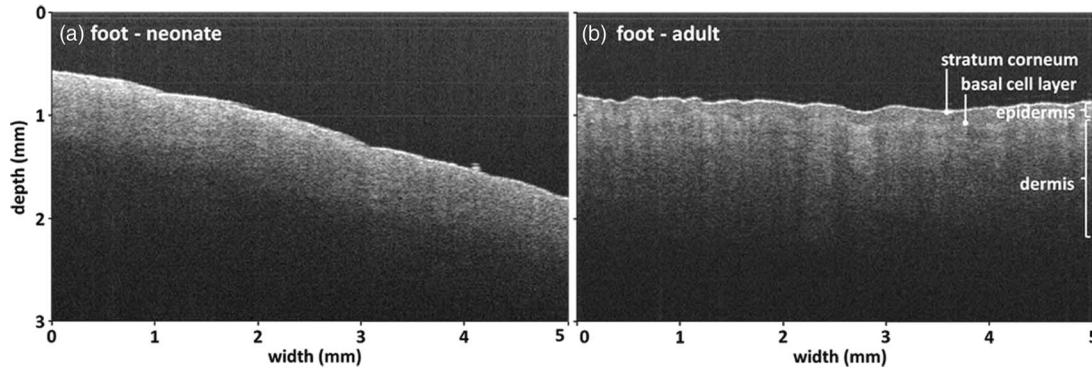
**Fig. 7** Median values of  $\mu_a$  versus wavelength at (a) the sternum (upper graphs) and (b) the foot (lower graphs) for all measurements (dashed lines, Table 1) and measurements within the validity limits (solid lines). Difference is made between skin types (type 1: Caucasian, type 2: Mediterranean, and type 3: Negroid).



**Fig. 8** Measured values for  $\mu_a$  at  $\lambda = 600$  nm versus postnatal age at the sternum, for all patient measurements within the validity limits (filled symbols) and outside the validity limits (open symbols). Difference is made between skin types (type 1: Caucasian, type 2: Mediterranean, and type 3: Negroid).

3 (Table 1). For the sternum, a larger difference in  $\mu_a$  can be observed between the three skin types, if we analyze the median values of  $\mu_a$  for all measurements (dotted lines, no elimination of measurements). This difference cannot be observed at the foot, which is likely to be due to the lack of melanin at this measurement site (plantar side of the foot). The results presented by the dotted lines in Fig. 7 need to be interpreted with caution, because our model of analysis is not valid for these measurements. The presented values for  $\mu_a$  are likely to be larger, considering the underestimation of  $\mu_a$  for values beyond the  $\mu_a/\mu_s' \leq \frac{3}{4}$  validity limit [Fig. 2(b)]. Hence, these results should be interpreted qualitatively (e.g., relative values, trends) rather than quantitatively.

The dependency of  $\mu_a$  at  $\lambda = 600$  nm on postnatal age is shown in Fig. 8 at the sternum for each skin type. Also, for these results, the difference is made between the measurements where the validity limits are valid (closed symbols), and not valid (open symbols). A (qualitative) analysis of all measurements per skin type, yields a high correlation between the  $\mu_a$  at 600 nm for skin type 3 and the postnatal age (Spearman  $r^2 = 0.77$ ,  $p < 0.05$ ). This can be ascribed to the stimulation of the production of melanin after birth.<sup>12</sup> The production of melanin for patients with skin



**Fig. 9** OCT scans (1310 nm) of the skin at the plantar side of the foot of a neonate (skin type 1, gestational age: 25 weeks, postnatal age: 56 days) and an adult (skin type 1, age: 25 years).

types 2 and 1 is lower, resulting in less or no correlation with postnatal age (Spearman  $r^2 = 0.18$  and  $0.07$ , respectively,  $p < 0.05$ ). Similar trends in  $\mu_a$  versus postnatal age were observed for the forehead and hand. The  $\mu_a$  of the foot for patients with skin type 3 did not depend as much on age (Spearman  $r^2 = 0.43$ ,  $p < 0.05$ ) as for the other locations, due to the lower melanin production at the measurement site (plantar side of the foot). Similar results were obtained at other wavelengths.

### 3.2.3 OCT images of neonatal skin

Figures 9(a) and 9(b) show typical OCT scans at 1310 nm of the skin at the foot of a patient (skin type 1, gestational age: 25 weeks, postnatal age: 56 days) and an adult (skin type 1, age: 25 years). No distinct layers can be observed in the skin of the neonate, which seems to extend beyond the imaging depth of approximately 1 mm. The skin of the adult shows more optical contrast between the individual skin layers and structures. Images at the sternum, forehead, and hand gave similar results, as well as images for other patients and adults (results not shown).

## 4 Discussion

In this article, we presented measurements of the optical properties of neonatal skin and assessed the influence of age and skin type on a large group of patients. This new knowledge on neonatal skin optical properties can aid in the development of optical techniques in neonatology. For instance, the measured values of  $\mu_a$  and  $\mu_s'$  can be used as input to Monte Carlo simulations of photon distributions inside the skin, which provide insight in, e.g., optimal design for optical diagnostic probes.

We used a spatially resolved, steady state diffuse reflectance spectroscopy setup and a steady state diffusion model to obtain the absorption and reduced scattering coefficient of the skin. Our method of analysis was validated in a phantom study (Sec. 3.1), by measuring  $\mu_s'$  and  $\mu_a$  of phantoms with known optical properties in the range of those of neonatal skin. We were able to measure  $\mu_a$  with an accuracy of 15% and  $\mu_s'$  with an accuracy of 10%, provided that the measurements were within the validity limits of the diffusion model ( $\mu_a/\mu_s' \leq \frac{3}{4}$  and  $(\mu_a + \mu_s') > 0.8 \text{ mm}^{-1}$ ). Other studies that analyze the spatially diffuse tissue reflectance with a diffusion model, typically allow more constrained validity limits,<sup>7,8</sup> thereby eliminating more measurements. Reasons for this difference may be the choice for larger uncertainty margins in the limits of validity and other

study design, such as measurement geometry and method of analysis. If, for instance, we do not apply the scatter power fit on the first outcome of the diffusion model fit to the measured reflectance and merely look at  $\mu_{a,1st}$  and  $\mu_{s',1st}$  (Sec. 2.3), the value of  $\beta$  will be considerably lower. This use of spectral constraints in diffusion model based analysis was investigated before by Kim et al.<sup>13</sup> for source-detector separations similar to ours, and was thought to be the cause of extended validity limits, comparable to the limits found in this study. Measuring optical properties at small source-detector separations ( $< 1 \text{ mm}$ ) is usually associated with nondiffuse photon distributions and requires adjustments with respect to common diffusion model based studies, either in measurement geometry<sup>14</sup> and/or method of analysis.<sup>14,15</sup> In this study, the diffusion model of Farrell et al. was modified to a semi-empirical model by correcting for the initial underestimation of the measured  $\mu_{s',2nd}$  [Fig. 2(a)]. This correction contributes to the validity of our analysis, as shown in our phantom study. We speculate that the initial underestimation of  $\mu_{s',2nd}$  may be caused by the back reflection of photons into the medium at the aluminum probe surface, which is not accounted for in the boundary conditions of the diffusion model.<sup>10</sup> This may result in a more diffuse photon distribution for the relatively short source-detector separations in our setup. Fortunately, the underestimation is consistent and therefore does not affect our determination of  $\mu_s'$ , since the measured  $\mu_s'$  agrees very well with the expected value for both Intralipid [Fig. 2(c)] and polystyrene spheres after correction.

By using the diffusion model to analyze the optical properties of neonatal skin from the patient measurements, we implicitly assume that the probed volume consists of skin only, and is semi-infinite and homogeneous. It is known from literature that (preterm) neonatal skin exhibits no to little distinct variations between individual skin layers<sup>16</sup> and is therefore expected to be optically more homogeneous than adult human skin.<sup>17</sup> This difference in homogeneity between neonatal and adult skin is clearly reflected in the OCT images of Figs. 9(a) and 9(b). The thickness of neonatal skin is not well reported in literature, but is expected to range between 0.65 and 1.5 mm.<sup>16,18</sup> Since the transition of the dermis into the subcutaneous tissue could not be observed in any of the OCT images of neonatal skin, we conclude that either no optical contrast exists between the dermis and the subcutaneous tissue (i.e., the optical coefficients are similar), or the skin thickness is larger than the imaging depth of the OCT system (approximately 1 mm). Therefore, we

conclude that we measure the optical properties of skin only, since the probing depth of our system was assessed to be  $\sim 1$  mm for a phantom comparable to neonatal skin (Sec. 3.1.2). Based on similar OCT images for other locations and patients (Sec. 3.2.3), we expect that our assumptions on homogeneity and semi-infinity are justified for all reported patient measurements. To further verify these assumptions, the optical properties would need to be measured at different depths in the tissue, which requires a small measurement volume from which the exact location inside the skin can be controlled. A potentially suited technique for this purpose is low-coherence spectroscopy (LCS),<sup>19,20</sup> although its performance *in vivo* has not yet been tested. LCS has the additional possibility of measuring the nonreduced scattering coefficient  $\mu_s$ , instead of the reduced scattering coefficient  $\mu_s'$  that is influenced by the scattering anisotropy  $g$  of the skin [ $\mu_s' = \mu_s(1 - g)$ ].<sup>20</sup>

The optical properties of the skin of 60 patients were measured at the forehead, sternum, hand, and foot. Comparison of the optical properties between the four measurement locations resulted in no significant differences. The spread of the optical properties is narrow for every location (Fig. 5), ranging maximally  $1.5 \text{ mm}^{-1}$  between the 5th and 95th percentile of all measurements. However, this spread may be skewed, since the selection of patient measurements based on the validity limits results in a reduction of measurements (Table 1). As a consequence, the lowest  $\mu_s'$  values and the highest  $\mu_a$  values are neglected in our presentation of the optical properties of neonatal skin (Figs. 5 and 6). Figure 7 shows that the presented median values for  $\mu_a$  of patients with skin type 3 should therefore be interpreted with caution, because  $\mu_a$  changes considerably if the validity limits are neglected. Again, we would like to emphasize that the optical properties outside the validity limits should receive qualitative, rather than quantitative interpretation. For patients with skin types 1 and 2, very little measurements are lost and the presented values of  $\mu_a$  can be trusted.

In line with the results of Saidi,<sup>4</sup> the measured absorption coefficient spectra seem to mainly contain the contribution of oxygenized and deoxygenized hemoglobin, bilirubin, and melanin. Laboratory analysis of sampled blood for a selection of patients yielded total hemoglobin levels in the range of 5 to 13 mmol/L and total serum bilirubin levels in the range of 40 to 400  $\mu\text{mol/L}$ . The concentration of hemoglobin and bilirubin in the investigated tissue volume is affected by vessel density and bilirubin extravasation, and is therefore expected to be lower than in whole blood. The magnitude of the measured  $\mu_a$  agrees well with the results of Saidi, who reported a range of approximately  $0.2$  to  $1.3 \text{ mm}^{-1}$  between 450 and 600 nm. More differences are found when comparing the measured values of  $\mu_s'$  between this study and Saidi et al.<sup>4,5</sup> The reported average values of  $\mu_s'$  by Saidi between 450 and 600 nm are considerably higher ( $\sim 2\text{--}4 \text{ mm}^{-1}$ )<sup>4</sup> than the median values of  $\mu_s'$  presented in this study (Fig. 5), and the spread of the data of Saidi is larger (standard deviation of  $\sim 3.6 \text{ mm}^{-1}$  at 450 nm). Another remarkable difference exists in the dependency of  $\mu_s'$  on gestational maturity. Whereas we found no to weak correlation between  $\mu_s'$  and gestational maturity [Fig. 6(a)], Saidi et al. found much stronger correlation, which they ascribed to an increase of collagen fiber density in the skin during maturation.<sup>5</sup> Although small differences exist between the investigated patient populations and skin areas (abdomen in the study of Saidi), and not all of these results were

published in peer reviewed journals,<sup>4</sup> it is most likely that the observed differences in  $\mu_s'$  are caused by unavoidable sample preparation procedures<sup>6</sup> for the *in vitro* tissue study of Saidi. However, it is expected that  $\mu_s'$  will increase at a certain age outside the investigated age range in this study, since  $\mu_s'$  is considerably higher for adult skin ( $3$  to  $9 \text{ mm}^{-1}$ , measured *in vitro* between 450 to 600 nm).<sup>21</sup>

The measured values of  $\mu_a$  for neonatal skin are comparable to adult skin ( $0.2$  to  $1 \text{ mm}^{-1}$  between 450 and 600 nm).<sup>21</sup> The absorption coefficient at 600 nm did not depend on gestational maturity, but did depend on postnatal age for patients with skin types 2 and 3 (Fig. 8). This can be ascribed to the stimulated production of melanin after birth.<sup>12</sup> It is not expected that the suggested linear relation in Fig. 8 will hold for all ages, because the increase of  $\mu_a$  will level off at a certain age, which is approximately 6 months for neonates with a dark skin type.<sup>12</sup> Since melanin is located in the epidermal layer of the skin, the skin will be an optically layered medium for pigmented skin areas in neonates with high postnatal age. The homogeneity shown in the OCT scans of Fig. 9 will therefore be lost. The reader should keep this in mind when using the presented optical properties, as well as the underestimation of  $\mu_a$  for patients with a dark skin type. An increase of absorption due to tanning of the skin has also been described for neonates (gestational age  $\geq 35.5$  weeks) with skin type 1, receiving photo therapy.<sup>22</sup> Although the majority of our patient population with skin type 1 received photo therapy during the measurement period, no increase of  $\mu_a$  at 600 nm was observed for these patients [Fig. 6(b)].

## 5 Conclusion

We measured *in vivo* the absorption and reduced scattering coefficients of neonatal skin for a large group of patients with varying gestational age, postnatal age, and skin pigmentation. The optical properties did not differ significantly between different locations on the body (forehead, sternum, hand, and foot). No to very weak correlation was observed between the optical properties and gestational maturity, but a strong dependency of the absorption coefficient on postnatal age existed for patients with skin type 3 (Negroid), which can be ascribed to the production of melanin after birth.

Knowledge of the optical properties of neonatal skin is invaluable when developing new, or improving existing optical diagnostic and therapeutic procedures that are used at the neonatal intensive care. We therefore hope that the results presented in this study can contribute to the future development and improvement of these techniques.

## Acknowledgments

We thank Astrid Newsum and Dagmar Ouweeneel for their help in performing the patient measurements. This research is funded by personal grants in the Vernieuwingsimpuls program (MCGA: AGT07547) by the Netherlands Organization of Scientific Research (NWO) and the Technology Foundation STW.

## References

1. K. Grohmann, M. Roser, B. Rolinski, I. Kadow, C. Müller, A. Goerlach-Graw, M. Nauck, and H. Küster, "Bilirubin measurement for neonates: comparison of 9 frequently used methods," *Pediatrics* **117**, 1174–1183 (2006).

2. J. W. Salyer, "Neonatal and pediatric pulse oximetry," *Respir. Care* **48**, 386–398 (2003).
3. M. Kaplan, V. Gold, C. Hammerman, A. Hochman, D. Goldschmidt, H. J. Vreman, and D. K. Stevenson, "Phototherapy and photo-oxidation in premature neonates," *Biol. Neonate* **87**, 44–50 (2005).
4. I. S. Saidi, "Optical properties of neonatal skin," *Light Transport in Neonatal Skin*, MS thesis, Rice University, Houston (1990).
5. I. S. Saidi, S. L. Jacques, and F. K. Tittel, "Mie and Rayleigh modeling of visible-light scattering in neonatal skin," *Appl. Opt.* **34**, 7410–7418 (1995).
6. R. Graaff, A. C. M. Dassel, M. H. Koelink, F. F. M. de Mul, J. G. Aarnoudse, and W. G. Zijlstra, "Optical properties of human dermis *in vitro* and *in vivo*," *Appl. Opt.* **32**, 435–447 (1993).
7. R. M. P. Doornbos, R. Lang, M. C. Aalders, F. W. Cross, and H. J. C. M. Sterenborg, "The determination of *in vivo* human tissue optical properties and absolute chromophore concentrations using spatially resolved steady-state diffuse reflectance spectroscopy," *Phys. Med. Biol.* **44**, 967–981 (1999).
8. M. G. Nichols, E. L. Hull, and T. H. Foster, "Design and testing of a white-light, steady-state diffuse reflectance spectrometer for determination of optical properties of highly scattering systems," *Appl. Opt.* **36**, 93–104 (1997).
9. D. Arifler, C. MacAulay, M. Follen, and R. Richards-Kortum, "Spatially resolved reflectance spectroscopy for diagnosis of cervical precancer: Monte Carlo modeling and comparison to clinical measurements," *J. Biomed. Opt.* **11**, 064027 (2006).
10. T. J. Farrell, M. S. Patterson, and B. Wilson, "A diffusion theory model of spatially resolved, steady-state diffuse reflectance for the noninvasive determination of tissue optical properties," *Med. Phys.* **19**, 879–888 (1992).
11. H. J. van Staveren, C. J. M. Moes, J. van Marle, S. A. Prahl, and M. J. C. van Gemert, "Light scattering in Intralipid-10% in the wavelength range of 400 to 1100 nm," *Appl. Opt.* **30**, 4507–4514 (1991).
12. R. J. Walsh, "Variation in the melanin content of the skin of New Guinea natives at different ages," *J. Invest. Dermatol.* **42**, 261–265 (1964).
13. A. Kim, R. Mathieu, F. Dadani, and B. C. Wilson, "A fiberoptic reflectance probe with multiple source-collector separations to increase the dynamic range of derived tissue optical absorption and scattering coefficients," *Opt. Express* **18**, 5580–5594 (2010).
14. S. Tseng, C. Hayakawa, J. Spanier, and A. J. Durkin, "Investigation of a probe design for facilitating the uses of the standard photon diffusion equation at short source-detector separations: Monte Carlo simulations," *J. Biomed. Opt.* **14**, 054043 (2009).
15. N. Rajaram, R. H. Nguyen, and J. W. Tunnell, "Lookup table-based inverse model for determining optical properties of turbid media," *J. Biomed. Opt. Lett.* **13**, 050501 (2008).
16. Y. B. Chiou and U. Blume-Peytavi, "Stratum corneum maturation – a review of neonatal skin function," *Skin Pharmacol. Physiol.* **17**, 57–66 (2004).
17. J. Welzel, "Optical coherence tomography in dermatology: a review," *Skin Res. Technol.* **7**, 1–9 (2001).
18. P. H. Hoeger, "Physiology of neonatal skin," Chapter 2 in *Textbook of Pediatric Dermatology*, J. Harper, A. Oranje, N. Prose, Eds., pp. 42–47, Blackwell Publishing, Oxford, United Kingdom (2006).
19. N. Bosschaart, M. C. G. Aalders, D. J. Faber, J. J. A. Weda, M. J. C. van Gemert, and T. G. van Leeuwen, "Quantitative measurements of absorption spectra in scattering media by low-coherence spectroscopy," *Opt. Lett.* **34**, 3746–3748 (2009).
20. N. Bosschaart, D. J. Faber, T. G. van Leeuwen, and M. C. G. Aalders, "Measurements of wavelength dependent scattering and backscattering coefficients by low-coherence spectroscopy," *J. Biomed. Opt. Lett.* **16**, 030503 (2011).
21. E. Salomatina, B. Jiang, J. Novak, and A. N. Yaroslavsky, "Optical properties of normal and cancerous human skin in the visible and near-infrared spectral range," *J. Biomed. Opt.* **11**, 064026 (2006).
22. L. L. Randeberg, E. B. Roll, L. T. N. Nilsen, T. Chrystensen, and L. O. Svaasand, "*In vivo* spectroscopy of jaundiced newborn skin reveals more than a bilirubin index," *Acta Paediatrica* **94**, 65–71 (2005).