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### Three dimensional modeling of bruise evolution for improved age determination

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# 6

## Reflectance spectroscopy of bruises measured in time

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### **Abstract**

Clinical applicability of spectroscopy combined with bruise modeling may be improved if bruises on the same anatomical position have similar values of model parameters.

We first investigate if a group of bruises located on the medial arm and measured once, at variable ages of presentation, show similar spectroscopic properties as a single bruise measured in time. Second, we investigate if spectroscopic properties vary between anatomical positions on the body.

We first found similar time behavior of groups of bruises measured once. Secondly, we found no spectral differences between medial and lateral arm bruises.

Therefore, a single set of bruise parameters measured on an anatomical position can also be used to model bruises on different subjects but on the same anatomical position. This finding would improve clinical applicability, as it can reduce the number of required measurements.

## Introduction

Recent advances in bruise research have shown that the combination of bruise modeling and spectroscopy is essential for accurate age determination [1-3]. Clinical applicability of this combination depends on several factors, such as the frequency of required measurements (preferably only one measurement is necessary). We previously speculated that knowledge of model parameters for specific anatomical positions would allow bruise characterization by a single measurement, and we showed that model parameters were similar in groups of medial arm bruise, lateral arm bruises and lateral leg bruises (Chapter 5). In this paper we investigate if spectroscopic properties and parameters derived from remittance spectra such as hemoglobin or bilirubin content, are also similar within groups of bruises.

We investigate spectroscopic properties (such as hemoglobin and bilirubin content) and their relation with the age of the bruise. First we compare the ratio of the concentrations of hemoglobin over bilirubin in time obtained from a single bruise followed in time to the ratio obtained from a group of several bruises of different ages, measured once, on the same anatomical position. Second we use this ratio to compare different anatomical positions; the medial arm, lateral arm and lateral leg bruises.

## Methods

### *Bruise database*

We previously described a large database of bruises that were (mostly) measured once (Chapter 5) (table 6.1).

Subjects		Male	Female
Number of subjects		124	144
Average Age		32.7 y (9-66 y)	32.6 y (9-80 y)
Fitzgerald skin type	1	5 (4.0%)	3 (2.3%)
	2	31 (25.0%)	37 (27.8%)
	3	39 (31.5%)	48 (36.1%)
	4	41 (33.1%)	26 (19.6%)
	5	2 (1.6%)	4 (3.0%)
	6	6 (4.8%)	15 (11.3%)
Use of alcohol at time of origin		37 (29.8%)	28 (22.6%)
Use of blood thinners		7 (4.86%)	6 (4.17%)
Bruises			
Number of bruises		180	354
Average age of bruise at measurement		4.0 d (0.17-35 d)	3.3 d (0.04-18 d)
Body location	Head	24 (13.3%)	15 (4.2%)
	Eye	61 (33.9%)	24 (6.8%)
	Arm	36 (20%)	104 (29.4%)
	Rump	15 (8.3%)	32 (9.0%)
	Leg	28 (15.6%)	134 (37.9%)
	Other	16 (8.9%)	45 (12.7%)

Table 6.1. Overview of all subjects and bruises

We here use that same database, and extract 3 groups of bruises. These are the same as in Chapter 5: the first group contains 30 medial arm bruises, the second contains 42 lateral arm bruises, and the third contains 51 lateral leg bruises, all on women. An overview of the 3 groups of selected bruises is given in table 6.2. We also use a *single* lateral arm bruise followed in time (described previously in [1] as bruise 1)

Group	Anatomical position	Number of bruises	Average age of bruise at measurement
1	Medial side of arm	30	3.3 d (0.7-13.6 d)
2	Lateral side of arm	42	4.6 d (1.3-13.6 d)
3	Lateral side of leg	51	4.4 d (0.5-18.1 d)

Table 6.2. Overview of the 3 groups of bruises used in the analyses: 30 medial arm bruises (first group), 42 lateral arm bruises (second group) and 51 lateral leg bruises (third group), all on women.

*Spectroscopic measurements and data analysis*

Optical reflection spectra were measured with a fiber based reflectance probe. A light source (Ava-Hal, Avantes) provided white light (430-950nm) to a fiber bundle of six 450 μm fibers (Ocean Optics), interoptode distance 600 μm. A seventh fiber, located in the

middle of the bundle, collected the remitted light from the bruise. The detection fiber was coupled into a spectrograph (USB4000 Ocean Optics), which was coupled into a computer to gather the data. The probe was surrounded by a plastic frame, to create a larger surface area to distribute the pressure with which the probe was placed on the bruise. Because spatial differences of hemoglobin and bilirubin content are present in bruises [1, 4], measurements were done in the center of the bruise and at different edges of the bruise. Three reference measurements on healthy skin surrounding the bruise were taken and averaged.

Spectroscopic analysis was performed using an in-house developed program in LabVIEW (National Instruments, Austin TX, USA) and is based on Lambert-Beer's law. We write the remitted signals from the bruise and healthy tissue as:

$$\begin{aligned} I(\lambda)_{bruise} &= I_0(\lambda) * e^{-(\mu_s + \mu_{a,bruise})d_{eff}} \\ I(\lambda)_{healthy} &= I_0(\lambda) * e^{-(\mu_s + \mu_{a,healthy})d_{eff}} \end{aligned} \quad (1)$$

Here we assume that the input intensity  $I_0$ , the scattering coefficient  $\mu_s$ , the effective path length  $d_{eff}$  and melanin contribution are equal for both the bruise and healthy tissue. All parameters in the exponent are wavelength-dependent (omitted for clarity). In this case, we can calculate the differential absorption from:

$$\ln\left(\frac{I(\lambda)_{healthy}}{I(\lambda)_{bruise}}\right) = (\mu_{a,healthy} - \mu_{a,bruise}) * d_{eff} = \Delta\mu_a * d_{eff} \quad (2)$$

The differential absorption contains contributions from bilirubin bound to albumin and free bilirubin (both only present in the bruise but not in healthy skin) [5-6], and differential absorption between bruise and healthy skin of oxygenated hemoglobin and deoxygenated hemoglobin:

$$\Delta\mu_a = \Delta\mu_{a,HbO_2} + \Delta\mu_{a,Hb} + \Delta\mu_{a,BiliBound} + \Delta\mu_{a,BiliFree} \quad (3)$$

Considering the molar absorption spectra  $\epsilon(\lambda)$  of these compounds ( $\text{mm}^{-1}/(\text{mol/L})$ ) and their concentration  $c$  ( $\text{mol/L}$ ) we can re-write equation 3 to:

$$\Delta\mu_a = \Delta c_{HbO_2} \epsilon_{HbO_2} + \Delta c_{Hb} \epsilon_{Hb} + \Delta c_{BiliBound} \epsilon_{BiliBound} + \Delta c_{BiliFree} \epsilon_{BiliFree} \quad (4)$$

Using a constrained General Least Squares (GLS) fitting algorithm, the measured  $\Delta\mu_a$ -spectra were fitted to the reference molar absorption spectra. The fit had 5 independent variables  $\alpha_1 \dots \alpha_5$ , where  $\alpha_1$  corresponds to  $\Delta c_{HbO_2} \times d_{eff}$ ;  $\alpha_2$  corresponds to  $\Delta c_{Hb} \times d_{eff}$ ;  $\alpha_3$  corresponds to  $c_{BiliBound} \times d_{eff}$ ;  $\alpha_4$  corresponds to  $c_{BiliFree} \times d_{eff}$ ; and  $\alpha_5$  is a constant offset. For each fit, the coefficients  $\alpha_1 \dots \alpha_4$  for the chromophores, their standard errors, mutual dependencies, 95% confidence intervals were calculated, as well as the goodness of fit ( $R^2$ ). After fitting, we combine the hemoglobin and bilirubin

## Chapter 6

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coefficients into two numbers:  $\alpha_{Hb} = \alpha_1 + \alpha_2$  and  $\alpha_{Bili} = \alpha_3 + \alpha_4$ . Using these coefficients to compare subsequent measurements will be difficult due to day to day variation from placement induced pressure differences [7], and temperature dependent differences in skin perfusion (less perfusion and thus lower concentrations can be expected at lower temperatures [8]). Therefore, for either the center or the edge location of the bruise, we take the ratio of the  $\alpha_{Hb}$  and  $\alpha_{Bili}$ , which is related to the physiological concentration as follows:

$$\frac{\alpha_{Hb}}{\alpha_{Bili}} = \frac{\Delta c_{Hb}}{c_{Bili}} \quad (5)$$

Taking this ratio will also cancel out any dependence on  $d_{eff}$  (under the current assumptions).

### *Comparison of a single bruise measured in time to multiple bruises measured once*

We use the single lateral arm bruise followed in time and evaluated the  $\alpha_{Hb}/\alpha_{Bili}$  versus the age of the bruise. We compared the results from this single arm bruise followed in time to the lateral arm bruises (group 2 from table 3), which we measured once at their variable bruise ages at presentation.

### *Comparison between anatomical positions*

We compare  $\alpha_{Hb}/\alpha_{Bili}$  for the 3 groups of bruises to search for differences between anatomical positions.

## Results

### *Comparison of a single bruise measured in time to multiple bruises measured once*

For the single lateral bruise measured on several days, a few of the spectra of the center location are shown in figure 6.1. This figure shows that the day to day variation in spectra is such that a clear relation with the age of the bruise cannot be seen.

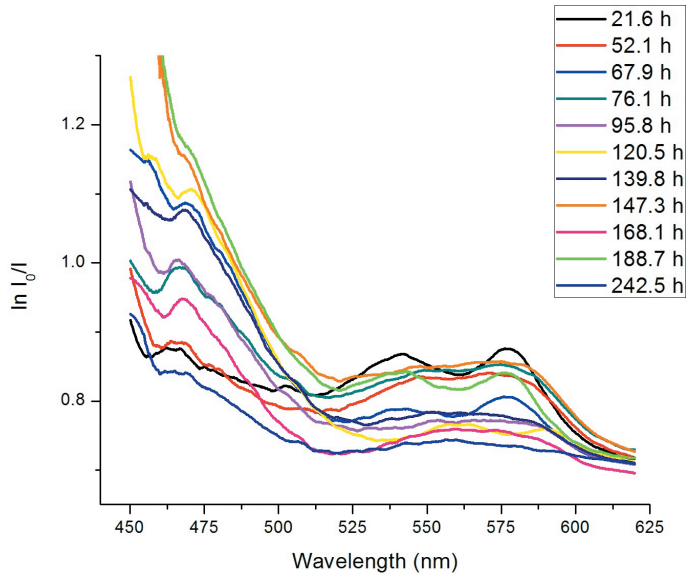


Figure 6.1. Spectra measured at the center location of a single bruise followed in time.

When we obtain the time dependent relation of the  $\alpha_{\text{Hb}}/\alpha_{\text{Bili}}$  ratio, we do see a clear relation with age, especially in the first 100 h (figure 2A, where fits with an  $R^2 < 0.7$  are excluded). No clear difference between the center and edge location is visible. Figure 6.2B shows the  $\alpha_{\text{Hb}}/\alpha_{\text{Bili}}$  ratio of the center and edge locations of the 42 lateral arm bruises of various ages and measured once (fits with an  $R^2 < 0.7$  are excluded). Interestingly, the relation of the  $\alpha_{\text{Hb}}/\alpha_{\text{Bili}}$  ratio as a function of the age of the bruise for this heterogeneous group of bruises all measured once is similar to the relation for a single bruise measured over time (figure 6.2B versus figure 6.2A respectively).

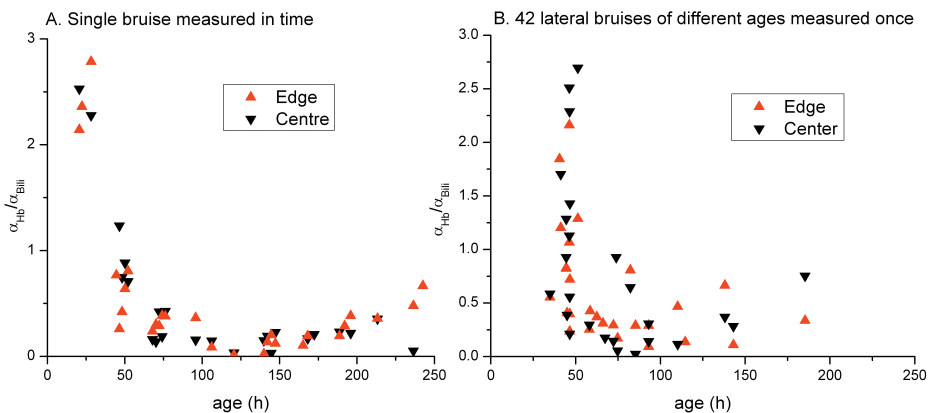


Figure 6.2 A. Ratio of hemoglobin coefficient over bilirubin coefficient over time for a single lateral bruise. B. Ratio of hemoglobin coefficient over bilirubin coefficient over time for 42 lateral arm bruises of variable ages.

*Comparison between anatomical positions*

Figure 6.3 shows the  $\alpha_{\text{Hb}}/\alpha_{\text{Bili}}$  ratios for the edge location of the 3 groups (center location gave same results, not shown). This figure shows that the temporal behavior of the  $\alpha_{\text{Hb}}/\alpha_{\text{Bili}}$  ratio for medial arm bruises, lateral arm bruises and lateral leg bruises is similar.

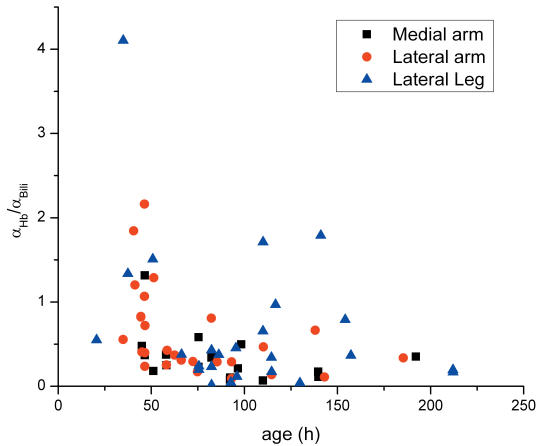


Figure 6.3. Ratio of hemoglobin coefficient over bilirubin coefficient over time for the edge location of the 3 groups of bruises of variable ages.

## Discussion

In this study we showed that bruises located on the same anatomical position have similar temporal behavior of spectroscopic properties. Together with our previous conclusion that model parameters are similar in bruises grouped by their anatomical position (Chapter 5), we can conclude that using the same set of parameters to model a bruise of unknown age can be performed using a set of parameters from other bruises on the same anatomical position. This finding would greatly improve the clinical applicability of age determination of bruises using spectroscopy and bruise modeling, as frequency of measurements can be reduced to a single measurement.

We previously hypothesized that a difference in diffusivity and other parameters may occur between the medial and lateral side of the arm, but rejected this hypothesis based on the group averages of the parameters (Chapter 5). Based on the temporal behavior of the spectroscopic properties of the two groups we investigated in this paper, this conclusion is reaffirmed.

Visually, a difference between hemoglobin and bilirubin concentrations in the center and edge is present. Spectroscopic measurements confirmed this difference for the fitted coefficients of hemoglobin and bilirubin; the determined concentrations were larger in the center than in the edge (not shown), however, the difference was not

observed in the  $\alpha_{\text{Hb}}/\alpha_{\text{Bili}}$  ratio of these fitted coefficients (figure 6.2). This  $\alpha_{\text{Hb}}/\alpha_{\text{Bili}}$  ratio can also be modeled using our 3D model. Our current method to determine the age of the bruise is based on matching simulated hemoglobin and bilirubin areas to measured hemoglobin and bilirubin areas [1]. Future research should focus on combining these ratios and the areas of hemoglobin and bilirubin in a method to determine the age of the bruise. This combination may reduce the frequency of measurements and thus increase the clinical applicability.

One advantage of using healthy skin measurements as a reference in the spectroscopic analysis is that the influence of skin color on the fit will be small, and therefore we did not distinguish between the different skin types (table 6.1). However, a higher concentration of melanin in the skin will cause a lower probing depth of the light, and future research may indicate that the thickness of the models top layer of the dermis should be adjusted for varying melanin concentrations.

### **Conclusion**

We have shown that bruises located on the same anatomical position have similar time evolution of spectroscopic properties. When we combine these findings with our previous conclusion that model parameters are similar in bruises grouped by their anatomical position (Chapter 5), we can state that using the same set of parameters to model a bruise of unknown age can be performed using a set of parameters from other bruises on the same anatomical position. Clinical applicability of age determination of bruises using spectroscopy and bruise modeling would greatly benefit from this finding, as frequency of measurements can be reduced to a single measurement.

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