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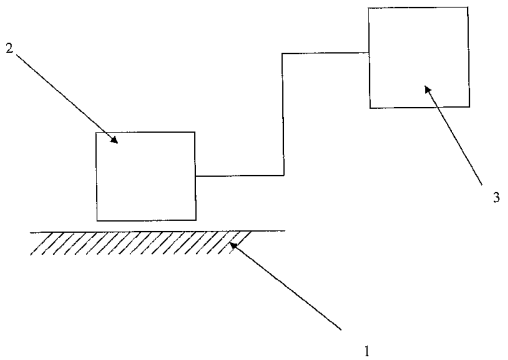
(54) **Title:** DETERMINING THE AGE OF SKIN BRUISES



(57) **Abstract:** A method of estimating the age of a haematoma involves measuring skin reflectance of both normal and bruised skin. A model is provided that relates haemoglobin and bilirubin content with time after injury, and is used to estimate the haemoglobin and bilirubin distributions as a function of depth, for different times after injury. A photon transport model is then used to simulate reflectance spectra corresponding to the estimated haemoglobin and bilirubin distributions at different times after injury. The photon transport model also takes various input parameters calculated from the measured spectra such as melanin content and blood oxygenation. The age of the haematoma is found by comparing the measured and simulated reflectance spectra and determining the time that gives the best fit. Such a method can be implemented using an integrating sphere (2) and a suitably programmed computer (3). The integrating sphere (2) is placed opposite the skin (1) and collects reflectance spectra of both normal and bruised skin. The computer (3) calculates the various parameters required for the photon transport model from the reflectance spectra. The computer (3) also estimates haemoglobin and bilirubin distributions at different times after injury. The computer (3) then simulates reflectance spectra at different times after injury using the photon transport model. Finally, the measured spectrum and simulated spectra are compared in order to estimate the age of the bruise.

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Determining the age of skin bruises

The present invention relates to determining the age of skin bruises based upon their reflectance spectra.

Determining the age of injuries on a victim's body is an important aspect of forensic medicine, for example in cases of abuse. Visual assessment is most commonly used to estimate the age of a bruise, as disclosed for example in "The aging of bruises; a review and study of the colour changes with time", Forensic Sci Int, 50:227-238, 1991, Langlois & Gresham. Bruises may be observed directly or photographs may be inspected. However, interpretation of the finding is purely empirical and relies strongly of the skills and experience of the observer, and might be disturbed by factors like e.g. ambient lighting or photograph quality. The uncertainty in the result is therefore comparatively large.

Several authors have discussed the problem of determining the age of bruises, for example "Estimation of the age of bruising", Arch Dis Child, 74(1): 53-55, 1996, Stephenson & Bialas. In this article a blind observer estimated the age of 50 bruises. The estimates were only correct for 24 of the bruises, with the estimates being wrong for 20 of the bruises. Six of the bruises could not be aged due to colours not previously reported in the literature. As such, it was concluded that the error rate using traditional methods, in terms of the proportion not within 24 hours of the correct age, can be about 50 %.

A bruise or skin haematoma is caused by blunt force, resulting in bleeding from subcutaneous vessels. (In the case of superficial bruises there may be bleeding from dermal vessels which gives a characteristic bright red appearance, but these are not considered here.) The subcutaneous vessels can continue to bleed some time after the impact, and the bruise develops during the first 24-48 hours. The immediate tissue response to traumatic injury involves an acute inflammatory reaction initiated by the trauma. This reaction causes recruitment of neutrophils and macrophages from the vascular system. Macrophages and neutrophils engulf both erythrocytes and free haemoglobin molecules and initiate the heme oxygenase system to break down the haemoglobin and produce biliverdin, bilirubin, and hemosiderin.

The visual appearance of skin is characterized by light backscattered from the epidermis and from dermis down to about 600-800 μm depth from the basal layer. Thus, the visibility of the haematoma is dependent on the haemoglobin and bilirubin densities at a depth typically less than about 0.6 mm. The bluish colour of a bruise is caused by extravascular haemoglobin diffusing into this region after subcutaneous vessel damage, whilst the subsequent production of bilirubin causes bruises to appear yellowish. Since the transport velocity in a diffusion process is strongly dependent on transport distance, the dermal thickness is a very important factor in the visibility development of bruises and is thus important when estimating the age of the bruise. Thus, larger skin thickness results in a slower developing blue colour, due to the larger diffusion length. For example, a subcutaneous bleeding in regions around the eye, where the skin is very thin, shows up fast whereas subcutaneous bleedings in the palm of the hand or under the sole of the foot, where the dermis is very thick, usually do not show up at all. The dermal thickness can therefore have an impact on the accuracy of the age of a bruise estimated from visual inspection.

The description of colour development in bruised areas differs considerably in the literature. As an example, in "The aging of bruises; a review and study of the colour changes with time" (referenced above), it is suggested that a yellow-ish colour can be observed after 18 hours, whereas in "How accurately can bruises be aged in abused children?", Pediatrics, 97(2):254-257, 1996, it is reported that yellow colour has been found between 7 days and 2 weeks after injury. Furthermore, the individual ability to perceive yellow colour declines with age, as disclosed in "The perception of yellow in bruises", J Clin Forensic Med., 2004 Oct; 11(5):257-9.

Thus, there is a need for an objective, easily manageable method to date bruises.

Reflectance spectroscopy has over recent years been developed as a useful tool for characterization of dermal lesions and discolorations such as, e.g. purpura after laser treatment of port-wine stains (flammeus naevus) and hypo- or hyperpigmentation, see for example "Tissue parameters determining the visual appearance of normal skin and port wine stains", Lasers Med Sci, 10:55-65, 1995, Svaasand et al. This technique has also proven valuable for evaluation of skin

bilirubin content for newborn with jaundice and measurements of dermal blood volume fraction and oxygenation.

In "Spectrophotometric evaluation of the colour of intra and subcutaneous bruises", Int J Legal Med, 113:343-348, 2000, Bohnert et al, reflectance spectroscopy was used to study the depth and colour of contusions post mortem. It was found that shallow fresh bruises appear bright red, and deeper injuries show a more bluish colour.

Reflectance spectroscopy of bruises in vivo has also been carried out, as disclosed, for example, in "The practical application of reflectance spectrophotometry for the demonstration of haemoglobin and its degradation in bruises", J Clin Pathol, 57(4):355-359, 2004, Hughes et al. In this paper, reflectance spectroscopy is used to analyse the presence of haemoglobin and haemoglobin degradation products in bruises. In "Optical classification of bruises", Proceedings of SPIE, vol 5312, p54-64, 2004, Randeberg et al, bruises are analysed using reflectance spectroscopy. Particular parameters that change with time are identified as being important in determining the age of a bruise, for example bilirubin density.

Thus, reflectance spectroscopy has been successfully used to characterize bruises and identify parameters which may help in estimating the age of a bruise. However, the prior art has failed to provide an objective analytical model from which the age of a skin bruise can be estimated.

Accordingly, in a first aspect, the present invention provides a method for estimating the age of a haematoma, comprising the steps of: obtaining a measured reflectance spectrum of a haematoma; simulating a plurality of reflectance spectra as a function of time using a model relating haemoglobin and/or bilirubin content with time after injury; and determining the age of the haematoma by comparing the measured and simulated reflectance spectra.

Thus, the haemoglobin and/or bilirubin contents corresponding to different times after the injury that caused the bruise may be estimated and used to predict the reflectance spectra that would be measured at those times at the site of injury. These can be compared with the measured spectrum and the age of the bruise estimated as the time giving the best fit between measured and simulated data. In one

embodiment the time is iterated and reflectance spectra simulated until a good fit is obtained between the measured and simulated data.

The model could be unitary and directly simulate the reflectance spectra. However it is highly preferred that the model comprises two components: the first component simulating the development of a bruise with time, and the second component simulating the reflectance spectra. The first component generally relates haemoglobin and/or bilirubin content with time, and preferably relates haemoglobin and/or bilirubin density (as defined below) with time. This enables haemoglobin and/or bilirubin densities to be estimated at different times after injury.

Generally, the second component comprises a photon transport model that simulates reflectance spectra using the estimated haemoglobin and/or bilirubin contents. Such models are known and a preferred model is described in more detail below.

Consequently, viewed from another aspect, the invention provides a method for estimating the age of a haematoma, comprising the steps of: obtaining a measured reflectance spectrum of a haematoma; using a model relating haemoglobin and/or bilirubin density with time after injury to estimate the haemoglobin and/or bilirubin density in the bruise at different times after injury; simulating the reflectance spectra expected at different times after injury using the estimated haemoglobin and/or bilirubin densities; and determining the age of the haematoma by comparing the measured and simulated reflectance spectra.

The term 'haematoma' used throughout the specification is to be understood to include a skin bruise. The term "density" as used above and elsewhere means the quantity in terms of moles/m^3, or some term proportional to it (it will be appreciated that since this is dealing with fluids the density is constant, and thus the density is proportional to the volume fraction).

Thus, according to the present invention, the age of a bruise can be objectively determined using a model.

The invention also extends to an apparatus for carrying out the method and so, viewed from a further aspect, the invention provides an apparatus for estimating the age of a haematoma, comprising a means for inputting a measured reflectance spectrum of a haematoma; a means for simulating a plurality of reflectance spectra

as a function of time using a model relating haemoglobin and/or bilirubin content with time after injury; and a means for determining the age of the haematoma by comparing the measured and simulated reflectance spectra.

The reflectance spectrum of the haematoma can be measured in any known way. For example, a fibre probe or an integrating sphere arrangement may be used with a spectrophotometer. The reflectance spectrum may be measured several times and an average value obtained. As such, the apparatus may also further comprise a spectrometer or other device for obtaining the measured spectra. The means for simulating and determining preferably comprise data processing apparatus such as a suitably programmed computer.

It is envisaged that the apparatus may comprise a hand-held apparatus that can be used conveniently by a clinician or scientist. Such a device may carry out all of the features of the method of the invention, or it may be in communication with a remote unit that performs data processing functions.

The model relating haemoglobin and/or bilirubin contents with time is preferably a mathematical model developed from first principles. Such a model can describe the development of a bruise in an analytical manner. In one preferred embodiment, the model relates only haemoglobin content with time. However it is particularly preferred that the model relates both haemoglobin and bilirubin with time since this should provide a more accurate age estimate. In order to develop such a preferred mathematical model, the inventors have considered the physics and biochemistry behind the changing skin appearance of a bruise.

The development of the colour of a bruise can be analysed in terms of haemoglobin and its breakdown products. Haemoglobin is a major chromophore in blood, and bruises are coloured initially by haemoglobin and secondarily by haemoglobin breakdown products such as bilirubin. A trauma causing localized vessel damage may rapidly result in a pool of blood in subcutaneous tissues, driven by arterial/venous pressure, which is subsequently transported deeper into the tissue. Haemoglobin will be transported into the dermis by diffusion of whole and haemolysed blood from the subcutaneous layer. This will be followed by clearance by lymphatic flow, macrophage activity or conversion to breakdown products such

as biliverdin and bilirubin (see Figure 13 for a schematic representation of this process).

In order to create a suitable model relating haemoglobin and/or bilirubin content with time, the above processes can be modelled analytically. Figure 14 schematically illustrates the processes it is preferred to model. Preferably this is done using Darcy's law of convection (which describes liquid flow in percolative media) and Fick's law of diffusion, as detailed in the Appendix. As such, this model may be considered a 'diffusion model', and is occasionally referred to as such below.

It has been found that a useful model need only take into account the diffusion along the vertical axis (i.e. the axis into the bruise), but a more general model can be used if desired. In addition, it is preferred to consider the tissue in which the bruise is formed in terms of a finite number of discrete layers. The preferred forms of the invention below use a two-layer model, although three, four five or more layers may be used if desired and this may improve accuracy. The number of layers used in the diffusion model is a separate issue to the number of layers used in the photon transport model.

The model relating haemoglobin and/or bilirubin content with time preferably comprises an equation relating the spatial distribution of haemoglobin content, in particular the density, with time. A suitable equation giving the density of haemoglobin as a function of depth and time is:

$$N_H(x,t) = N_{H0}\{\sum_{n=0}^{\infty}[(-1)^n erfc(\frac{1}{2}\frac{(2n+1)d-x}{\sqrt{D_H t}}) + (-1)^n erfc(\frac{1}{2}\frac{(2n+1)d+x}{\sqrt{D_H t}})]\}e^{-\frac{t}{\tau_H}}$$

(Equation 15 from the Appendix)

where:

N_{H0} is the initial subcutaneous density (i.e. mol/unit volume) of haemoglobin,

x = depth, i.e. distance from the epidermal/dermal junction (basal layer)

t = time after introduction of the bruise,

d = dermal thickness,

n= counting variable

τ_H = haemoglobin relaxation time, i.e. time required for reduction of the density to 1/e, and

D_H = haemoglobin diffusivity.

For short times after induction of the bruise, the first terms in this sum provide an adequate approximation, and the expression can be simplified as:

$$N_H(x,t) = N_{H0}\{erfc(\frac{1}{2}\frac{d-x}{\sqrt{D_H t}}) + erfc(\frac{1}{2}\frac{d+x}{\sqrt{D_H t}})\}e^{-\frac{t}{\tau_H}}$$

(Equation 17 from the Appendix)

The model also preferably comprises an equation relating the spatial distribution of bilirubin content, in particular the density, with time. A suitable equation giving the density of bilirubin as a function of depth and time is:

$$N_B(x,t) = \int_{\tau=0}^{t}\int_{\xi=0}^{d} N_H(\xi,\tau)\frac{(e^{-\frac{(x-\xi)^2}{4D_B(t-\tau)}} + e^{-\frac{(x+\xi)^2}{4D_B(t-\tau)}})e^{-\frac{t-\tau}{\tau_{BR}}}}{2\tau_B\sqrt{\pi D_B(t-\tau)}}d\xi d\tau$$

(Equation 19 from the Appendix)

Where:

τ_B = haemoglobin to bilirubin relaxation time, i.e. time required for converting 1/e = 0.37 of the haemoglobin molecules to bilirubin,

τ_{BR} = bilirubin relaxation time, i.e. the time required for reduction of the bilirubin density to 1/e = 0.37,

D_B = bilirubin diffusivity,

ξ = distance integration variable, and

τ = time integration variable.

When diffusion of bilirubin can be neglected this expression reduces to:

$$N_B(x,t) = e^{-\frac{t}{\tau_{BR}}}\int_{\tau=0}^{t}\frac{N_H(x,\tau)e^{\frac{\tau}{\tau_{BR}}}}{\tau_B}d\tau$$

(Equation 21 from the Appendix)

The equation giving the spatial distribution of haemoglobin density as a function of time may more generally give the density in terms of the subcutaneous density of the haemoglobin, the dermal thickness, the haemoglobin diffusivity and the haemoglobin relaxation time.

The equation giving the spatial distribution of bilirubin density as a function of time may more generally give the density in terms of the haemoglobin distribution, the bilirubin relaxation time, the generation of bilirubin relaxation time and the dermal thickness.

The model relating haemoglobin and/or bilirubin density with time may alternatively comprise an equation relating the average density of haemoglobin and an equation relating the average density of bilirubin with time. In this approach, in both cases the density is averaged over the entire dermis. However, this can be extended to multiple layers with a different value for each layer. It should also be appreciated that the number of layers used in the model of the bruise need not be the same as used in the photon transport model. Thus, the bruise can be modelled first, and then the result may be divided into further layers in order to consider the optical properties.

A suitable equation giving the average density of haemoglobin as a function of time, which is valid for short times after the injury, is

$$\bar{N}_H(t) = \frac{2N_{H0}}{d}\sqrt{\frac{D_H t}{\pi}}e^{-\frac{t}{\tau_H}}$$

(Equation 23 from the Appendix)

A suitable equation giving the average density of bilirubin is:

$$\bar{N}_B(t) = \frac{N_{H0}\tau_{BR}\tau_H}{\tau_B(\tau_H - \tau_{BR})d}\{2\sqrt{\frac{D_H t}{\pi}}e^{-\frac{t}{\tau_H}} - erf(\sqrt{\frac{(\tau_H - \tau_{BR})t}{\tau_{BR}\tau_H}})\sqrt{\frac{D_H\tau_{BR}\tau_H}{\tau_H - \tau_{BR}}}e^{-\frac{t}{\tau_{BR}}}\}$$

(Equation 24 from the Appendix)

It is particularly preferred that the model relating haemoglobin and/or bilirubin density with time takes into account the thickness of the dermis. This improves accuracy because, as mentioned previously, the diffusion velocity of haemoglobin/bilirubin (and thus the density) depends strongly on dermal thickness. Dermal thickness is different at different parts of the body, and varies on an individual basis depending for example on age, gender and smoking habit. As such,

the accuracy of the model will depend on the accuracy of the measurement of the dermal thickness.

Dermal thickness can be estimated, but is more preferably measured by any suitable method, for example high frequency ultrasound. It may also be measured using optical coherence tomography (OCT). Preferably, the skin thickness is measured concurrently with the reflection measurement.

As mentioned above, simulated reflectance spectra are generally calculated using a photon transport model. This may also be called a reflectance model. A suitable analytic photon transport model based on the diffusion approximation is the three layer model disclosed in "Tissue parameters determining the visual appearance of normal skin and port wine stains", Lasers med sci 10 (1): 55-65 MAR 1995, Svaasand et al.

This model is based on the optical diffusion approximation and describes two dermal layers on a region of semi-infinite extent. The first layer simulates the melanin containing epidermis, the second layer represents the upper part of dermis, and the last semi-infinite region represents the deeper parts of the dermis and subcutaneous adipose tissue. This simple model is adequate because reflected light from skin is constituted primarily of light backscattered from regions down to a depth of typically 0.6-0.8mm, whereas reflected light from deep dermal regions is quite negligible. Intravascular haemoglobin (occasionally referred to below as the background haemoglobin), which is the predominant optical absorber in dermis, is modelled as a uniformly distributed chromophore within each of the dermal layers. The other chromophores are also assumed to be uniformly distributed within each layer.

In order to simulate the reflectance spectra of bruised skin, this photon transport model estimates light absorbed by the bruised skin. This is preferably a combination of both the background absorption (i.e. the absorption of normal skin) and the absorption caused by the additional chromophores in the bruise. The preferred form of photon transport model models normal skin and then adds the additional blood volume fraction (from the haemoglobin) and haemoglobin breakdown products (such as bilirubin) to the dermal layers. The densities of haemoglobin and bilirubin are most preferably calculated using equations 17 and 21

respectively. (The blood volume fraction includes only the haemoglobin, the other chromophores have their own volume fractions in the optical model.)

As such, the preferred photon transport model uses information about both the normal skin and the bruise itself and thus preferably reflectance spectra should be measured for normal skin as well as bruised skin.

The preferred parameters used by the photon transport model for normal skin are: melanin content, dermal blood volume fraction (i.e. the haemoglobin) and blood oxygenation. These parameters are preferably determined using the reflectance spectra for normal skin as follows.

The melanin content may be determined by calculating the melanin index of the measured spectrum and of a simulated spectrum (see 'A theoretical and experimental study of light absorption and scattering by in vivo skin', Dawson et al., Phys Med Biol 1980;25:695-709). The melanin content in the simulated spectrum is iterated until the melanin indices of the two spectra substantially match.

The blood volume fraction may be estimated in a corresponding manner by calculating the erythema index (a measure of redness), and iterating the blood volume (also see 'A theoretical and experimental study of light absorption and scattering by in vivo skin', as above). All the parameters are fitted one by one since the wavelength bands used to determine the parameters overlap. Preferably, the initial value of the blood volume is taken to be 1% (or approximately such) in both dermal layers.

From the melanin (pigmentation) content (i.e. the amount of melanin in the skin) and the known physical properties of melanin, the melanin absorption can be calculated using the model given by Svaasand et al in the above-mentioned paper "Tissue parameters determining the visual appearance of normal skin and port wine stains", and in "Characterisation of layered tissue structures with diffusely propagating photon density waves", Spott. T., Doctoral thesis, NTNU, Trondheim, Norway, 1999. Preferably, the initial melanin absorption is (at least approximately) 500 m^{-1} at 694 nm.

Blood oxygenation can be determined from the ratio of the blood absorption at an isosbestic point and a nonisosbestic point ('Wave propagation and scattering in random media, volume 1', Ishimaru, New York Academic Press, 1978). The

equation was modified by Spott et al. ('Application of optical diffusion theory to transcutaneous bilirubinometry', SPIE Europto Series 1997; 3195:234-245) to calculate the oxygenation from the derived dermal absorption coefficient:

$$OS = \frac{\mu_{Hb}(\lambda_1) - \mu_{HbO2}(\lambda_2) \cdot \frac{\mu_a(\lambda_1)}{\mu_a(\lambda_2)}}{\mu_{Hb}(\lambda_1) - \mu_{HbO2}(\lambda_1)}$$

where:

OS is the oxygen saturation in percent,

μ_a is the measured dermal absorption coefficient,

μ_{Hb} and μ_{HbO2} are the specific absorption coefficients of deoxygenated and oxygenated blood, respectively; and

λ_1 and λ_2 are a nonisosbestic and an isosbestic point, respectively.

In order to estimate blood oxygenation directly from the reflectance spectrum, the measured absorption coefficients can be substituted with 1/R1 and 1/R2, where R1 and R2 are the reflectance at λ_1 and λ_2.

By choosing wavelengths in the yellow and red part of the spectrum the average oxygenation down to about 600 μm can be determined. By choosing wavelengths in the blue/green range the oxygenation value computed will represent the oxygenation in the more superficial part of the skin due to the short penetration depth in this wavelength region. In one preferred embodiment, the chosen wavelengths are λ_1/λ_2 = 660nm/805nm (yellow/red) and λ_1/λ_2 = 542nm/548nm (blue/green). This is useful since in the preferred photon transport model the oxygenation for each of the dermal layers is needed. However, the blood oxygenation for these layers in normal skin will be relatively low; it is more important to obtain a good estimate of the oxygenation due to the haemorrhage (extra-vascular) as discussed below.

As mentioned above, the preferred photon transport model also requires certain parameters for bruised skin. These may be selected from melanin content, blood oxygenation, dermal blood volume fraction, blood volume fraction due to the haemorrhage (i.e. the haemoglobin) and the bilirubin volume fraction. The dermal

blood volume fraction is the total blood volume in the skin, including both the 'background' (i.e. that present in normal skin) and the blood volume fraction due to the haemorrhage. Generally however, the 'background' is small (e.g. 1%) in comparison with the bruise (e.g. 35%) and thus the total dermal blood volume fraction is almost equal to the volume fraction due to the haemorrhage for most bruises.

The melanin content can be found in the same manner as for normal skin, but using the reflectance spectrum of the bruise instead of that for normal skin. However, it can be adequate to assume that the melanin content for bruised skin is the same as that for normal skin.

The blood oxygenation can be determined from the equation above for OS. The measured absorption coefficient is found from the reflectance spectrum of the bruise by using the photon transport model inversely after fitting the input parameters (melanin, oxygenation and dermal blood volume) as described above.

The dermal blood volume fraction can be found in the same manner as for normal skin, but using the reflectance spectrum of the bruise instead of that for normal skin.

These values of blood oxygenation and dermal blood volume fraction are a combination of the intra- and extra-vascular blood contributions (i.e. the 'background' and 'bruise' contributions), and can be used as a starting point in the iteration to fit the extravascular oxygenation.

The blood volume fraction due to the haemorrhage and the bilirubin volume fraction are found from the haemoglobin and/or bilirubin densities calculated using the model of the invention. It is the time dependence of these quantities that enables spectra to be simulated for different times after injury. As such, the haemoglobin and/or bilirubin densities need to be calculated for different values of time after injury.

In the preferred photon transport model the blood volume fraction (haemoglobin density) and bilirubin density is required for both the upper dermis and deeper dermis layers. Preferably, the haemoglobin density is calculated separately for each dermal layer and the result is averaged over the thickness of the respective layer. This can be done by averaging equation 15 or 17 over each layer.

Similarly, the bilirubin density in the layers is preferably determined by averaging equation 20 or 21 over each layer.

Alternatively, the haemoglobin and/or bilirubin densities can be found as an average over the entire dermis from, for example, equations 23 and 22. These averages could then be used for both layers. This method is however less preferred since the density varies continuously over the depth and thus an average value over the whole dermis would not be representative for, e.g., the upper dermis.

In order to increase the accuracy of the photon transport model, the number of layers could be increased. However this is at the cost of processing time since it would then be necessary to calculate haemoglobin and/or bilirubin densities for more layers.

The volume fraction of blood can be found from the haemoglobin density using the following equation:

$$v = \frac{N_H}{N_{Hblood}}$$

Where: N_H = density of haemoglobin as calculated by model

N_{Hblood} = density of haemoglobin in whole blood

The normal concentration of haemoglobin in blood is 150g/litre and the molecular weight in grams is 64500. Thus, the density of haemoglobin in whole blood, $N_{Hblood} = (150/64500) \times 10^3$ mol/m^3 (see http://omlc.ogi.edu/spectra/hemoglobin/index.html 'Prahl S., Optical Absorption of Haemoglobin'). There is a separate and analogous equation for bilirubin.

The initial subcutaneous density of haemoglobin N_{H0} essentially corresponds to the initial volume fraction of the blood pool, v_0. This can be estimated from the colour and extent of the bruise.

A measured value for the volume fraction of blood due to the haemorrhage can be obtained from the reflectance spectra by calculating the dermal blood volume fraction for the bruise and normal skin using the photon transport model (as discussed above). The difference between the two values will be the contribution from the bruise. This can be refined through a process of iteration. This value is then preferably compared to the blood volume fraction simulated by the model to

check whether the simulation is reasonable. This feedback is important as it enables the simulation calculations to be iteratively adjusted.

Skin thickness is a required parameter for both the photon transport model, and when estimating the haemoglobin and/or bilirubin densities. In the latter case, if, as is preferred, the haemoglobin and/or bilirubin is averaged over the thickness of each of the two dermal layers, the thickness of each separate layer is needed. Preferably, the thickness of the upper dermal layer is assumed to be 20% of the total skin thickness. The skin thickness can be assumed, but more preferably is measured using, for example, high frequency ultrasound as mentioned previously.

The photon transport model preferably uses the haemoglobin absorption coefficient and the light scattering properties within the skin. The haemoglobin absorption coefficient can be based on spectra given in "Visible and near infrared absorption spectra of human and animal haemoglobin determination and application", W.G. Zijlstra et al, VSP, Zeist, The Netherlands, 2000. The scattering properties may be assumed to be known and can be modelled using data from "Mie and Rayleigh modelling of visible-light scattering in neonatal skin", Saidi et al, Appl Opt 1995; 34:7410-7418, scaled to fit adult skin: "In vivo spectroscopy of newborn skin reveals more than a bilirubin index", Randeberg et al, Acta Paediatrica 2005;94:65-71.

As discussed above, the preferred photon transport model models the skin as having three different layers. From the preferred input parameters as discussed above, preferably, different parameters will be used in modelling each layer. The parameters for the different layers may be found in different ways and have different values. A summary of the parameters used in a preferred embodiment are given in Figure 15. In this embodiment, it can be seen for example that melanin is only used in modelling the epidermis, and that the same value is used for both normal and bruised skin. It can also be seen that the blood volume fraction due to the haemorrhage is used in modelling the upper dermis and deeper dermis, but not the epidermis. This is because blood due to the haemorrhage does not generally diffuse into the epidermis.

Once the parameters required by the photon transport model have been determined, the photon transport model can be used to simulate spectra for different

values of time (i.e. using the haemoglobin and/or bilirubin densities calculated at different times), in a manner known in the art.

Another approach to the optical model is to use the Monte Carlo approach. This is a numerical technique where statistical probabilities are used to consider the paths taken by photons. Other numerical techniques, such as finite elements can also be used.

Once the spectra have been simulated, the measured spectrum can then be compared with each of the reflectance spectra simulated for different values of time. The age of the haematoma can be determined as the time value that gives the simulated spectrum that most closely fits the measured spectrum. This can be found, for example, by numerical analysis or curve fitting. Therefore the value of time is essentially iterated until a 'best fit' is achieved between simulated and measured spectra. In order to reduce processing time, instead of comparing the complete measured spectrum with a complete simulated spectrum, only one or more parts of the measured and simulated spectra may be compared. If no fit can be achieved at all, then it is preferable to vary the estimate of skin thickness in order to try to obtain a fit.

According to a further aspect, the present invention provides a method for estimating the age of a haematoma, comprising the steps of: performing reflectance spectroscopy on both a haematoma and normal skin in order to obtain measured reflectance spectra; calculating the blood volume fraction, blood oxygenation and melanin content for normal and/or bruised skin from the measured reflectance spectra; estimating the haemoglobin and/or bilirubin density as a function of time after injury using a mathematical model; inputting the blood volume fraction, blood oxygenation and melanin content of normal and/or bruised skin and the haemoglobin and/or bilirubin density into a photon transport model; obtaining simulated reflectance spectra for different values of time after injury from the photon transport model; comparing the measured spectrum with each of the simulated reflectance spectra; and determining the age of the haematoma as the time value that gives the simulated spectrum that most closely fits the measured spectrum.

Instead of determining the age of a haematoma by comparing simulated reflectance spectra with a measured spectrum, the age can also be determined by evaluating the content of haemoglobin/bilirubin in the bruise from the measured spectrum. Thus, according to yet another aspect, the present invention provides a method for estimating the age of a haematoma, comprising the steps of: performing reflectance spectroscopy on a haematoma in order to obtain a dataset of measured reflectance spectra; determining haemoglobin and/or bilirubin content in the haematoma from the reflectance spectra; and estimating the age of the haematoma using a mathematical model relating the haemoglobin and/or bilirubin content with time after injury.

The invention also provides a method for estimating the age of a haematoma, comprising the steps of: performing reflectance spectroscopy on a haematoma in order to obtain a measured reflectance spectrum; estimating the density of haemoglobin and/or bilirubin in the haematoma from the reflectance spectrum; and estimating the age of the haematoma using a model relating the density of haemoglobin and/or bilirubin with time after injury.

The model is preferably a mathematical one based on first principles in line with the discussion above. Thus, the spatial distribution of haemoglobin and bilirubin in the bruise can be determined from reflectance spectroscopy combined with a mathematical modelling of light distribution in the dermis (e.g. a photon transport model). The haemoglobin and/or bilirubin in the bruise at different times after injury can be estimated from the model and these compared with the actual haemoglobin/bilirubin found in the bruise. The age is the time giving the best fit. Thus, the combination of modelling the light distribution in the dermis and modelling the spatial distribution of the chromophores versus time after injury enables a quantitative determination of the age of the bruise.

Alternatively, the model relating haemoglobin and/or bilirubin distribution with time may enable the age to be directly calculated from haemoglobin/bilirubin measurements. Thus the haemoglobin/bilirubin distributions estimated from the spectra can be input into the model to directly determine age.

The invention also provides a method for estimating the age of a haematoma, comprising the steps of : performing reflectance spectroscopy on a haematoma in

order to obtain a dataset of measured reflectance spectra; estimating the value of a time varying physical quantity of the haematoma from the reflectance spectra; and estimating the age of the haematoma from a mathematical model relating the physical quantity with time after injury.

As described above, the age of a haematoma can be determined either using a comparison of simulated spectra with a measured spectrum, or by finding the density of haemoglobin/bilirubin from the measured spectrum.

Therefore, according to another aspect, the invention more broadly provides a method for estimating the age of a haematoma, comprising the steps of : performing reflectance spectroscopy on a haematoma in order to obtain a measured reflectance spectrum; and estimating the age of the haematoma using the measured reflectance spectrum and a model relating a time varying physical quantity of the haematoma with time after injury.

The time-varying quantity is preferably haemoglobin density and/or bilirubin density.

The invention also extends to apparatus arranged to carry out any of the above methods.

The inventors have recognised that very localized damage, such as e.g. caused by a sharp stone or needle, will often result in very localized damage to the blood vessels. A local inflammatory action is initiated by the trauma and causes recruitment of neutrophils and macrophages from the vascular system. The macrophage activity is enhanced in the central zone of the wound where the damage is more extensive and the biological response is strong. The inventors have observed that this often results in a whitish spot in the dermal region just above the damaged subcutaneous vessel. Therefore, if a substantially white area is determined to be present towards the centre of the bruise, it can be concluded that strong localised damage has occurred. By analysing the shape of the whitish area, the shape/geometry of the object that caused the bruise can be evaluated. For example, damage to a vessel by puncturing it with a needle will result in a centrally whitish spot, whereas e.g. a blow from a cane results in a whitish central line along the line of damage.

Therefore, preferably, the methods of determining the age of a skin bruise as described above further comprise the step of analyzing the reflectance spectra to determine if a substantially white area is present towards the centre of the bruise, in order to assist in determining the object that caused the bruise.

Furthermore, this effect of macrophages depleting the haemoglobin distribution can be included in the model relating haemoglobin and/or bilirubin density with time. For example, when deriving the preferred equations as given in the appendix, the time constants can be adjusted according to the higher macrophage activity and thereby higher haemoglobin turnover. The macrophage activity can be characterized as a contribution to the haemoglobin relaxation time:

$$\frac{1}{\tau_H} = \frac{1}{\tau_L} + \frac{1}{\tau_B} + \frac{1}{\tau_M} + ...$$

Where τ_L, τ_B are the relaxation times due to lymphatic drainage and conversion to bilirubin, respectively. The macrophage activity as characterized by τ_M is the time required to reduce the haemoglobin density to 1/e = 0.37 by macrophage action alone.

Following on from the above, another aspect of the present invention provides a method of determining the nature of an object that has caused a bruise, comprising determining if a substantially white area is present towards the centre of the bruise. The method may further comprise performing reflectance spectroscopy on a haematoma in order to obtain a dataset of reflectance spectra; and analyzing the reflectance spectra to determine if a region of substantially uniform reflectivity over all wavelengths is present. A 'substantially white' area generally means a pale, whitish region.

An apparatus may also be provided for determining the age of a haematoma, wherein the apparatus is arranged to carry out any of the various methods described above.

In a further aspect, the present invention provides an apparatus for determining the age of a haematoma, comprising: a light source; a light receiver; a

spectrophotometer; and a processor, wherein the light source is directed towards the skin having the haematoma, the light receiver receives light reflected from the skin; the spectrophotometer carries out spectral measurements on the reflected light and the processor determines the age of the haematoma from the measured reflectance spectrum and a mathematical model relating a time varying physical quantity of the haematoma with time after injury.

Preferably the light source is also directed towards normal skin in order to obtain spectral measurements of the normal skin.

The light source and receiver may be combined into a single unit such as a fibre optic probe. This uses a mixture of illuminating and detecting optical fibres arranged in a bundle. A light-integrating sphere may also be used to emit and receive light.

Typically, the model is implemented in software which is run on a computer. Preferably the model comprises two components, with the first component comprising an equation relating the density of haemoglobin and/or an equation relating the density of bilirubin with depth and time after injury. The second component is preferably a photon transport model that enables the processor to simulate reflectance spectra as a function of time using the haemoglobin and/or bilirubin densities. The processor can then determine the age of the haematoma by comparing the simulated spectra with the measured spectrum.

Alternatively, the processor may determine the density of haemoglobin and/or bilirubin in the haematoma from the measured spectrum, and determine the age of the haematoma using the density data and the equations relating haemoglobin and/or bilirubin density with time.

The calculations necessary to fit measured data to simulated data are not time consuming and could potentially be implemented on a chip that could be used in a measuring device for use in forensic examinations or in clinical settings.

The invention is preferably implemented by means of a computer using software that contains instructions for carrying out the method(s) described above. Thus, the invention extends to a software product carrying such instructions, whether in physical form, e.g. as a disc, or in downloadable form, e.g. available over the internet. As such, in an another aspect, the invention provides a software

product comprising instructions which when executed by a computer cause the computer to estimate the age of a haematoma by simulating a plurality of reflectance spectra as a function of time using a model relating haemoglobin and/or bilirubin content with time after injury; and comparing the simulated reflectance spectra with a measured reflectance spectrum obtained of the haematoma.

The invention furthermore extends to both a method of manufacturing such software products and a method of providing such software products to a remote location by means of transmitting data to a computer at that remote location.

In strongly pigmented skin, melanin will act as a filter decreasing the light penetration depth into the skin. This may have an effect on the accuracy of the system. This problem can be solved by using near infrared light in the measurement system instead of white light, since melanin absorption falls off strongly with increasing wavelength.

The above described methods can be used both on living persons, for example determining the age of bruises in child abuse cases, and can also be used post mortem. In this situation, it is well-known that other chromophores will appear and these can be taken into account. In addition, it can be used to assist in determining time of death by analysing the characteristic bruises caused by blood pooling in the body after death.

Thus, devices operating according to the invention may be used by forensic scientists, by police or medical personnel who need to gather evidence about a victim of assault.

A further medical application is the analysis of surgical wounds where it is important to check for post-operative bleeding. It is normal for there to be a haematoma at the site of a surgical wound, but the age of this should correspond to the time of surgery. If the haematoma's age does not correspond to surgery, then this may be an indication of fresh bleeding. In this context, because of the normal bleeding, an accurate age measurement will probably not be possible, but in this situation this is not important. An indication that the bruise is "too young" will provide the necessary indication.

Preferred embodiments of the present invention will now be described by way of example only and with reference to the accompanying drawings, in which:

Figure 1 shows an apparatus for carrying out the method according to a preferred embodiment of the present invention;

Figure 2 is a flow diagram illustrating the steps in determining the age of a bruise according to a preferred embodiment of the present invention.

Figure 3 is a graph illustrating an example of the time taken for blood to perfuse over a particular distance in subcutaneous muscle, as calculated by equation 7;

Figure 4 is a graph illustrating an example of normalised dermal haemoglobin density vs. distance from the basal layer (mm) and time after injury, according to the mathematical model of a preferred embodiment of the present invention;

Figure 5 is a graph illustrating a further example of normalised dermal haemoglobin density vs. distance from the basal layer (mm) and time after injury, according to the mathematical model of a preferred embodiment of the present invention;

Figure 6 is a graph illustrating another example of normalised dermal haemoglobin density vs. distance from the basal layer (mm) and time after injury, according to the mathematical model of a preferred embodiment of the present invention;

Figure 7 is a graph illustrating an example of normalised average haemoglobin and bilirubin densities versus time, calculated according to the mathematical model of a preferred embodiment of the present invention;

Figure 8 shows simulated reflectance spectra demonstrating the effect of different dermal thicknesses;

Figure 9 shows simulated reflectance spectra demonstrating the effect of different values of haemoglobin diffusivity.

Figures 10a and 10b give a comparison of measured and simulated reflectance spectra for two different patients;

Figure 11 gives a number of the parameters used when simulating reflectance spectra to evaluate an embodiment of the invention, and gives the error in the estimated age;

Figure 12 show the whitish spots that can occur in a bruise;

Figure 13 illustrates the chemical/physiological processes that occur when a bruise is formed;

Figure 14 illustrates the processes modelled in order to create the preferred model relating haemoglobin and bilirubin density with time; and

Figure 15 gives the parameters used in the photon transport model according to an embodiment of the invention.

Figure 1 illustrates an apparatus that can be used to determine the age of a skin bruise. It comprises an integrating sphere 2 placed opposite skin 1, the integrating sphere 2 being connected to a computer 3. The integrating sphere is modified to a 20mm aperture to avoid loss of (red) light backscattered from deeper skin layers. The wavelength scale is calibrated using the oxy- and deoxyhaemoglobin absorption peaks. A suitable integrating sphere is the ISP-REF and SD2000, by Ocean Optics, Duiven, the Netherlands.

The method of determining the age of the skin bruise using such an apparatus is shown in the flow diagram of Fig 2.

The dermal thickness of the injured area is measured using a high frequency ultrasound probe (not shown). This information is input to the computer 3.

The integrating sphere is placed opposite the bruise and then normal skin to collect reflectance spectra of both bruised and normal skin in the 400-1400 nm wavelength range. The integrating sphere is held gently against the skin during measurement to avoid blanching due to pressure from the sphere. The measurements are input to the computer 3.

The computer calculates the input parameters required for the photon transport model as discussed above. The blood oxygenation, dermal blood volume fraction and melanin content for normal skin are calculated using the reflectance spectra for normal skin in the preferred manner discussed previously.

The blood oxygenation is calculated for bruised skin using the reflectance spectra for bruised skin. The melanin content of bruised skin is taken to be the same as for normal skin. The dermal blood volume fraction is calculated for bruised skin as discussed previously.

The photon transport model also requires the volume fraction of blood due to the haemorrhage (the haemoglobin) and the volume fraction of bilirubin for both the

upper and deeper dermis. In order to find these, the computer estimates the density of haemoglobin and bilirubin as a function of depth and time after injury using the preferred mathematical model given by equations 17 and 21 respectively in the Appendix, the measured value of dermal thickness and values inputted for haemoglobin diffusivity, subcutaneous density of haemoglobin, haemoglobin relation time, haemoglobin to bilirubin relaxation time and bilirubin relaxation time. The densities are then averaged over the depth of each layer. In other embodiments other equations as derived in the Appendix relating density with time may be used. The volume fractions are found from the densities as previously discussed.

The blood volume fraction calculated from the model is checked against the measured blood volume fraction to ensure the calculated blood volume fraction is reasonable. This feedback is important as it enables the simulation calculations to be iteratively adjusted.

As an example, the normalised extravascular dermal haemoglobin density versus distance from the basal layer and time after injury (as calculated by equation 17) is shown in Fig 4. The dermal thickness and haemoglobin relaxation time have been set to $d = 1.5mm$ and $\tau_h = 5days$ respectively. The subcutaneous haemoglobin density is normalized to unity, i.e. N_{H0}. In this example, it can be seen that the maximum haemoglobin in the basal layer occurs about four days after injury, and that this value slowly reduces to about half the value after ten days.

A further example is illustrated in Fig 5. In this case, a thick dermis is assumed, and the dermal thickness and haemoglobin relaxation time are set to $d = 4mm$ and $\tau_h = 5days$ respectively. It can be seen that the maximum haemoglobin in the basal layer is insignificant, and thus subcutaneous bleedings do not show up as blue-ish coloured bruises. This phenomenon is well known from forensic medicine because torturers often beat their victims at such thick skinned locations so damages do not show up on visual inspection.

An example of enhanced macrophage activity is illustrated in Fig 6. The haemoglobin relaxation time is reduced to τ_h=1day and the dermal density is d=1.5mm. A very small amount of haemoglobin is located in the basal layer after 1-2 days, but the value relaxes thereafter rapidly to zero. This phenomenon can be

observed as white spots in the dermal region just above a damaged subcutaneous vessel. The high macrophage activity this region engulf the haemoglobin molecules very efficiently, and the blue colour is suppressed.

Curves showing the normalised average haemoglobin and bilirubin densities v. time calculated using equations 23 and 24 are illustrated in Figure 7. The curves show that the average haemoglobin density peaks before the maximum in the bilirubin density. A crossover can be seen where the bilirubin density becomes larger than the haemoglobin. In the data selected, the bruise will have a predominant bluish appearance until 3-4 days after injury, whereas the bruise will appear yellowish after 9-10 days.

The photon transport model models the skin in three different layers and different input parameters are used for the different layers. A summary of the input parameters required, how they are found and the assumptions that are made in a particularly preferred embodiment of the invention is given in Figure 15. Once the input parameters have been found, the photon transport model is used to simulate reflectance spectra as a function of time.

The measured spectrum is compared with each of the simulated reflectance spectra for different values of time, and the age of the haematoma is determined as the time value that gives the simulated spectrum that most closely fits the measured spectrum.

Simulated reflection spectra found using the above method have been evaluated, and found to depend strongly on particular input parameters. Typical simulated reflection spectra are shown in Figs 8 and 9. For these curves the blood volume fraction of the subcutaneous reservoir of blood was v_0=0.4. The blood volume fraction of intravascular blood in the upper layer (epidermis) was 0.002 and its oxygen saturation was 0.4. In both dermal layers the extravascular blood volume fraction was 0.01 with oxygenation 0.8. The oxygenation of extravascular haemoglobin is strongly depleted by metabolism and oxygen saturation was therefore set to 0.2. The haemoglobin relaxation time was set to 5 days. A skin pigmentation typical for Caucasian skin was used, i.e. melanin absorption coefficient at 694nm is $\mu_{a,mel} = 500\ m^{-1}$.

The effect of varying dermal thickness is shown in Figure 8. It can be seen that the simulated reflection spectra depend heavily on the dermal thickness used. The effect is that the bruise appears to be older or more recent depending on the epidermal and dermal thickness chosen. When the skin thickness value is decreased the bruise appears to be older, and when it is increased the bruise appears to be younger. This is because if the skin thickness is assumed to be smaller than the real skin thickness, the model will give a fit for a shorter time than the actual time after injury. This is due to the fact that the reflectance spectrum is strongly influenced by the skin thickness through the diffusion time. This effect was especially visible during the first three days after injury. For large dermal and epidermal thicknesses, e.g. dermis-3mm and epidermis=0.5mm, the haemoglobin did not reach visibly detectable skin depths (approximately 600μm) before decomposing to bilirubin. Thus, using the correct skin thickness is important to obtain an accurate age determination, and should be measured e.g. by ultrasound.

The mathematical model discussed above and derived in the Appendix depends on the assumption that the development of a bruise is a diffusion driven process. This is supported by the fact that injured vessels stop bleeding within a short time after impact, altering the pressure gradients in the wounded area. Deep bruises have been found to appear bluish, while more superficial bruises appear red. The bruises studied by the inventors were visually detectable 1-2 days after injury and were mostly bluish. This can be seen as evidence for a diffusion process rather than a fast pressure driven flow, since such a process would have made the bruises appear faster. The value of haemoglobin diffusivity used in the model is preferably extrapolated from a given value for myoglobin diffusion in muscle tissue and is scaled according to the difference in size between haemoglobin and myoglobin.

Changing the haemoglobin diffusivity affects the simulated haemoglobin distribution and thereby the simulated reflection spectra. Reflection spectra simulated as three days post injury are shown in Fig 9. The epidermal and dermal thicknesses are 0.1 and 1mm respectively. A haemoglobin diffusivity of $D_H=3 \times 10^{-12}\ m^2s^{-1}$ resulted in a fully developed bluish bruise at day two. A diffusivity of $D_H=1.2 \times 10^{-11}\ m^2s^{-1}$ gave a fully developed bruise within 24 hours,

whereas $D_H=1x10^{-13}$ m^2s^{-1} did not result in a fully developed bruise at all since the blood was broken down before it reached the optically detectable skin layers.

The mathematical model used in the preferred embodiment of the invention has been verified using experimental data. Reflection spectra in the 400-850nm-wavelength range were collected from normal and bruised skin using an integrating sphere set-up. All bruises in this study developed within 2-3 days after injury, depending on the nature of the injury and the skin thickness at the injured site.

When testing the model, the values of the haemoglobin and bilirubin relaxation times were assumed to be the same for all patients. This is probably acceptable for a homogeneous population, but not necessarily for a heterogeneous one.

Measured and simulated reflection spectra for two different patients are shown in Figs 10a and 10b. The spectra were fitted by iterating the time after injury, the blood content in the blood pool and the oxygen content in the blood pool. The value of skin thickness was varied slightly on an individual basis. It can be seen that the experimental data and simulated data agree well. The discrepancy that can be observed between simulated and measured spectra in Fig 10a for the wavelength region above 700nm is probably due to the measurement technique. The integrating sphere was held gently against the skin to avoid blanching, and this might have caused loss of light from the aperture. This effect is therefore especially visible during measurements on curved regions such as the forearm in this case.

Simulation parameters and error in the estimated age compared to true age of the bruises for twenty-six different patients are given in Fig 11. In this study the parameters given in Figure 15 were used. It can be seen that the present method dates bruises with an accuracy of approximately three hours for injuries less than a day old. For injuries up to ten days old the estimated error is 0±0.5 days. This is a considerable improvement over prior art methods. The error depends strongly on the values of time constants used in the model. For injuries less than a day old, the bilirubin density has not yet reached detectable levels, thus only haemoglobin contributes to the reflection spectra. Most of the included samples were in the range of one to ten days old, consequently the values of the time constants could be

estimated with sufficient accuracy. The narrow range of age in the experimental setup improves the statistics.

In about one third of subjects a clear white spot could be observed after localized trauma caused by inserting an intra venous catheter or syringe. An example of such a white spot is given in Fig 12. The photo is taken two days after injury. The bruise was located at the volar side of the left lower arm of a patient. The spot developed during the first two days after injury and increased in size with time. The effect can be observed in traumatic injuries where there is substantial crushing of the tissue.

Appendix

Blood distribution in bruised tissues – percolative flow

A crushing type of tissue injury will result in bleeding from damaged vessels. In most bruises damages are confined to smaller vessels such as arterioles and venules. A localized damage may rapidly result in a pool of blood in subcutaneous tissues, resulting in a transport of whole and haemolysed blood in intercellular space which subsequently is transported deeper into tissue. Blood will flow easily along the interspaces between muscle bundles and membranes as well as along and across muscle fibres. The flow across membranes is much less. The driving force of this flow will be the pressure of damaged veins or arteries together with gravitational effects.

A simple model of this transport could be as given by the phenomenological Darcy's law:

$$j_i = -K_{ik}\frac{\partial p}{\partial x_k} \qquad 1$$

Where j_i is the components of the blood flux vector, p is the local pressure, x_i is the spatial coordinate of a Cartesian system and K_{ik} is the hydraulic conductivity tensor.

An order of magnitude estimate of the time required for subcutaneous transport of whole and haemolysed blood can be found by considering flow in an isotropic porous medium. In isotropic tissues the hydraulic conductivity tensor can be written $K_{ik} = K\delta_{ik}$, where δ_{ik} is the Kronecker unit tensor. The above law can then be expressed,

$$\vec{j} = -Kgradp \qquad 2$$

Where K is the hydraulic conductivity of whole blood.

The blood can be considered as an incompressible liquid, thus the continuity of the blood flux vector can be expressed,

$$\frac{\partial j_i}{\partial x_i} = 0 \qquad 3$$

In a homogeneous isotropic tissue with a lesion of spherical geometry the flow outside the lesion can be expressed,

$$j = \frac{Q}{4\pi r^2} \qquad 4$$

Where Q is the total discharge rate of blood from the lesion and r is the distance from its centre.

The pressure p at an arbitrary position r from source can be expressed using Eqs. 2 and 4 as:

$$p = \int_r^\infty \frac{Q}{4\pi K r^2} = \frac{Q}{4\pi K r} \qquad 5$$

Where the pressure at infinity is set equal to zero.

The time Δt required for blood to flow from a pool of radius a and saturate a volume of radius c is given by:

$$\Delta t = \frac{4\pi}{3Q}(c^3 - a^3)\rho \qquad 6$$

Where ρ is the porosity of the tissue, i.e., the fractional volume of tissue that is filled with extravascular blood.

Thus, the time can also be expressed using equations 5 and 6 as:

$$\Delta t = \frac{(c^3 - a^3)\rho}{3aK\Delta p} \qquad 7$$

Where Δp is the pressure difference between the blood-pool and the region of radius c.

Equation 7 is also valid for flow from a sub-dermal hemispheric pool of blood perfusing radially into subcutaneous tissues, such as in the case of a subcutaneous bleeding.

The corresponding expression in the case of linear flow along a channel of constant cross-section, such as in the space between two membranes, is:

$$\Delta t = \frac{L\rho}{K\Delta p} \qquad 8$$

Where L is the length of the channel and Δp is the pressure drop along the channel.

Values of the hydraulic conductivity across the abdominal muscle may increase with abdominal pressure, and are in the range $K = 0.1 - 0.6 \cdot 10^{-12}\ m^4 / Ns$ for pressures from 2-20 mmHg. The component of the hydraulic conductivity along the dermis/subcutaneous junction, i.e. parallel to the membranes may be in the region $K = 0.1 - 0.5 \cdot 10^{-10}\ m^4 / Ns$. As such, the component of the hydraulic conductivity along the muscle fibre and membranes can be significantly larger than the corresponding value across these structures.

An example of the transport time in subcutaneous muscle Δt can be found from Equation 7 and is shown in Fig 3. The hydraulic conductivity is set to $K = 10^{-11}\ m^4 / Ns$, the porosity $\rho = 0.1$ and $\Delta p = 2.6kPa(20mmHg)$. The pressure, which here corresponds to a typical venous pressure, can be much higher if arteries/arterioles are damaged. Furthermore, if perfusion over larger distances is considered, the gravitational contribution to the pressure gradient must be considered.

The results give the time required for blood to perfuse from a pressurized region of 5 mm diameter to a distance of up to 30 mm. The diameters of typical bruises are in the range 20-60 mm, and it can thus be concluded that the subcutaneous perfusion of blood will take place within a few hours. The use of Darcy's law, which is based on quasi-static conditions, is a coarse simplification of the dynamics. However, it is good enough to predict a proper order of magnitude of the time scale for forming a subcutaneous pool of extravascular haemoglobin.

Δt is used to give an order of magnitude when making assumptions in the derivation of the diffusion equations. It can be seen that $\Delta t \ll$ diffusion time and thus the first phase of the haemorrhage is so fast that it is acceptable to 'start the clock' in modelling the diffusion process when the sub-dermal haemoglobin source is in place.

Transport of haemoglobin in dermis

The extravascular transport of haemoglobin in tissue can be expressed by convection and diffusion, using equation 1:

$$j_i = -D_{ik}\frac{\partial N}{\partial x_k} - \xi K_{ik}\frac{\partial p}{\partial x_k} \qquad 9a$$

where j_i is the component of the haemoglobin flux vector, D_{ik} is the haemoglobin diffusivity tensor, K_{ik} is the Darcy constant (hydraulic conductivity tensor) for whole blood, N_H is the density of molecular haemoglobin and ξ is the volume fraction of haemoglobin in whole blood, i.e. $\xi = 0.14 - 0.15$ corresponding to a haemoglobin density of $140 - 150\ g/l$.

One of the important roles of the skin is to prevent body fluids from being lost by seepage. It is therefore reasonable to assume that the convective flow of haemoglobin in dermis is negligible, and that the extravascular transport of haemoglobin is dominated by diffusion. The stratum corneum and the upper part of the epidermis, which are composed of very densely packed keratinocytes, are assumed to form diffusion barriers with zero diffusivity. The diffusion of haemoglobin into dermis can then be modelled as flow in a planar model where the boundary condition at the basal layer is zero flux.

As mentioned above, the diffusion process is much slower than the process for distribution of blood in subcutaneous tissue, and thus the sub-dermal haemoglobin source can be assumed to be instantaneously established at time t=0. Extravascular dermal haemoglobin can then be considered as transported into dermis by diffusion from a subcutaneous reservoir of whole and haemolysed blood. Extravascular dermal haemoglobin can, of course, also be caused by seepage of whole blood from damaged dermal blood vessel, e.g. from damage to the superior vascular plexus. However, such bleedings will result in a fast developing reddish colour of the skin, whereas subcutaneous bleeding will give rise to a bluish coloration that develops over time.

When the Darcy term in Eq.9a is neglected the transport flux vector $\vec{j}_H$ in an isotropic medium becomes:

$$\vec{j}_H = -D_H \, grad N_H \qquad 9$$

where D_H is the haemoglobin diffusivity.

The continuity of haemoglobin in a medium with loss can be expressed,

$$div\vec{j}_H = -\frac{\partial N_H}{\partial t} - \frac{N_H}{\tau_H} \qquad 10$$

Where t is time and τ_H is the haemoglobin relaxation time.

The relaxation time is the time required for the haemoglobin density to be reduced to 1/e of the initial density. This is determined by several mechanisms such as drainage by the lymphatic system, conversion to bilirubin/biliverdin or consumption by macrophages. The relaxation time can be expressed,

$$\frac{1}{\tau_H} = \frac{1}{\tau_L} + \frac{1}{\tau_B} + \frac{1}{\tau_M} + ... \qquad 11$$

Where $\tau_L, \tau_B \; and \; \tau_M$ are the relaxation times due to lymphatic drainage, conversion to biliverdin/bilirubin and to macrophage activity, respectively.

The diffusivity of myoglobin in skeletal muscle cell may be for example $1.2 \cdot 10^{-11} m^2/s$. The molecular weight of haemoglobin (64 000 Dalton) is approximately four times as large as that of myoglobin (17 000 Dalton). Therefore the diffusivity of haemoglobin in skin should be less than that in muscle, and may be for example around $D_H = 3 \cdot 10^{-12} m^2/s$.

The relaxation time may be in the range of $\tau_H = 1.7 - 2.6 \cdot 10^5 \, s (2 - 5 days)$. However, the relaxation may be significantly less in a region of 5-10 mm around damaged vessels. Here, a whitish region is formed about 0.5-1 day after injury, and this region expands with time. This phenomenon, which is assumed caused by macrophages from the damaged vessel, reduces the haemoglobin relaxation time to $\tau_H = 0.2 - 0.4 \cdot 10^5 \, s (0.25 - 0.5 days)$.

Transport and generation of bilirubin in dermis

Diffusion of bilirubin in dermis can be expressed by a transport flux vector $\vec{j}_B$:

$$\vec{j}_B = -D_B grad N_B \qquad 12$$

Where D_B is the bilirubin diffusivity and N_B is the density of molecular bilirubin.

The transition from biliverdin to bilirubin is fast compared to other timescales of the system. Therefore, the generation of bilirubin can be characterized by the time τ_B, which characterizes the haemoglobin to bilirubin/biliverdin transition.

The continuity of bilirubin can be expressed,

$$div\vec{j}_B = -\frac{\partial N_B}{\partial t} - \frac{N_B}{\tau_{BR}} + \frac{N_H}{\tau_{BG}} \qquad 13$$

Where τ_{BR} is the bilirubin relaxation time, τ_{BG} is the bilirubin generation time, i.e. $\tau_{BG} = \tau_B / n$ where n= no. of bilirubin molecules generated per decomposed haemoglobin molecule.

Bilirubin diffusion is expected to play a less important role as compared to haemoglobin diffusion during the first days after injury. The reason is that haemoglobin diffusion results from a very steep density gradient in the dermal/sub-dermal junction shortly after the injury, whereas bilirubin mostly is generated from a haemoglobin distribution that is smoothened out after diffusion. If diffusion of bilirubin is neglected, Eq.13 is simplified to,

$$\frac{\partial N_B}{\partial t} = -\frac{N_B}{\tau_{BR}} + \frac{N_H}{\tau_{BG}} \qquad 14$$

The bilirubin distribution discussed above only takes into account bilirubin diffused from its site of generation. Contributions from subcutaneous transport of bilirubin by percolative or lymphatic flow followed by diffusion into dermis will give additional values. These effects can be analysed along the same principles as used for haemoglobin distribution.

Relationship between haemoglobin/bilirubin density and time after injury

The diffusion of haemoglobin into dermis can be modelled as flow in a planar model. Epidermis is composed of very densely packed keratinocytes that form a diffusion barrier. The boundary condition at the basal layer can therefore be taken as zero flux. The sub-dermal haemoglobin source can, since the diffusion process is much slower than the process for distribution of blood in subcutaneous tissue, be taken to be instantaneously established at time t=0.

The spatial and temporal distribution of haemoglobin in dermis $N_H(x,t)$ can be derived from equations 9 and 10 to be:

$$N_H(x,t) = N_{H0}\{\sum_{n=0}^{\infty}[(-1)^n erfc(\frac{1}{2}\frac{(2n+1)d-x}{\sqrt{D_H t}}) + (-1)^n erfc(\frac{1}{2}\frac{(2n+1)d+x}{\sqrt{D_H t}})]\}e^{-\frac{t}{\tau_H}}$$

(15)

Where N_{H0} is the subcutaneous density of haemoglobin, x is the distance from the basal layer and d is the dermal thickness.

The relaxation time of dermal haemoglobin and haemoglobin in the subcutaneous region are both assumed to be τ_H. Epidermis, which is the outer protective layer of skin, is composed of very densely packed keratinocytes. This layer constitutes a barrier and the diffusivity is much less that in dermis. The epidermal diffusivity in the model is therefore set equal to zero.

The corresponding value for the average haemoglobin density $\overline{N}_H(t)$ in dermis can be expressed by the integration of Eq.15 over the dermis:

$$\overline{N}_H(t) = 2N_{H0}\sqrt{\frac{D_H t}{d^2}}\{\frac{1}{\sqrt{\pi}} - 2\sum_{n=1}^{\infty}(-1)^n \frac{dnerfc(\frac{dn}{\sqrt{D_H t}})\sqrt{\pi} - e^{-\frac{d^2n^2}{D_H t}}\sqrt{D_H t}}{\sqrt{\pi D_H t}}\}e^{-\frac{t}{\tau_H}} \quad 16$$

The series in Eq.15 converges rapidly, and for time scales where $t < 4d^2 / D_H$ the first term gives satisfactory accuracy. In the case of dermal thickness in the range of 1-2mm and with a haemoglobin diffusivity of D_H=3 x 10^{-12} m^2/s, this timescale corresponds to 4-6 days.

$$N_H(x,t) = N_{H0}\{erfc(\frac{1}{2}\frac{d-x}{\sqrt{D_H t}}) + erfc(\frac{1}{2}\frac{d+x}{\sqrt{D_H t}})\}e^{-\frac{t}{\tau_H}} \quad 17$$

The first of these terms represent diffusion into a semi-infinite medium and the second term represents a mirror source ensuring zero flux at the basal layer.

The corresponding approximation for the average dermal haemoglobin distribution follows from integration of Eq.17:

$$\bar{N}_H(t) = \frac{2N_{H0}}{d\sqrt{\pi}}\{\sqrt{D_H t} - \sqrt{D_H t}e^{-\frac{d^2}{D_H t}} + d\sqrt{\pi}erfc(\frac{d}{\sqrt{D_H t}})\}e^{-\frac{t}{\tau_H}} \quad 18$$

The corresponding solution for bilirubin distribution in presence of diffusion in dermis can be established by forming a Green's function from the haemoglobin distribution, together with a mirror-distribution that establishes the criterion of no bilirubin transport into the epidermis.

$$N_B(x,t) = \int_{\tau=0}^{t}\int_{\xi=0}^{d} N_H(\xi,\tau)\frac{(e^{-\frac{(x-\xi)^2}{4D_B(t-\tau)}} + e^{-\frac{(x+\xi)^2}{4D_B(t-\tau)}})e^{-\frac{t-\tau}{\tau_{BR}}}}{2\tau_{BG}\sqrt{\pi D_B(t-\tau)}}d\xi d\tau \quad 19$$

Where $N_H(\xi,\tau) = N_H(x \Rightarrow \xi, t \Rightarrow \tau)$ from Eq. 15 or 17.

Diffusion into dermis of bilirubin formed from subcutaneous haemoglobin can be found by adding a corresponding term to Eq.19:

$$N_B(x,t) = \int_{\tau=0}^{t}\int_{\xi=0}^{\infty}[N_H(\xi,\tau)H(d-\xi) + N_{H0}e^{-\frac{\tau}{\tau_H}}H(\xi-d)]\frac{(e^{-\frac{(x-\xi)^2}{4D_B(t-\tau)}} + e^{-\frac{(x+\xi)^2}{4D_B(t-\tau)}})e^{-\frac{t-\tau}{\tau_{BR}}}}{2\tau_{BG}\sqrt{\pi D_B(t-\tau)}}d\xi d\tau \qquad 20$$

where $H(x)$ is the Heaviside unit step function.

If all diffusion of bilirubin can be neglected the solution of Eqs. 19 and 20 can be simplified as:

$$N_B(x,t) = e^{-\frac{t}{\tau_{BR}}}\int_{\tau=0}^{t}\frac{N_H(x,\tau)e^{\frac{\tau}{\tau_{BR}}}}{\tau_{BG}}d\tau \qquad 21$$

Where $N_H(x,\tau) = N_H(x,t \Rightarrow \tau)$ follows from Eqs. 15 or 17.

When diffusion within the dermis is present but diffusion across the dermal/subcutaneous tissue border layer is neglected, the average bilirubin density can be expressed as:

$$\overline{N}_B(t) = e^{-\frac{t}{\tau_{BR}}}\int_{\tau=0}^{t}\frac{\overline{N}_H(\tau)e^{\frac{\tau}{\tau_{BR}}}}{\tau_{BG}}d\tau \qquad 22$$

Where $\overline{N}_H(\tau) = \overline{N}_H(t \Rightarrow \tau)$ follows from Eq.18.

There are no analytical solutions to the integrals of equations 21 and 22. However, for small values of time, i.e. $t < d^2 / D_H$, equation 18 can be approximated as:

$$\overline{N}_H(t) = \frac{2N_{H0}}{d}\sqrt{\frac{D_H t}{\pi}}e^{-\frac{t}{\tau_H}} \qquad 23$$

and from equations 22 and 23, the corresponding value for the average bilirubin density then becomes:

$$\bar{N}_B(t) = \frac{N_{H0}\tau_{BR}\tau_H}{\tau_B(\tau_H - \tau_{BR})d}\{2\sqrt{\frac{D_H t}{\pi}}e^{-\frac{t}{\tau_H}} - erf(\sqrt{\frac{(\tau_H - \tau_{BR})t}{\tau_{BR}\tau_H}})\sqrt{\frac{D_H\tau_{BR}\tau_H}{\tau_H - \tau_{BR}}}\, e^{-\frac{t}{\tau_{BR}}}\} \qquad 24$$

5

Claims

1. A method of estimating the age of a haematoma, comprising the steps of: obtaining a measured reflectance spectrum of a haematoma; simulating a plurality of reflectance spectra as a function of time using a model relating haemoglobin and/or bilirubin content with time after injury; and determining the age of the haematoma by comparing the measured and simulated reflectance spectra.

2. A method as claimed in claim 1, wherein the model calculates the amount of chromophores in the haematoma.

3. A method as claimed in claim 1 or 2, wherein the model is a first-principle mathematical model.

4. A method as claimed in claim 1 2 or 3, wherein the model takes into account the thickness of the dermis.

5. A method as claimed in any preceding claim, wherein the model comprises a first component that simulates the development of a bruise with time and a second component that simulates the reflectance spectrum of the simulated bruise.

6. A method as claimed in any preceding claim, further comprising the step of obtaining a reference spectrum from a region adjacent to the haematoma and using this spectrum in the simulation of the simulated spectra.

7. A method as claimed in any preceding claim, wherein the model relates the haemoglobin density with depth in the dermis and time after injury and/or the bilirubin density with depth in the dermis and time after injury.

8. A method as claimed in claim 7, wherein the model comprises an equation relating the haemoglobin density with depth and time, and wherein the density is

also given in terms of: the subcutaneous density of the haemoglobin, the dermal thickness, the haemoglobin diffusivity and the haemoglobin relaxation time.

9. A method as claimed in claim 7 or 8 wherein the model comprises an equation relating the bilirubin density with depth and time, and wherein the density is also given in terms of: the haemoglobin distribution, the bilirubin relaxation time, the generation of bilirubin relaxation time and the dermal thickness.

10. A method as claimed in claim 7, wherein the mathematical model comprises an equation relating the haemoglobin density with depth and time, which is at least approximately given by:

$$N_H(x,t) = N_{H0}\{erfc(\frac{1}{2}\frac{d-x}{\sqrt{D_H t}}) + erfc(\frac{1}{2}\frac{d+x}{\sqrt{D_H t}})\}e^{-\frac{t}{\tau_H}}$$

wherein N_H is the haemoglobin density

N_{H0} is the subcutaneous density of haemoglobin

d is the dermal thickness

D_H is the haemoglobin diffusivity

t is time

τ_H is the haemoglobin relaxation time

x is the depth in the dermis

11. A method as claimed in 7 or 10, wherein the mathematical model comprises an equation relating the bilirubin density with depth and time, which is at least approximately given by:

$$N_B(x,t) = e^{-\frac{t}{\tau_{BR}}} \int_{\tau=0}^{t} \frac{N_H(x,\tau)e^{\frac{\tau}{\tau_{BR}}}}{\tau_B} d\tau$$

wherein N_B is the bilirubin density

N_H is the haemoglobin density

t is time

τ_B is the generation of bilirubin relaxation time

τ_{BR} is the bilirubin relaxation time

x is the depth in the dermis

12. A method as claimed in any of claims 1 to 6, wherein the model relates the average density of haemoglobin with time after injury and/or the average density of bilirubin with time after injury.

13. A method as claimed in claim 12, wherein the model comprises an equation relating the average density of haemoglobin with time, wherein the average density is also given in terms of: the subcutaneous density of the haemoglobin, the dermal thickness, the haemoglobin diffusivity and the haemoglobin relaxation time.

14. A method as claimed in claim 12 or 13 wherein the model comprises an equation relating the average density of bilirubin with time, wherein the average density is also given in terms of: the subcutaneous density of bilirubin, the bilirubin relaxation time, the haemoglobin relaxation time, the generation of bilirubin relaxation time, the dermal thickness and the haemoglobin diffusivity.

15. A method as claimed in claim 12, wherein the mathematical model comprises an equation relating the average density of haemoglobin with time, which is at least approximately given by: $\bar{N}_H(t) = \frac{2N_{H0}}{d}\sqrt{\frac{D_H t}{\pi}}e^{-\frac{t}{\tau_H}}$

wherein N_H is the average density of haemoglobin

N_{H0} is the subcutaneous density of haemoglobin

d is the dermal thickness

D_H is the haemoglobin diffusivity

t is time

τ_H is the haemoglobin relaxation time

16. A method as claimed in 12 or 15, wherein the mathematical model comprises an equation relating the average density of bilirubin with time, which is given by:

$$\bar{N}_B(t) = \frac{N_{H0}\tau_{BR}\tau_H}{\tau_B(\tau_H - \tau_{BR})d}\{2\sqrt{\frac{D_H t}{\pi}}e^{-\frac{t}{\tau_H}} - erf(\sqrt{\frac{(\tau_H - \tau_{BR})t}{\tau_{BR}\tau_H}})\sqrt{\frac{D_H\tau_{BR}\tau_H}{\tau_H - \tau_{BR}}}\, e^{-\frac{t}{\tau_{BR}}}\}$$

wherein N_B is the average bilirubin density

N_{H0} is the subcutaneous density of haemoglobin

d is the dermal thickness

D_H is the haemoglobin diffusivity

t is time

τ_H is the haemoglobin relaxation time

τ_B is the generation of bilirubin relaxation time

τ_{BR} is the bilirubin relaxation time

17. A method as claimed in any preceding claim, further comprising analysing the reflectance spectra to determine if a substantially white area is present towards the centre of the bruise.

18. A method of determining the nature of an object which has caused a bruise, comprising determining if a substantially white area is present towards the centre of the bruise.

19. A method as claimed in claim 18, further comprising:

performing reflectance spectroscopy on a haematoma in order to obtain a dataset of reflectance spectra; and

analyzing the reflectance spectra to determine if a region of substantially uniform reflectivity over all wavelengths is present.

20. A method for estimating the age of a haematoma, comprising the steps of:

performing reflectance spectroscopy on a haematoma and the region adjacent to the haematoma in order to obtain a dataset of measured reflectance spectra;

calculating the blood volume fraction, blood oxygenation and melanin content from the measured reflectance spectra for normal and/or bruised skin;

estimating the haemoglobin and/or bilirubin concentration as a function of time after injury using a mathematical model;

inputting the blood volume fraction, blood oxygenation and melanin content of normal and/or bruised skin and the haemoglobin and/or bilirubin concentration into a photon transport model;

obtaining simulated reflectance spectra as a function of time from the photon transport model; and

comparing the measured spectrum with each of the simulated reflectance spectrums for different values of time; and

determining the age of the haematoma as the time value that gives the simulated spectrum that most closely fits the measured spectrum.

21. A method for estimating the age of a haematoma, comprising the steps of:

performing reflectance spectroscopy on a haematoma in order to obtain a dataset of measured reflectance spectra;

determining haemoglobin and/or bilirubin content in the haematoma from the reflectance spectra; and

estimating the age of the haematoma using a mathematical model relating the haemoglobin and/or bilirubin content with time after injury.

22. An apparatus for estimating the age of a haematoma, wherein the apparatus carries out the method as claimed in any of claims 1 to 21.

23. An apparatus for estimating the age of a haematoma, comprising a means for inputting a measured reflectance spectrum of a haematoma; a means for simulating a plurality of reflectance spectra as a function of time using a model relating haemoglobin and/or bilirubin content with time after injury; and a means for determining the age of the haematoma by comparing the measured and simulated reflectance spectra.

24. A system for determining the age of a haematoma, comprising:

a light source;

a light receiver;

a spectrophotometer; and

a processor,

wherein the light source directs light towards the haematoma of the skin, the light receiver receives the light reflected from the skin, the spectrophotometer carries out spectral measurements on the reflected light, and the processor is arranged to process the reflected spectral measurements, wherein the processor determines the age of the haematoma from the measured reflectance spectrum and a model relating a time varying physical quantity of the haematoma with time after injury.

25. A system as claimed in claim 24, wherein the light source and light receiver are combined into a single unit optical probe.

26. A system as claimed in claim 24, wherein the light source and light receiver are combined into a light-integrating sphere.

27. A system as claimed in claim 24, 25 or 26 wherein the model comprises an equation relating the haemoglobin density and/or an equation relating the bilirubin density with depth in the dermis and time after injury.

28. A system as claimed in any of claims 24 to 27, wherein the processor simulates reflectance spectra as a function of time using the model, and determines the age of the haematoma by comparing the simulated spectra with the measured spectrum.

29. A system as claimed in any of claims 24 to 27, wherein the processor determines the density of haemoglobin and/or bilirubin in the haematoma from the measured spectrum, and determines the age of the haematoma using the density data and the model.

30. A software product comprising instructions which when executed by a computer cause the computer to carry out the method of any of claims 1 to 21.

31. A software product comprising instructions which when executed by a computer cause the computer to estimate the age of a haematoma by simulating a plurality of reflectance spectra as a function of time using a model relating haemoglobin and/or bilirubin content with time after injury; and comparing the simulated reflectance spectra with a measured reflectance spectrum obtained of the haematoma.

32. A software product as claimed in claim 30 or 31 wherein the software product is a physical data carrier.

33. A software product as claimed in claim 30 or 31 wherein the software product comprises signals transmitted from a remote location.

Fig 1

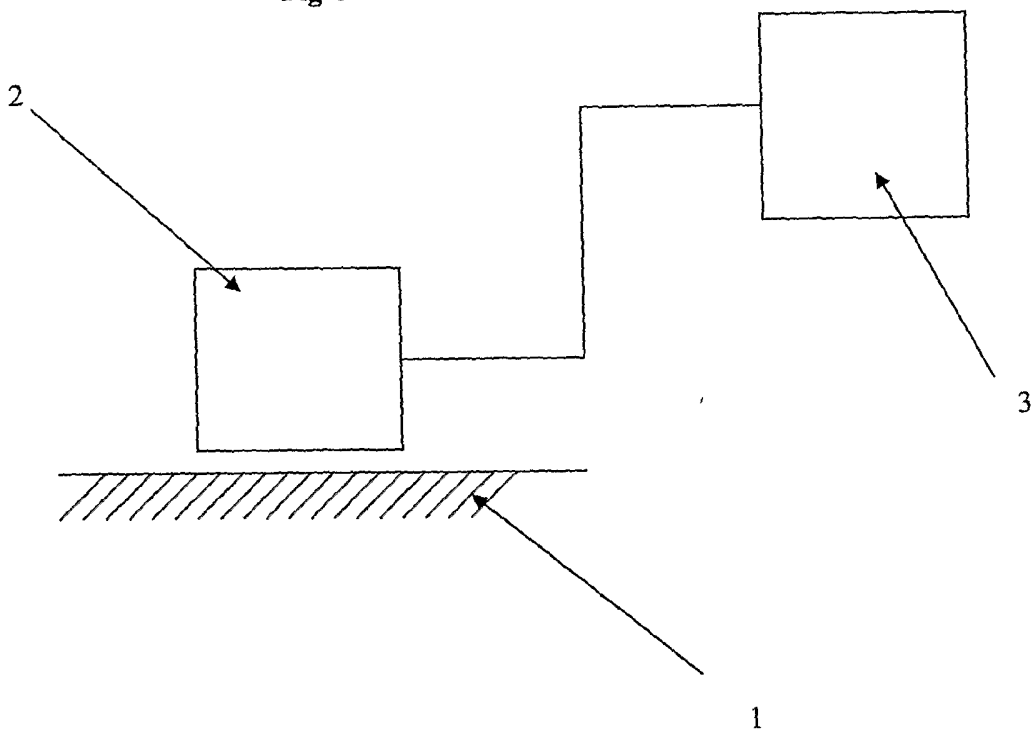
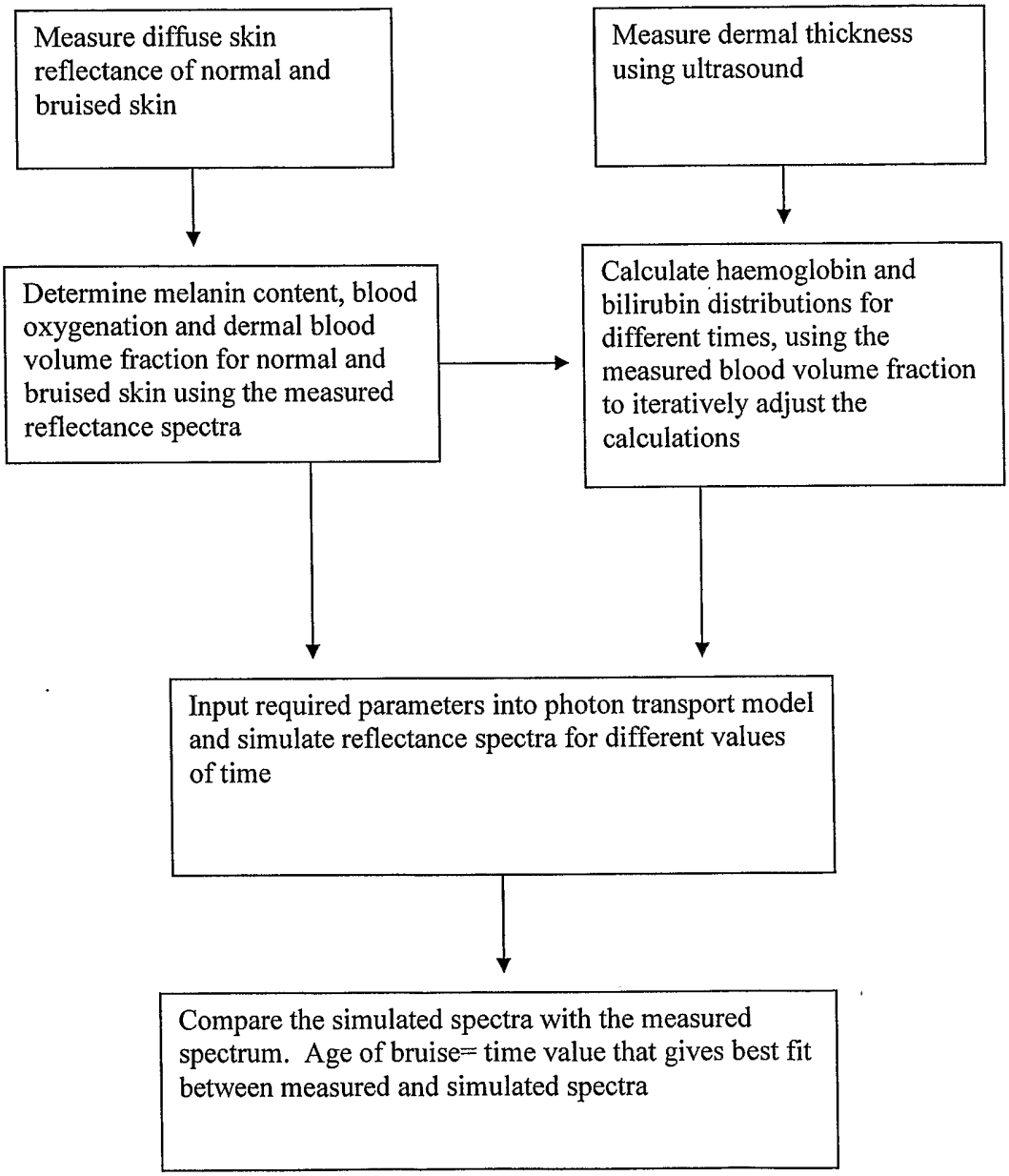


Fig 2


Measure diffuse skin reflectance of normal and bruised skin
Measure dermal thickness using ultrasound
Determine melanin content, blood oxygenation and dermal blood volume fraction for normal and bruised skin using the measured reflectance spectra
Calculate haemoglobin and bilirubin distributions for different times, using the measured blood volume fraction to iteratively adjust the calculations
Input required parameters into photon transport model and simulate reflectance spectra for different values of time
Compare the simulated spectra with the measured spectrum. Age of bruise= time value that gives best fit between measured and simulated spectra

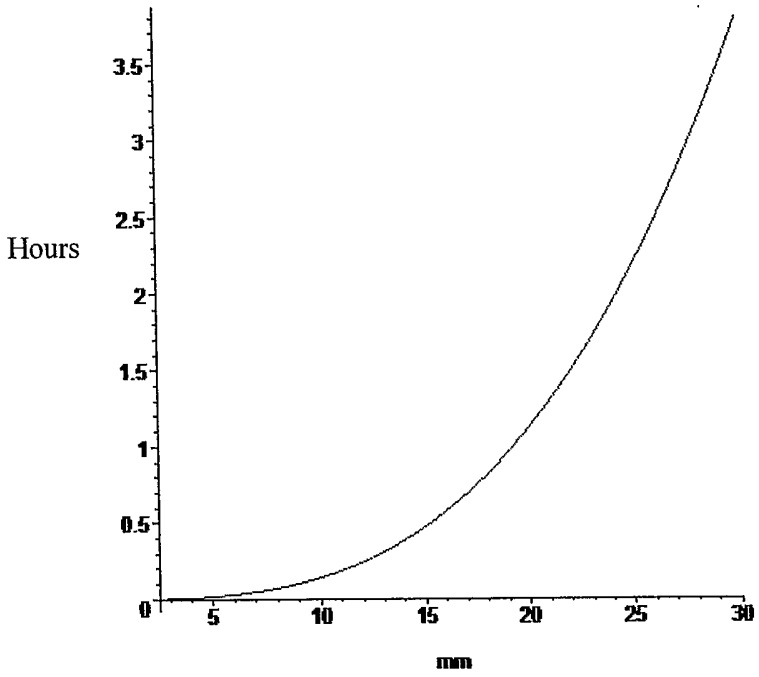


Fig. 3 Time (hours) for blood to perfuse over a distance (mm).
$K = 1 \cdot 10^{-11}\ m^4 / Ns, \quad \Delta p = 2.6kPa(20mmHg), \quad a = 2.5mm, \quad \rho = 0.1$

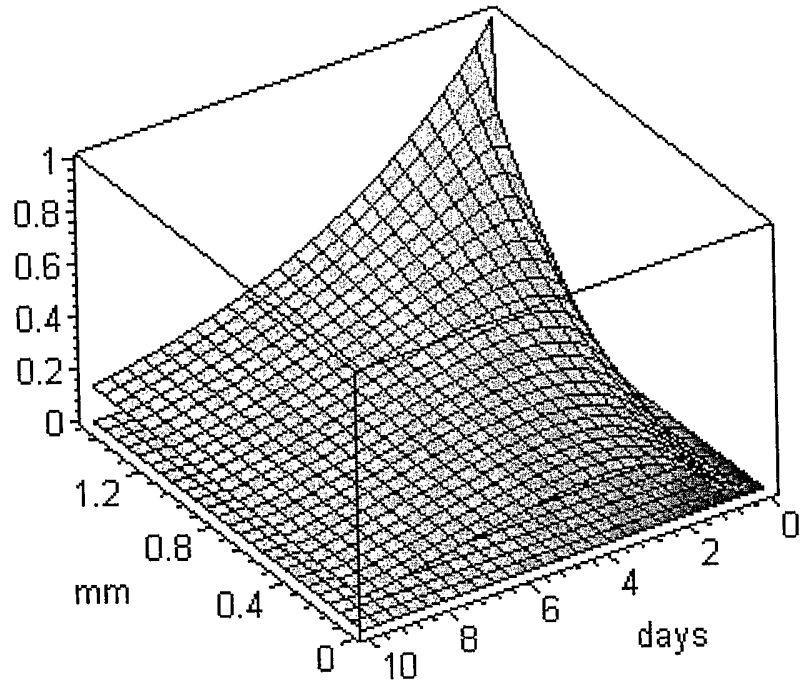


Fig.4 Normalised dermal hemoglobin density vs. distance from the basal layer (mm) and time after injury (days)

$N_{H0}=1, d=1.5mm, D_H=3\cdot10^{-12}m^2/s, \tau_H=5days$

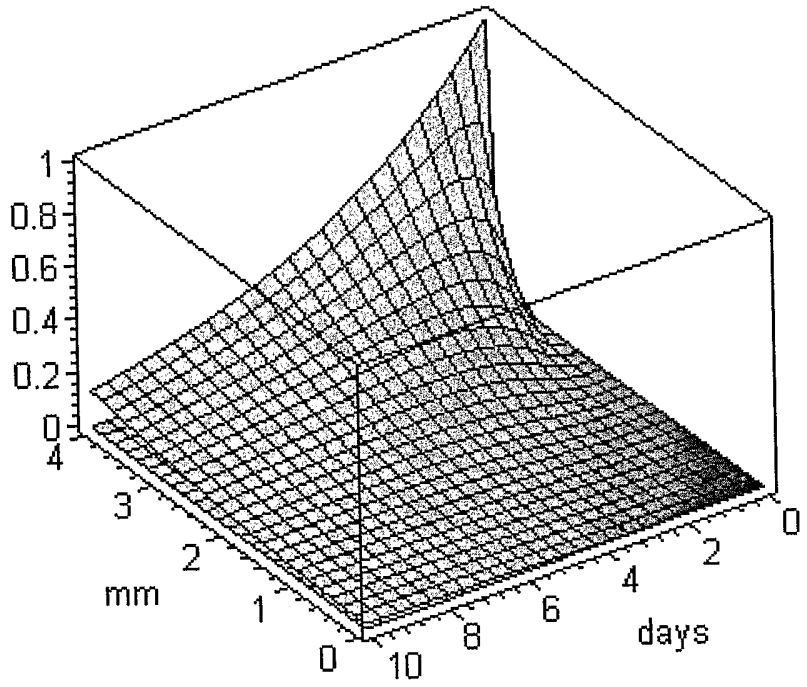


Fig.5 Normalised dermal hemoglobin density vs. distance from the basal layer (mm) and time after injury (days)

$N_{H0} = 1, d = 4mm, D_H = 3 \cdot 10^{-12} m^2 / s, \tau_H = 5days$

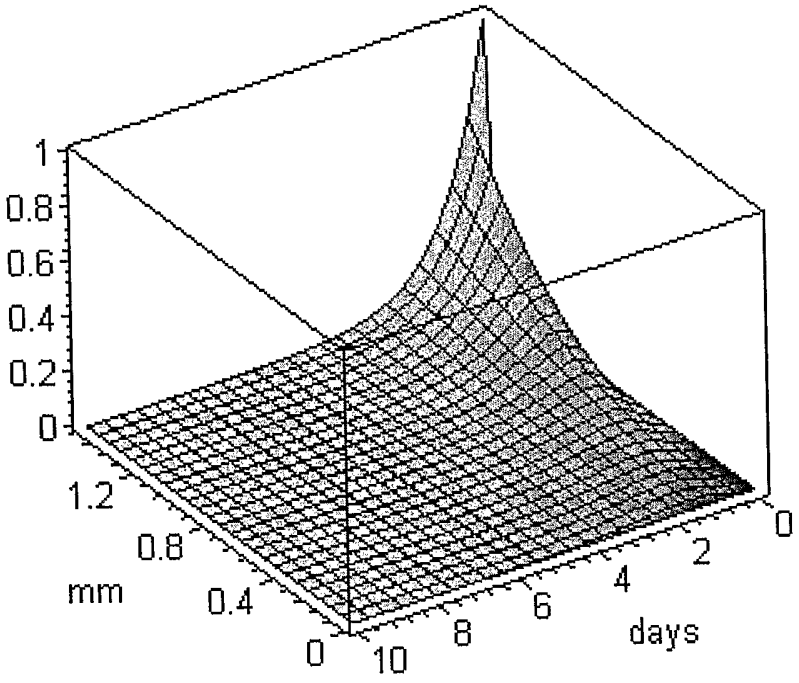


Fig.6 Normalised dermal hemoglobin density vs. distance from the basal layer (mm) and time after injury (days)
$N_{H0}=1, d=1.5mm, D_H=3\cdot10^{-12}m^2/s, \tau_H=1day$

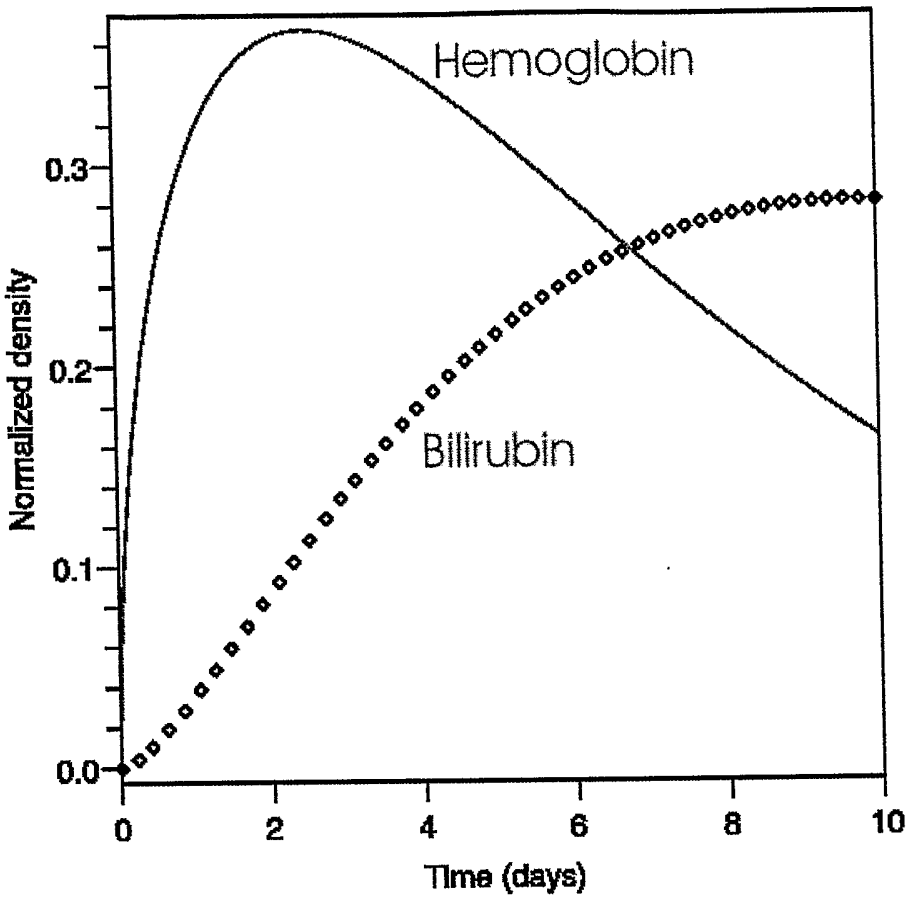


Fig.7 Normalised average hemoglobin density v. time after injury

Skin thickness d = 1.5mm, $D_H = 3 \times 10^{-12}$ m^2/s, $D_B=0$, $\tau_H = 5$ days, $\tau_{BG} = 6$ days, $\tau_{BR} = 10$days

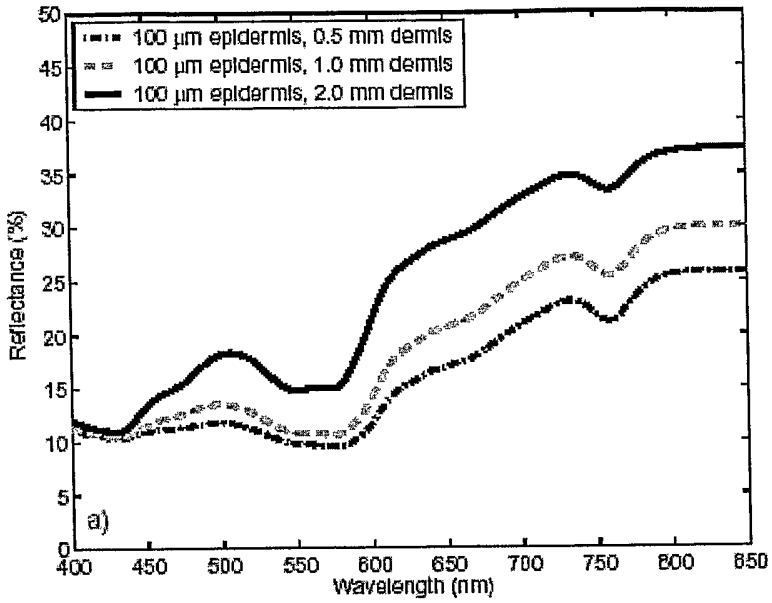


Figure 8. Simulated reflectance spectra for caucasian skin showing the effect of varying the dermal thickness, 3 days after injury.

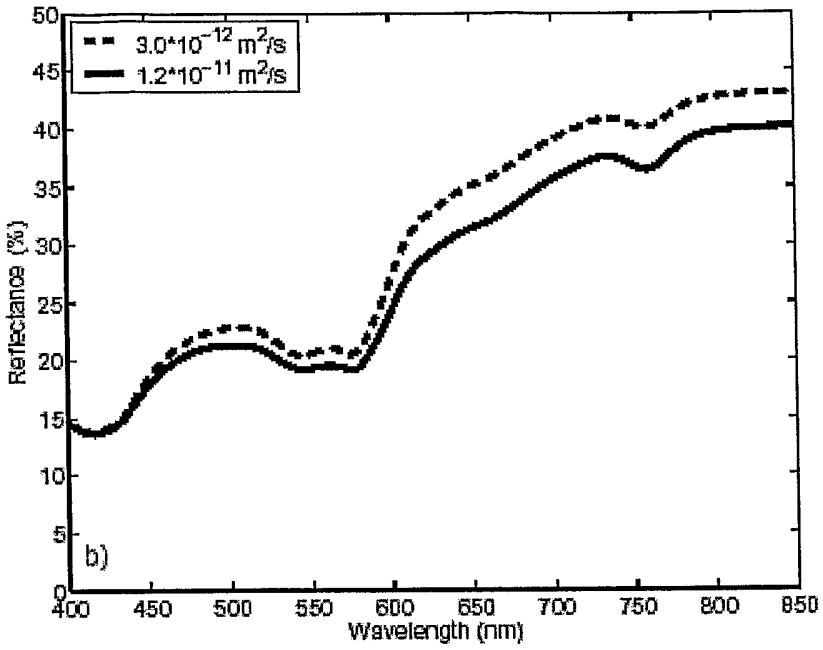


Figure 9. Simulated reflectance spectra for Caucasian skin demonstrating the effect of varying hemoglobin diffusivity, 3 days after injury.

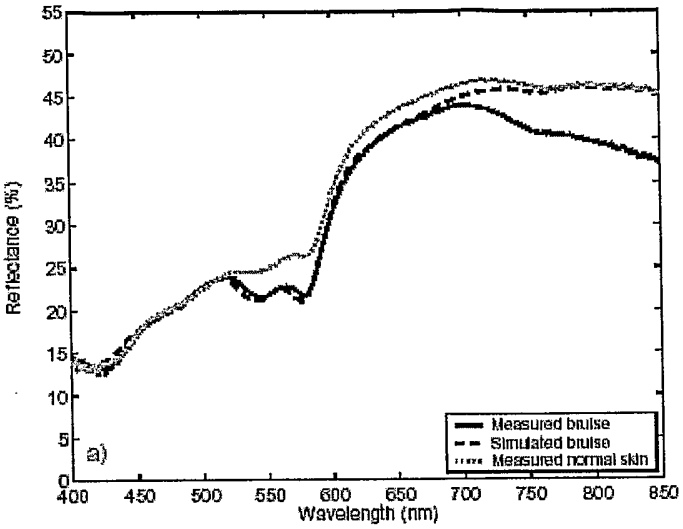


Figure 10a. Measured and simulated reflectance spectra from patient no. 8

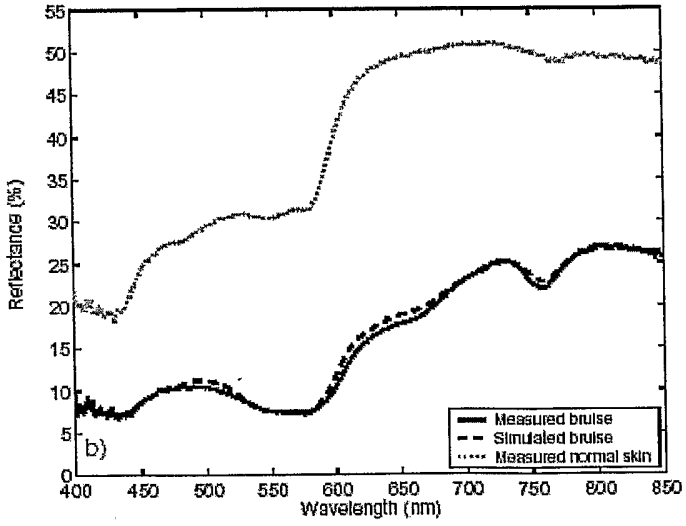


Figure 10b. Measured and simulated reflectance spectra from patient no. 1.

Figure 11. Fitting parameters and results

T = true age of injury
Δt = error in estimated age relative to true age
Background blood volume in dermis was assumed to be 0.01 in all cases
Background dermal oxygenation (layers 2 and 3) was assumed to be 0.8
N_{H0} = initial volume fraction of the blood pool
$\mu_{a,mel}$ = melanin absorption per meter at 694 nm wavelength

Patient	Oxygenation		Skin thickness		N_{H0}	$\mu_{a,mel}$	T	Δt
	Layer 1	Injury	dermis (mm)	epidermis (μm)				
1	0.4	0.4	1.5	120	0.8	490	5 d	0 d
2	0.4	0.2	1.0	100	0.5	460	3 d	+ 1 d
3	0.3	0.5	0.8	50	0.55	580	2 d	0 d
4	0.3	0.8	1.0	100	0.3	500	1 h	+ 1 h
5	0.4	0.4	1.0	100	0.9	475	7 d	0 d
6	0.3	0.3	0.8	30	0.6	500	6 d	- 1 d
7	0.4	0.8	1.0	100	0.4	300	12 h	0 h
8	0.4	0.8	1.2	100	0.3	500	4 h	0 h
9	0.4	0.5	1.5	100	0.3	495	2 d	-0.5 d
10	0.3	0.4	1.5	120	0.25	434	2 d	0 d
11	0.5	0.2	1.5	100	0.55	488	2 d	0 d
12	0.3	0.3	1.5	80	0.2	490	2 d	+1 d
13	0.3	0.3	1.0	80	0.35	484	6 d	-1 d
14	0.4	0.2	1.0	80	0.7	497	20 d	-6 d
15	0.5	0.8	1.0	100	0.2	498	4 h	+2 h
16	0.4	0.2	1.0	100	0.5	467	1 d	0 d
17	0.3	0.3	1.0	50	0.7	431	2 d	0 d
18	0.2	0.2	1.0	80	0.9	494	23 d	0 d
19	0.6	0.3	1.0	100	0.5	480	6 d	0 d
20	0.35	0.3	1.0	80	0.35	491	12 h	+6 h
21	0.5	0.5	1.0	80	0.6	488	4 h	-1 h
22	0.4	0.2	1.5	120	0.2	598	2 d	-1 d
23	0.4	0.2	1.0	100	0.4	479	1 d	0 d
24	0.2	0.3	1.0	80	0.65	469	2 d	0 d
25	0.5	0.2	1.0	50	0.9	418	3 d	0 d
26	0.3	0.2	1.5	120	0.6	491	3 d	0 d

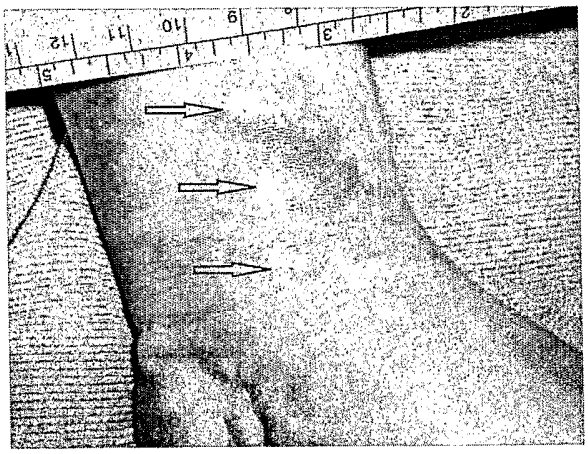


Figure 12. White spots in a bruise resulting from intra-vascular intervention.
The perforation sites can be seen in the center of the white zones. The photo was taken two days after injury. The patient was a 41 years old male (patient 3), and the bruise was located at the volar side of the left arm.

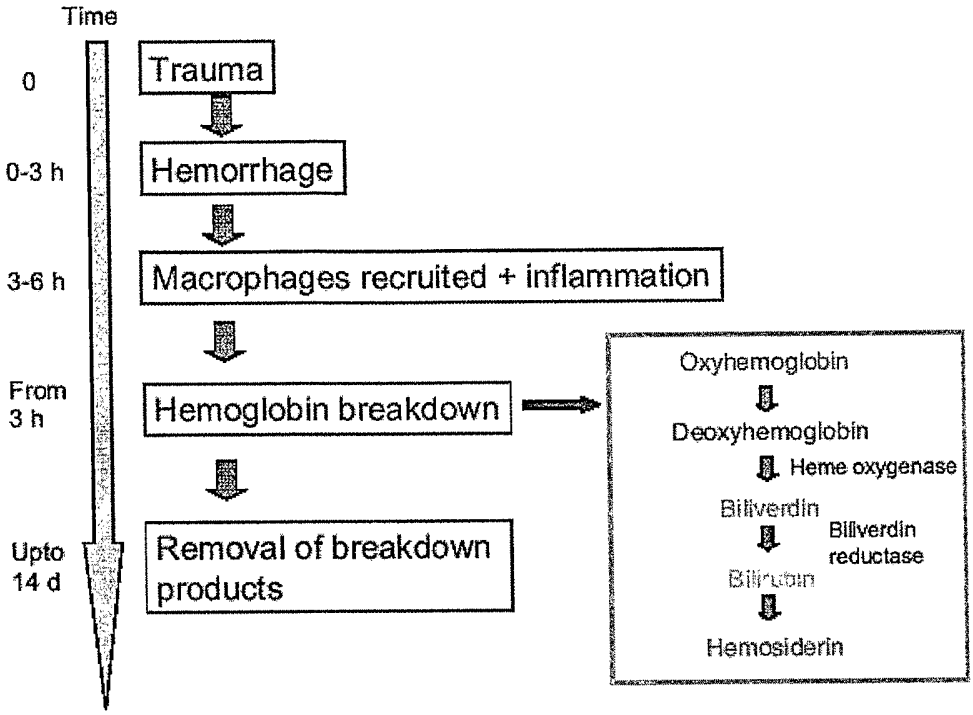


Figure 13. Schematic of chemical/physiological processes occurring when a bruise forms. The time scale shown is not absolute, and the values are chosen to indicate a typical example. Hemosiderin is formed from the iron released from hemoglobin, the color becomes visible in the last phase of the bruise.

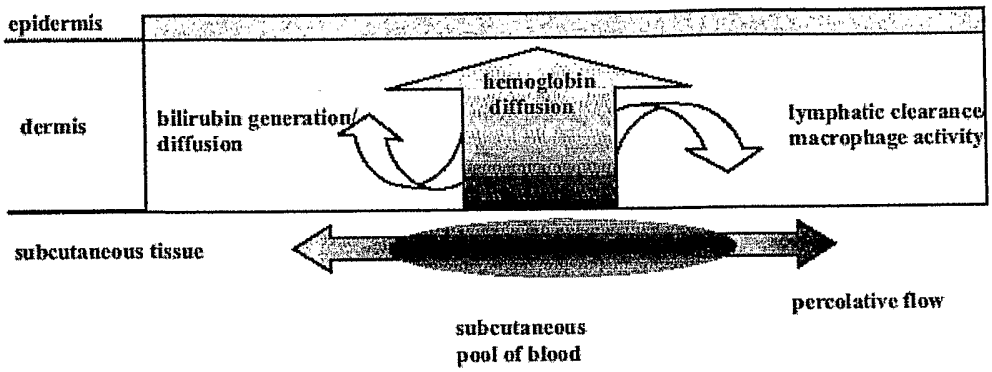


Figure 14. Processes modelled in order to create the preferred model relating hemoglobin and bilirubin density with time.

Layer	Normal skin	Bruise
Epidermis	Melanin from reflectance BVF 0.2% Oxygenation as upper dermis	Melanin and intravascular BVF as background Oxygenation as upper dermis
Upper dermis	BVF from reflectance Oxygenation from reflectance (λ_1/ λ_2 = 660nm/805nm)	Intravascular BVF 1%, with oxygenation from absorption (λ_1/ λ_2 = 542nm/548nm) + extravascular BVF estimated by diffusion model, with oxygenation given in Fig 11 or estimated from reflectance spectra. Bilirubin estimated by diffusion model
Deeper dermis	BVF 1% Oxygenation as upper dermis	Intravascular BVF 1% with oxygenation 80% + extravascular BVF estimated by diffusion model, with oxygenation given in Fig 11 or estimated from reflectance spectra. Bilirubin estimated by diffusion model.

Figure 15. Parameters used in a preferred embodiment of the photon transport model.

The thickness of upper dermis is assumed to be 20% of the total dermal thickness.
The thickness of dermis and epidermis were varied according to body site.
BVF = blood volume fraction.