Three dimensional modeling of bruise evolution for improved age determination
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Citation for published version (APA):
Stam, B. (2012). Three dimensional modeling of bruise evolution for improved age determination
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Summary of thesis

In this thesis we explored several aspects of bruise modeling in order to better understand the spatial and temporal behavior of bruises. This knowledge may lead towards a method for non invasive, accurate, objective age determination of bruises as aid in determining child abuse.

First, we built a 3D model that allowed bruises of all shapes and sizes, and used our model to explore the influence of factors such as bruise size and skin diffusivity on the healing of bruises (chapter 2). We were able to simulate spatial differences in bruises, e.g. between center and edge locations in bruises, and found that the development of a homogeneous, circular symmetric bruise is different on the edge than in the center, which is especially important when non homogeneous shaped bruises are simulated. This finding opened up a new method of age determination; in chapter 3 we showed how the color inhomogeneity in bruises can be used to determine the age of the bruise based on multiple measurements on a single bruise.

Next we looked at different input configurations of the subcutaneous blood pool at the onset of the bruise. A good estimation of not only the shape, but also the concentration distribution proved to be important (chapter 4), as kinetics greatly differ for e.g. an irregular shaped bruise that roughly resembles an ellipse and a smooth edged ellipse (figure 4.2).

For any model to be reliable, knowledge of the parameters in the model is required. In chapters 2 and 3 we showed that tissue specific parameters hemoglobin diffusivity, the concentration of Heme Oxygenase-1 and the relaxation time of bilirubin can be determined from multiple measurements of the areas of hemoglobin and bilirubin in the bruise. In chapter 5 we explored if group averages of these model parameters in three groups of bruises measured once were comparable. We showed that, within measurement uncertainty, no difference exists between these group averaged sets of parameters in medial and lateral arm bruises and lateral leg bruises. In chapter 6 we looked at differences in spectroscopic properties for these three groups of bruises. We first found comparable time behavior of groups of bruises measured once. Second, we found no spectral differences between medial and lateral arm bruises.

The recognition and handling of abuse in a clinical setting was discussed in chapter 7. We argued that having a multi-disciplinary team in each hospital ensures a better recognition and approach of child abuse.

In this discussion section we will further discuss some aspects of bruise modeling and look to the future of age determination of bruises.

Estimating the configuration of the initial blood pool

A good estimation of the shape, size and concentration distribution of the initial blood pool is important for accurate age determination of the bruise. As we showed in chapter 4, a gaussian concentration distribution in the initial blood pool results in different healing kinetics as compared to a homogeneous distribution. By following the bruise in time the irregular concentration distribution can be estimated and
accurate age determination achieved. In clinical, irregular bruises, the input shape, size and concentration distribution are estimated from the bruise at the time of first measurement. When using hyperspectral imaging the concentration distribution is obtained from the fits of the chromophores; figure 3.3 shows a cross section of the relative hemoglobin concentration maps at three different times. We use that same data from chapter 3, and now display the relative bilirubin concentration map for the 120.5 h old bruise (figure 8.1A). To display the differences in this relative concentration in 3D, we obtained a surface plot from this image (figure 8.1B)(compare to figure 3.3, where a cross section is shown) The increased bilirubin concentration is clearly visible in the higher intensity in the center of the bruise.

Figure 8.1. A. Relative bilirubin concentration map at 120.5 h. B. Surface plot of relative concentration map z-axis = fitted bilirubin coefficient.

Determining the concentration distribution from photographs is not possible e.g. due to differences in lighting on curved body parts, but can be obtained otherwise; as shown in figure 2.7A, the input shape determined from the photos is a collection of small point bruises. When this irregular shape with discrete hemoglobin points is used as initial blood pool, the point bruises approximately become gaussian shaped within hours due to diffusion, thus approximating an initial gaussian concentration distribution, and possibly allowing accurate age determination of the bruise.

Further aspects of bruise modeling

Point bruise convolution
Several times the question was raised whether a bruise of a certain shape can be represented by a convolution of the response to point bruises, i.e. the theory of Green’s functions. Although figure 4.1 already showed that different geometries give different healing kinetics, the point bruise convolution remained appealing from its simplicity. We approximate a delta function bruise by using a single element filled with
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hemoglobin as input in our model (0.0625 mm$^2$). We simulate the response with this point bruise, i.e. the point spread function, and convolute the point spread function over the shape of the hemoglobin blood pool at t=0 h of an elliptic bruise with a ratio of the major/minor axes of 4:1, and with the length of the major axis 50 mm. When we compare the behavior of the convolved bruise versus the fully simulated bruise (figure 8.2), we clearly see that for the convolved hemoglobin area goes to zero in 10 h, while the area of the fully simulated bruise go to zero after 530 h, The convolved bilirubin area grows rapidly to a maximum close to the maximum in the real ellipse, and goes to zero after 504 h, which is earlier than in the fully simulated ellipse (807 h).

![Figure 8.2. Pattern of healing for ellipse B (gray) and the convolution of the point bruise over ellipse B (black).](image)

**Age determination of older bruises**

In a clinical setting, bruises sometimes may be presented at considerable age, when hemoglobin in the bruise is already (almost) converted. As the shape of the bilirubin area has a direct relation with the shape of the former hemoglobin area, possibly an estimation of the bruise' age can be made using this phenomena. We test this for elliptic bruises with known parameters; we define 5 hemoglobin shapes with varying eccentricity (ratios of the minor/major axes 1:1, 2:1, 4:1, 6:1 and 8:1) and varying length axes (25, 50 and 75 mm), creating a set of 15 different hemoglobin shapes. Each shape is used as input in the model, and 15 simulations are performed. When we plot the relation between the ratio of the major/minor bilirubin axes at the point in time where hemoglobin is 0 and the bilirubin area at that same time point (figure 8.3A), we can determine the length of the major hemoglobin axis at t=0 h for an ‘unknown’ bruise; e.g. if the ratio of the major/minor bilirubin axes at t=(Hb=0) is 1.57, and the bilirubin area is 1750 mm$^2$, the major hemoglobin axis at t=0 h was 50 mm. Using figure 8.3B, where the ratio of the major/minor bilirubin axes at t=(Hb=0) is plotted for the various lengths of the major hemoglobin axis at t=0h, we can determine the ratio of the major/minor hemoglobin axes at t=0 h; e.g. if the ratio of the major/minor bilirubin axes at t=(Hb=0) is 1.57, and the length axis at t=0 h was 50 mm, the ratio of the major/minor hemoglobin axes at t=0 h was 1:2 for the ‘unknown’ bruise. Because
the ratio and length at t=0 h is now known, a simulation can be done with the known parameters and the unknown age of the bruise can be determined.

Clinically this may only be relevant if the method is not very sensitive to the model parameters. After all, if the lines in figure 8.3 would shift when e.g. the diffusivity changes, determining the age of a bruise of unknown parameters would not be possible. To test this, we create a new set of simulations for the shapes with input length of the major hemoglobin axis 50 mm with the diffusivity 2 times higher, and add these results to figure 8.3, obtaining figure 8.4. Interestingly, the relation between the ratio of the major/minor bilirubin axes at t=(Hb=0) and the bilirubin area at t=(Hb=0) for the 50 mm hemoglobin input length is very similar for the 2 diffusivities (figure 8.4A). But, when we now attempt to determine the ratio of the major/minor hemoglobin axes at t=0 h, the higher diffusivity gives a different answer than the normal diffusivity; e.g. if the ratio of the major/minor bilirubin axes at t=(Hb=0) is 2.1 (rounded off), figure 8.4A shows that the length of the major hemoglobin axis at t=0 h was 50 mm. From figure 8.4B the ratio of the major/minor hemoglobin axes at t=0 h could have been 4:1 or 8:1 for the normal or higher diffusivity respectively. This indicates that knowledge of the parameter diffusivity is necessary for this method of age determination.
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Can the eccentricity be used in age determination?

However, if we do know the parameters (such as diffusivity) for a clinical, irregular shaped bruise, can we then determine the age of the bruise using an assessment of the eccentricity? We use the first bruise from chapter 3, a clinical bruise with irregular shapes edges roughly approximating an ellipse with length 4.5 cm and width 1cm at t(Hb=0) and known parameters (eccentricity 4.5). As one could argue that this clinical bruise is comprised of 2 separate ellipses (inlay in figure 8.5), we also use a subset of this bruise with eccentricity 2.86. We create a set of simulations with these parameters and various eccentricities which results in figure 8.5. When we now place the clinical bruise in this figure, we can appreciate that this clinical bruise cannot be approximated with an ellipse, in that the irregular edges have a large influence on the healing, again showing the need for a model that incorporates spatial variation.

Figure 8.4. A. Ratio of the major/minor bilirubin axes at t=(Hb=0) versus bilirubin area at the same time point for data set with different hemoglobin input lengths (grays). Black: data set of 50 mm hemoglobin input length with 2 times higher diffusivity. B. Ratio of the major/minor bilirubin axes at t=(Hb=0) versus ratio of the major/minor hemoglobin axes at t=0 h for data set with different hemoglobin input lengths (grays). Black: data set of 50 mm hemoglobin input length with 2 times higher diffusivity.
Confluent bruises

When a victim is struck twice in the same location with some time between the beatings, a confluent bruise is formed. In chapters 2 and 4 we very briefly stated that our model allows a second bruise to be inserted on top of another bruise halfway during the simulation, but that without more knowledge into the pathophysiology this may be difficult. Here we simulate a confluent bruise in a first attempt, and compare to cross shaped bruises to assess the error in age estimation if the confluidity is not recognized. We define an ellipsoid basic shape with a 4:1 ratio of the major/minor axes, and a 50 mm length of the major axis (same as bruise B in chapter 4). Next we use this shape to define 3 bruises; the first is a confluent cross shaped bruise (A), composed of identical ellipses, the first inflicted at t=0 h, and a second ellipse at t=48 h, perpendicular to the first. The second is cross shaped bruise (B), composed of two identical ellipses perpendicular to each other, both inflicted at t=0 h. The third is cross shaped bruise (C), composed of two ellipses perpendicular to each other, both at t=0 h, where one ellipse has a 50% lower initial hemoglobin concentration than the other to mimic that the concentration of the first ellipse is lower than the second ellipse when this is added. We simulate these bruises with a standard set of parameters as in chapter 4. The patterns of healing for the confluent cross shaped bruise A, the cross shaped bruise B and the inhomogeneous cross shaped bruise C are shown in figure 8.6. Although the maximum hemoglobin and bilirubin areas are the same for bruises A and B, i.e. the bruises with equal hemoglobin content, the pattern of healing differs, especially after 500 h, indicating that a cross shaped bruise cannot describe the behavior of confluent cross shaped bruises. In addition, even the pattern of healing for second cross shaped bruise C with unequal concentrations of hemoglobin also differs from that of the confluent cross shaped bruise with equal hemoglobin content. These
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differences are important as they may be used clinically to aid distinguishing between two bruises inflicted at different times and a similar form of bruise inflicted at (virtually) one point in time.

Figure 8.6. Pattern of healing for confluent cross shaped bruise A (ellipse as input at t=0 h and perpendicularly at t=48 h) (black), cross shaped bruise B (two ellipses perpendicular to each other at t=0 h) (red), and cross shaped bruise C (two ellipses perpendicular to each other at t=0 h with inhomogeneous concentrations of hemoglobin) (blue).

Fiber measurements versus hyperspectral imaging
In order to aid in the diagnosis of child abuse, the technique to determine the age of bruises should be simple to use and cheap. Spectroscopy fills these requirements, and we have used photography and a simple fiber based spectroscopic system to measure the remittance spectra of all our bruises. From a fit of the measured spectra we can obtain hemoglobin and bilirubin fit coefficients, and subsequently a ratio of these fit coefficients. Chapter 6 showed an interesting temporal relation between hemoglobin and bilirubin in one location, which may be included in the age determination process. In our model simulations, we use hemoglobin and bilirubin concentrations, and can easily obtain the hemoglobin/bilirubin ratio in a single location. Comparing this simulated concentration ratio a fit coefficient ratio should be performed with caution, as this requires a translation of fit coefficients to actual concentrations. Encouragingly, our model gives a similar temporal relation for the hemoglobin/bilirubin ratio. At this stage however, the quality of the measured data is not sufficient to be used in a method to determine the age of the bruise. Accurate positioning of the probe between measurements is a prerequisite for further development of this method in the age determination of bruises. We can approximate accurate positioning of a fiber probe by defining a region in the spectral camera measurements and looking at the hemoglobin/bilirubin ratio in this region over time. In this way, we look at an average of a small region and possibly eliminate ‘positioning’ differences. We use the first bruise from chapter 3 and create a mask containing the pen stripes and a birthmark (thick red lines and dot in figure 8.7). Next we manually rotate and
The temporal behavior of the ratio of hemoglobin over bilirubin coefficients from the camera measurements is matched visually to the temporal behavior from the fiber measurements (figure 8.8). The difference in magnitude between the camera and fiber measurements can be attributed to different probed volumes; in a fiber measurement a small volume directly below the fiber is measured, where relatively much blue light is measured and much red light is lost. In a camera measurement all red and blue remitted light is measured. Another explanation lies is the difference of the fit procedure for these two types of measurements: for the fiber measurements healthy skin is used as a reference, where for the camera measurements a stack of white paper is used as reference.

These data show that although a clear temporal component is present, measurement techniques need to improve before this information can be useful in the determination of the age of the bruise. Interestingly, the quality of the fiber data is comparable to that of the hyperspectral imaging data, which may imply that the cheap fiber system could be clinically applied instead of the expensive hyperspectral imaging system. However, if future research would show that a “hyperspectral” imaging system that only uses 3 wavelengths is sufficient, this would reduce the cost of the system greatly, and one could think of an application where cheap filters are placed in front of a standard photo camera.
Combination of spectroscopy and bruise modeling

Although we showed that group averages of model parameters have similar values, previously determined parameters for a lateral arm bruise (chapter 3) lie outside this group range. Determining model parameters for each bruise individually may be necessary. A combination of spectroscopy and bruise modeling may lead to an improved age determination, as has been suggested by Hughes et al [1]. Current methods of age determination using modeling of bruises either ignore spectroscopic information (while taking spatial differences into account) [2], or ignore spatial differences (while taking spectroscopic information into account) [3]. We speculate that this combining these techniques could not only lead to improved age determination, but also provide a method to determine model parameters for individual bruises.

Here we make a first attempt to combine areas of hemoglobin and bilirubin and spectroscopic information, thus combining Chapters 5 and 6, in order to determine the 3 model parameters $D_{\text{Hb}}$, $[\text{HO}]$, and $\tau_{\text{B}}$ from single measurements from each bruise individually. We use 5 measured variables for each individual bruise: the $\alpha_{\text{Hb}}/\alpha_{\text{Bili}}$ ratio in three locations (from Chapter 6), and the hemoglobin and bilirubin areas (from Chapter 5). We indicate the locations of the spectroscopic measurements in the hemoglobin/bilirubin image (figure 8.9C).

Figure 8.8. Temporal behavior of the ratio of hemoglobin coefficient over bilirubin coefficient.

Figure 8.9. A. Photograph of 21.6 h old bruise located on lateral side of upper arm of a 27 year old woman (colors enhanced). B. Hemoglobin area determined from photograph and used as input in the subcutaneous layer at $t=0$h. C. Hemoglobin (red) and bilirubin (yellow) determined from photograph, overlap of hemoglobin and bilirubin indicated in orange. Black boxes indicate locations of measurements.
From a simulation with our model we also obtain these 5 variables, and thus we can determine the model parameters $D_{\text{Hb}}$, [HO] and $\tau_B$ for each bruise individually as follows; using a simplex fitting algorithm these 3 parameters in the simulation were varied until the simulated hemoglobin and bilirubin areas and the three simulated $\alpha_{\text{Hb}} / \alpha_{\text{Bili}}$ ratios in the measurement locations most closely matched the measured areas and $\alpha_{\text{Hb}} / \alpha_{\text{Bili}}$ ratios. As the determination of the bilirubin area from the photographs had a larger error than the other 4 variables, this was given a lower weight in the simplex fit, so that the fit was less dependent on the bilirubin area. A schematic of this optimization procedure is given in figure 8.10.

![Figure 8.10. Schematic of parameter optimization for individual bruises from a single measurement](image)

For validation of the method, we first use a model bruise with known parameters, and, second, a clinical bruise with known parameters.

**Validation model bruise (numerical phantom)**
A circular model bruise with a 2 cm diameter is defined with $D_{\text{Hb}} = 1.5 \times 10^{-9}$ m$^2$/h, [HO] = 5 mg/L and $\tau_B = 150$ h. A simulation is completed to an age of 10 h, and the $\alpha_{\text{Hb}} / \alpha_{\text{Bili}}$ ratios in three locations, as well as the hemoglobin and bilirubin areas, are obtained at 10 h. We then pretend that these 5 variables were measured in a bruise of unknown shape, size and with unknown parameters. We indicate the locations of the three $\alpha_{\text{Hb}} / \alpha_{\text{Bili}}$ ratios in the area image with a margin of 10 pixels (as in figure 8.9C), and determine a new input hemoglobin shape and size at $t=0$ h from the model photograph at 10 h (as in chapter 2). The simplex fit routine needed 35 iterations to converge to a
stable result: The parameters were determined to be $D_{Hb} = 1.52 \times 10^{-9}$ m$^2$/h (deviation 1.4%), $[HO] = 5.2$ mg/L (deviation 3.9%) and $\tau_B = 102$ h (deviation 32%).

Validation clinical bruise (with known parameters)
A clinical bruise located on the upper arm of a 27 year old woman was described previously [1], where the 3 parameters were determined by varying the parameters until the model’s hemoglobin and bilirubin areas matched the measured hemoglobin bilirubin areas for all measured time points: $D_{Hb} = 2 \times 10^{-9}$ m$^2$/h, $[HO] = 15$ mg/L and $\tau_B = 40$ h. Although we did obtain measurements at various times from this bruise, for this analysis we only use the single measurement at 21.6 h. We determine the subcutaneous hemoglobin input blood pool, and the hemoglobin and bilirubin areas from the image at 21.6 h. Using our fiber based system, we measure the remittance spectra of three locations, and determine the $\alpha_{Hb}/\alpha_{Bili}$ ratios. We indicate the locations of the measurements in the hemoglobin and bilirubin image (figure 8.9C). The parameters for this clinical bruise cannot be determined because the range of simulated $\alpha_{Hb}/\alpha_{Bili}$ ratios in the indicated locations is too large; 0.001-10.4, while the measured ratios at 21.6 h are 2.5 for the center and 2.14 for an edge location (figure 5A). This large range is due to the large ranges of hemoglobin and bilirubin concentrations simulated; i.e. the range of simulated bilirubin in the center location is $1.21 \times 10^{-8} - 2.06 \times 10^{-5}$ μmol per compartment. We also attempt to perform this optimization in other arm bruises with unknown parameters, which also is not possible at this point.

The parameter determination from a single measurement is quite accurate for the model bruise. The uncertainty in the determination of $\tau_B$ is much larger because the bilirubin area, the only factor affected by $\tau_B$, was given a lower weight in the fit procedure. However, when we attempt the same procedure for a single measurement in a validation clinical bruise (with known parameters), we are not able to determine the parameters, as the range of simulated $\alpha_{Hb}/\alpha_{Bili}$ ratios is quite large, and cannot be optimized by varying the parameters. Nevertheless, we have previously shown that multiple area measurements in time do allow determining these parameters. The incorporation of spectroscopic information in the parameter determination for a single measurement is probably at this stage not possible because of two reasons. First, the indication of the measurement locations (figure 8.9C), in which the ranges of $\alpha_{Hb}/\alpha_{Bili}$ ratios are determined is not sufficiently precise; the indicated areas are quite large, and thus the ranges of $\alpha_{Hb}/\alpha_{Bili}$ ratios within these areas too. For future fiber measurements, a more accurate documentation of the exact measurement locations may lead to smaller ranges of simulated $\alpha_{Hb}/\alpha_{Bili}$ ratios. Second, the range of simulated $\alpha_{Hb}/\alpha_{Bili}$ ratios is much larger than measured $\alpha_{Hb}/\alpha_{Bili}$ ratios, a consequence of the large range in the simulated hemoglobin and bilirubin values. Increased or decreased values of the 3 parameters did not decrease the range of these simulated values, nor did imitating a larger measurement volume by taking the average of several compartments. We speculate that incorporating the concentration distribution of hemoglobin in the initial blood pool may improve this method (Chapter 4), as this
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causes smaller differences in initial hemoglobin concentration between compartments, and thus smaller differences during simulation, leading to a smaller range of simulated values. Determining the concentration distribution is possible when a hyperspectral imaging system is used, but unfortunately the quality of our photographs, although taken with a high quality camera and under controlled conditions, is not sufficient to obtain the concentration distribution. We believe that our method to determine bruise parameters from a single measurement has great potential, but requires further research.

Gravity
Gravity was so far not included in the model, although the model does allow having a diffusivity proportional to direction. Sagging of bruises is most visible in the skin around the eyes, but most often the bruises do not sag further than the edge of the loose tissue (figure 8.11). Loose tissue on other locations on the body (elastosis) is most often seen on older individuals, but may also occur due to sun damage or heavy smoking [4].

![Figure 8.11. Bruised eye of a 48 year old man. Bruise originated when falling off a mountain bike.](image)

In the majority of the 563 bruises we observed, gravity did not play a (visible) role, but in some its role was undeniable. E.g.; we observed a bruise with dimensions of about 12*12 cm located on the chest of a 46 year old male (figure 8.12). This bruise was formed when he was punched in the eye with a knuckle-duster (iron object), and in the days following this abuse the bruise sagged downwards towards his chest, where we observed the bruise at day 12. The bruising around the eye was completely resolved at this time.
If gravity is incorporated in the model, one needs to take into account that the direction in the body changes with position during sleep, walking, eating, etc. For example for a bruise located on a forearm, the direction changes as the arm hangs vertically alongside of the body during walking, but horizontally during typing, reading etc. These many uncertainties in the direction of gravitational pull may make modeling of these bruises cumbersome, but we can also use these bruises to assess the diffusivity of the hemoglobin and bilirubin by following the bruise over time. This will provide knowledge of the diffusion in loose tissue, and possibly the difference in diffusion between hemoglobin and bilirubin can be obtained.

**Future of bruise research**

For accurate, objective age determination of bruises to become possible several more steps need to be taken. The pathophysiology of bruises is not yet completely understood, although literature research has provided some clues. Histological images of bruises may improve our knowledge of the pathophysiology, as well as give insight into the degree of injury of the bruised tissue. The latter may be of importance in assessing the diffusivity; chromophores are expected to diffuse faster in injured tissue where normal barriers like retinacula have disappeared. Another important factor in the pathophysiology is the size difference between the hemoglobin/macrohage complexes and bilirubin that causes the bilirubin to diffuse faster. The exact mechanism is not yet known; the macrophages engulf both erythrocytes and free hemoglobin [5-6], indicating that some hemoglobin molecules can diffuse freely through the tissue before phagocytosis occurs. These free hemoglobin molecules are smaller than the macrophages, and thus their diffusion is faster, which may explain why the hemoglobin area in a bruise rapidly becomes larger at onset, and once sufficient macrophages
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are recruited and all free hemoglobin is phagocytosed, hemoglobin diffusion slows
down so the hemoglobin area ceases to grow further. However, this latter effect can
also be explained through the conversion of hemoglobin to bilirubin. After release of
the bilirubin into the interstitial fluid, the bilirubin molecules can diffuse freely or be
bound to albumin [7-10], before their clearing through the lymphatic system [11-12].
As albumin is a large protein, much larger than the free bilirubin, this also has an effect
on the speed of the diffusion. So far, the ratio between bound and unbound bilirubin in
the tissue is unknown, and can possibly be investigated using remittance spectroscopy,
because the conformation change in bilirubin when it binds to albumin causes
differences in the absorption spectrum [13-15]. Most importantly, the difference
in diffusion between the hemoglobin and bilirubin should be established. We have
assumed a 4 times larger diffusivity of bilirubin compared to hemoglobin. Although this
value was not confirmed otherwise, our model was able to capture some essentials
of bruise behavior. Here, we refer to the statement that mathematical models can
only be “a deliberate oversimplification that can serve as a point of departure for
understanding a much more complicated reality” (authors reply to [16]).

Conclusion

In conclusion, we were able to accurately determine the age of bruises utilizing the
tempo-spatial variations in bruises. A good estimation of the configuration of the
initial subcutaneous blood pool will be essential for accurate modeling and thus
accurate age determination. Recognizing that spatial variations in chromophore
concentration have a major impact in the modeling of bruises brings the process of
developing a method for accurate age determination of bruises closer to realization. A
combination of spectroscopy and modeling of bruises is the way forward.
References