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3D-perfusion analysis of burn wounds using hyperspectral imaging

Q1 Jörg Marotz [a,*], Torsten Schulz [a], Sebastian Seider [a], Diogo Cruz [c], Ahmed Aljowder [d], Dominik Promny [e], Georg Daeschlein [d], Thomas Wild [b,c,d], Frank Siemers [a]

[a] Klinik für Plastische und Handchirurgie und Brandverletztenzentrum, BG-Klinikum Bergmannstrost, D-06002 Halle (Saale), Germany
[b] University of Applied Science Anhalt, Institute of Applied Bioscience and Process Management, Germany
[c] Clinic of Plastic, Hand and Aesthetic Surgery, Medical Center Dessau, University of Applied Science Anhalt, Germany
[d] Clinic of Dermatology, Immunology and Allergology, Medical Center Dessau, Medical University Brandenburg "Theodor Fontane" Medical Center Dessau, Germany
[e] Klinik für Plastische, Wiederherstellende und Handchirurgie, Zentrum für Schwerbrandverletzte, Klinikum Nürnberg, Q2 D-90471 Nürnberg, Germany

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ABSTRACT

Background: Determination of the depth of burn wounds is still a challenge in clinical practise and fundamental for an optimal treatment. Hyperspectral imaging (HSI) has a high potential to be established as a new contact-free measuring method in medicine. From hyperspectral spectra 3D-perfusion parameters can be estimated and the microcirculatory of burn wounds over the first 72 h after thermal injury can be objectively described.
Methods: We used a hyperspectral imaging camera and extended data processing methods to calculate 3D-perfusion parameters of burn wounds from adult patients. The data processing results in the estimation of perfusion parameters like volume fraction and oxygenation of haemoglobin for 6 different layers of the injured skin. The parameters are presented as depth profiles. We analyzed and compared measurements of wounds of different degrees of damage and present the methodology and preliminary results.
Results: The depth profiles of the perfusion parameters show characteristic features and differences depending on the degree of damage. With Hyperspectral Imaging and the advanced data processing the perfusion characteristics of burn wounds can be visualized in more detail. Based on the analysis of this perfusion characteristics, a new and better reliable classification of burn degrees can be developed supporting the surgeon in the early selection of the optimal treatment.

* *Corresponding author.*
E-mail addresses: joerg.marotz@ipross.de (J. Marotz), diogo.cruz@klinikum-dessau.de (D. Cruz), ahmedaljowder@hotmail.com (A. Aljowder), dpromny@gmail.com (D. Promny), thomas.wild@woundconsulting.com (T. Wild), frank.siemers@bergmannstrost.de (F. Siemers).
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1. Introduction

Q3 External heat impact, by flames, liquids or hot surfaces, causes Q4 thermal injuries. The degree of damage depends on temperature and duration of impact. 1st degree burns (superficial) heal by conservative treatment, 3rd degree burns (fill thickness) have to be treated operatively, because the destroyed structure, microcirculation and denatured tissue has no more inherent healing potential. At 2nd degree burns (partial thickness) the physician has to estimate the residual healing potential to decide between conservative and operative treatment. We denote wounds that have to be treated operatively (according to modern standards of burn treatment) as 2nd degree B and the others as 2nd degree A. The reliable estimation of the degree of damage and the correspondent healing potential as early as possible is fundamental for an adequate treatment, optimized and efficient wound management and outlay and a functional and aesthetic optimized outcome for the patient.

The estimation of the degree of damage is usually still performed without any kind of objective aids or technical methods, just based on subjective impressions (colour), re-capillarisation tests, pain and personal experience. 1st degree and 3rd degree burns normally can be distinguished by experienced physicians, however 2nd degree wounds are difficult to estimate in the first three days. The literature states a reliability of approximately 40–60% [1] for the assessment of 2nd degree A versus 2nd degree B, strongly dependant on the experience of the physician.

At 2nd degree burns the estimation is impeded by a dynamic wound process in the first 2–3 days, frequently resulting in an increase of the degree of damage from A to B. Frequently at those wounds the treatment decision is deferred until the final wound state has been revealed after 3–5 days.

The extent of functionality distortion of the perfusion, from the superficial capillary system and the reticular system to the deeper arterial and venous vessels, represents a fundamental physiological basis for the healing potential of wounds. Guidelines for the treatment of burn wounds (ISBI or AWMF-DGV in Germany), demand for a long time the development and use of objective methods for the early and reliable estimation of burn depth (degree of damage).

In the last few decades Laser-Doppler Imaging (LDI) has been established as a measuring method [2–5]. With a penetration depth of the laser light of a few millimetres the method determines an averaged flow parameter describing the perfusion intensity.

Hyperspectral imaging (HSI, imaging remission or diffuse-reflectance spectroscopy) as a non-contact, uncomplicated imaging measuring method is actually an intensively developing area for diverse medical applications [6–9]. The penetration depth in biological tissue in the visible (VIS) and near infrared (NIR) spectral range enables information retrieval with high clinical value by the effect of specific scattering and absorption by the tissue components. Especially the perfusion can be assessed due to the prominence of the haemoglobin absorption in the remission spectra [10]. Thereby HSI represents a further measurement method for the analysis of the perfusion and microcirculatory disorder of burn wounds with potentially higher wound insight, a clear window with more information content. Usable for an objective and more differentiated assessment of the degree of damage resp. the healing potential [11–13,15,16].

The objectives of this work are

- The presentation of the measuring method "Hyperspectral Imaging" with respect to its use in the treatment of burn wounds,
- The basic evaluation of the information content of the measuring data with respect to the assessment of the degree of damage of burn wounds,
- The evaluation of the potential of this method for
  - Advanced analysis of the perfusion dynamics of burn wounds,
  - Development of an advanced burn degree classification as a decision support for an optimized clinical treatment.

More comprehensive studies based on representative amount of data are actually in work and should lead to the validated determination of optimized parameters and classification processes and their informative value.

2. Materials and methods

2.1. Hyperspectral measuring system

All measurements have been performed with the HSI-camera TIVITA® Tissue (Diaspective Vision GmbH, Germany). The camera is a compact measuring system certified for clinical use including an illumination unit [17,18]. Remission spectra are recorded in the spectral range from 500 to 1000 nm with a resolution of 5 nm, the measuring area is approx. 20–30 cm, standard image size is 640–480 pixel, the recording needs approx. 5 s.

The camera allows for a quick and uncomplicated measurement without the need for special measurement conditions except the avoidance of external light illumination to the analyzed area.

To ensure good qualitative and undisturbed measuring data, calibration and quality tests of the camera and the measuring data are regularly performed by the software as described in Ref. [9]. Data of insufficient quality are excluded from further processing.

2.2. Patient measurements

In a first series HSI measurements of burn wounds predominantly of 2nd degree have been measured directly after debridement (day 1) and subsequently at day 2 and 3. Actually 43 patients have been measured. Patients were included between 18 and 80 years, burned by flames or fluids and a maximal burn size corresponding to standard image size.

The clinical treatment includes primary wound cleaning, primary assessment and antiseptic dressing. After 24 h an assessment by a plastic surgeon took place and a conservative or operative therapy was established. For ambiguous assessments the decision is made after 48 h, in doubt the wound is treated operatively.

The clinical assessment and the treatment decision were not influenced by the measurement.

The data acquisition from patients was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of the Ärztekammer Sachsen-Anhalt, Germany (47/19). All patients have signed an informed consent.

2.3. Hyperspectral Imaging data analysis and processing

For the analysis of the perfusion of burn wounds a special self-developed data processing method has been used, described in detail in Ref. [11]. The corresponding software runs in parallel to the standard software of the TIVITA-camera and accesses the stored raw measuring data of the camera.

We developed an extended data processing calculating more realistic system parameters of the skin from the remission spectra by using an inverse model-based algorithm [11].

Essential features of the processing method:

- The skin resp. burn wound is modelled as a five-layer-system as described in Ref. [11]. Every layer is regarded as homogenous and is provided with the relevant components:
  - perfusion parameters: vHb denotes the relative volume fraction of total haemoglobin, $xHbO_2$ the oxygen saturation of haemoglobin;
  - additionally, layer 1 (stratum corneum, epidermis) contains melanin, Layer 2 (upper dermis: papillary or capillary system) and layer 3 (reticular dermis) collagen structure, layer 4 and 5 (deep dermis, subcutis): vH_2O and vFat denoting the volume fractions of water and fat., collagen structure and connective tissue. The parameters vH_2O and vFat are calculated from the layers 4 and 5.
- The remission spectrum contains biochemical, physiological (perfusion) and structural (layer structure) parts. A typical remission spectrum in the absorbance mode shows the known prominent absorption characteristics of oxygenated and deoxygenated haemoglobin [18], but in the details also contributions of other components. For a differentiated analysis it is essential to identify this parts and components in the spectrum.
- The remission spectrum refers to different measuring depths depending on the wavelength (heterogeneous spectrum); the partially heuristic inverse processing algorithm uses this dependence to determine the model parameters of the different layers. In the spectral region 570–620 nm the penetration depth changes very dynamically, so that layer 3 has to be subdivided in two layers (3a, 3b) to obtain stable results.
- In a strictly physical sense, the inverse algorithm is an approximate solution, but represents a consistent transformation of the spectral values to the model parameters. That means, the spectra can be completely reconstructed by the model and the spectral information is completely transformed into the model system. The uniqueness of the transformation ($S \leftrightarrow M$) is sufficiently assured by analysis of the parameter space and the numerical solution procedure.
- The 5-layer model parameters have the advantage of a much more realistic physiological interpretation than other customary procedures of tissue oximetry as well as the Laser-Doppler method, which normally refer to 1- or 2- layer models [19,20].
- The invers algorithm also determines the thickness of the layers, but only relative to the thickness of the first layer, which cannot be determined absolutely. As with most other remission optical methods the measuring depth/volume cannot be exactly determined.
- Adequacy of the modelling for burn wounds: the thermal impact emanating from the surface causes in the depth of the skin coagulation necrosis, damage of the capillaries and anoxia. The upper layers (epidermis and upper dermis, capillary system) are changed to a superficial denatured layer and a second layer with more or less disturbed perfusion functionality. The interpretation of the corresponding model parameters has to be adapted, but the basic structure of the model is preserved.

2.4. 3D-physiological perfusion analysis of burn wounds

The data processing determines the perfusion parameters vHb and $xHbO_2$ for every model layer and additionally parameters describing absorption and scattering by melanin (epidermis) and collagen (dermis) in normal skin, respectively by denatured components in the burned skin. For the burned skin these parameters can be used as measures of denaturation:

Absorbance $A = \ln\left(\frac{R}{I_0}\right) = s_0 + s_1 \bullet \lambda + L \bullet \sum_i \vartheta_i \bullet \varepsilon_i$, with R: remission intensity, I_0: incident intensity, s_0 and s_1: contributions to absorption and scattering by melanin, collagen or denatured components; ϑ_i: volume fraction and ε_i extinction coefficient of component i; L denotes a mean path length.

With our model for 6 layers (1, 2, 3a, 3b, 4, 5) 12 perfusion parameters (vHb, $xHbO_2$) and 12 "structure" parameters (s_0, s_1) are determined and additionally 2 parameters for the relative water and fat content (vH20, vFat), overall, 26 parameters.

The denaturation of the collagen matrix is associated with a change of the optical features especially the scattering. These changes are expressed in some respects by the structure parameters, but absorption and scattering parameters cannot be determined separately by HSI.

In the model also the presence of methaemoglobin [24–27] has been tested and the methaemoglobin has been explicitly incorporated int the model. But in no case spectral features could be identified possibly related to methaemoglobin, in contrast the reconstruction quality of the spectra by the model decreased with methaemoglobin in every case.

Primary aim of the presented analysis is the evaluation of the perfusion properties of burn wounds. These properties are depicted in the form of depth profiles (Fig. 1).

In these depth profiles all layers are represented as equally broad columns, because the layer thicknesses cannot be precisely determined. This representation has been proved to be the most informative.

The calculation of the parameter vHb includes an estimation of the different contributing volumes of the layers, but because this is a globally fixed estimation, the vHb are index values actually with the range [0–3], the $xHbO_2$-value-range is [0–1] corresponding to 0–100%.

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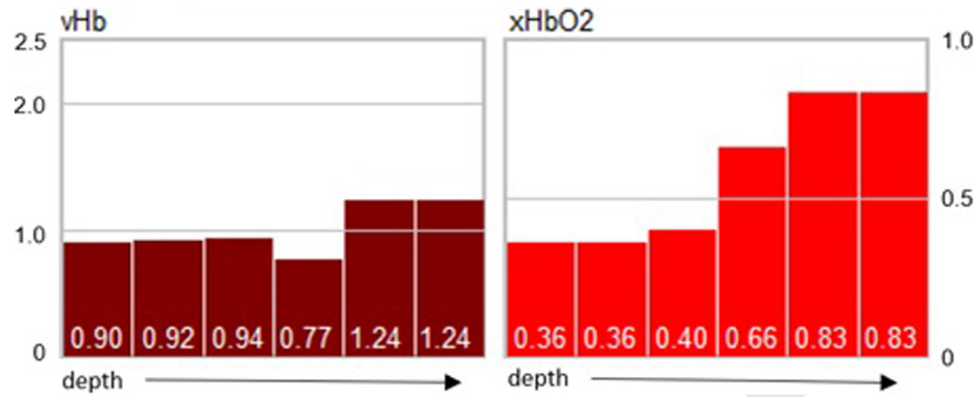

Fig. 1 – Depth profiles of vHb (left) and $xHbO_2$ (right) of a normal perfused hand.

The perfusion profiles have been proved to be physiologically plausible under different conditions, for instance in the well-known occlusion test [11]. The vHb value of Layer 1 results from the imperfect separation of the avascular outer layer and the perfused second layer in the actual processing algorithm.

$xHbO_2$ in the superficial layers (1 and 2) is approx. 0.36 due to oxygen consumption in the capillary system; $xHbO_2$ in the deep layers (5 and 6) is a mixture of arterial (approx. 0.98) and venous blood (0.36) from the capillary system; in the reticular system, the volume fraction of both arterial and venous blood are principally equal (in stationary states), so that $xHbO_2$ is the mean value of venous and arterial $xHbO_2$.

For a better overview in form of colour-coded parameter images, we calculated some secondary parameters from the primary vHb and $xHbO_2$ parameter of the 6 layers:

- vHb_1: mean blood volume of the superficial layers 1 and 2.
- vHb_2: mean blood volume of the deep layers 5 and 6.
- xHb_1: mean oxygenation of superficial layers 1 and 2.
- xHb_2: mean oxygenation of deep layers 5 and 6.

Additional we tested three "dynamic" parameters, which represent a blood flow under certain conditions:

- flow_1: describes the blood flow through the upper skin layers (→ capillary system) and are calculated under the boundary condition of a stationary state with respect to flow and oxygen consumption as $flow_1 \approx \frac{\upsilon_1 x_1}{x_a - x_1}$, with υ_1 = vHb_1, x_1 = xHb_1 and x_a = arterial oxygenation (≈0.98).
- flow_2: to have also a flow parameter for the deeper layers, $flow_2 \approx \frac{\upsilon_2}{x_a - x_2}$ has been defined with υ_2 = vHb_2 and x_2 = xHb_2. At a deeper damage and a corresponding distortion of the perfusion vHb_2 has lower values and xHb_2 decreases.
- *xRate*: oxygen consumption in the capillary system (related to the volume fraction vHb_1): $xRate \approx \Delta \upsilon x_1 (x_a - x_1)$, $\Delta\upsilon$ = vHb of layers 3 and 4.
- Additionally, the total blood volume fraction $\upsilon_0 = \frac{1}{6}\sum_{i=1}^{6} \upsilon Hb_i$ and the volume fraction water vH_2O as index parameters are included in the analysis.

All secondary parameters values are mapped to the range [0–1].

3. Results

3.1. *Perfusion analysis of burn wounds*

3.1.1. *Resulting perfusion profiles*

Fig. 2 shows typical perfusion profiles for different degrees of damage measured between 5 and 10 days after the thermal trauma at different patients, the degrees have been clinically determined in retrospect. 2nd degree A wounds have been treated conservatively and 2nd degree B operatively. For borderline classifications (2nd degree A ↔ B) the question if an operative treatment was adequate, cannot be answered with high certainty.

1st degree and 2nd degree A wounds (row 1 in Fig. 2) are characterized by extraordinarily high values of vHb in the superficial layers 1–3, high vHb values in the deeper layers, nearly normal $xHbO_2$-values in layers 1–3 and very high $xHbO_2$ values in the deeper layers. This represents the strong hyperaemic reaction (inflammatory response, vasodilation), triggered by the burn process: a strong blood influx into the deeper layers, proceeding up to the superficial layers through intact reticular vessels, a still functioning oxygen consumption in the capillary system ($xHbO_2$ superficial) and a significant higher portion of arterial blood in the deeper layers ($xHbO_2$ deep).

For 2nd degree burns (row 2 und 3 in Fig. 2) the total blood volume decreases, especially in the superficial layers, with the degree of damage. Because in the upper layers there is no more oxygen consumption in the distorted capillary system, $xHbO_2$ has still higher values, but decreases with increasing damage (ischaemic, stasis).

In 3rd degree wounds (row 4 in Fig. 2) the destruction of the upper skin layers is characterized by very low vHb values and significantly reduced vHb values in the deeper layers. The remaining blood have very low $xHbO_2$-values in the upper layers.

Fig. 3 shows the typical value distributions of the secondary parameters for different degrees of damage: blue points are

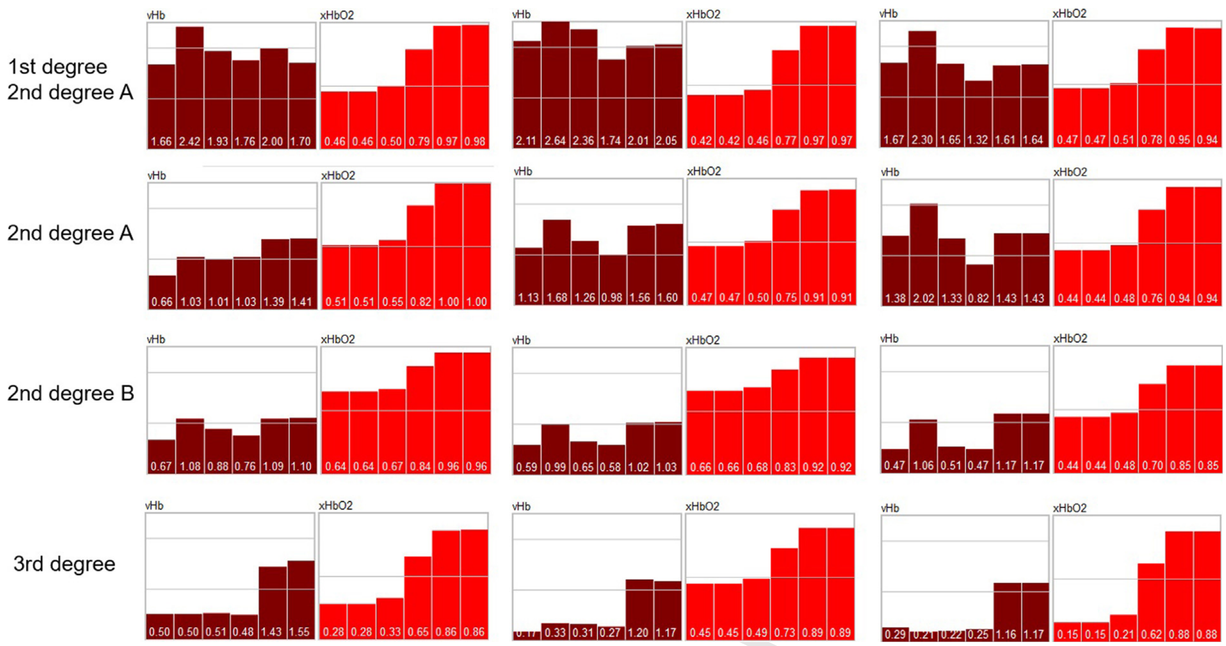

Fig. 2 – Depth profiles for different damage degrees from different patients, retrospectively determined degrees.

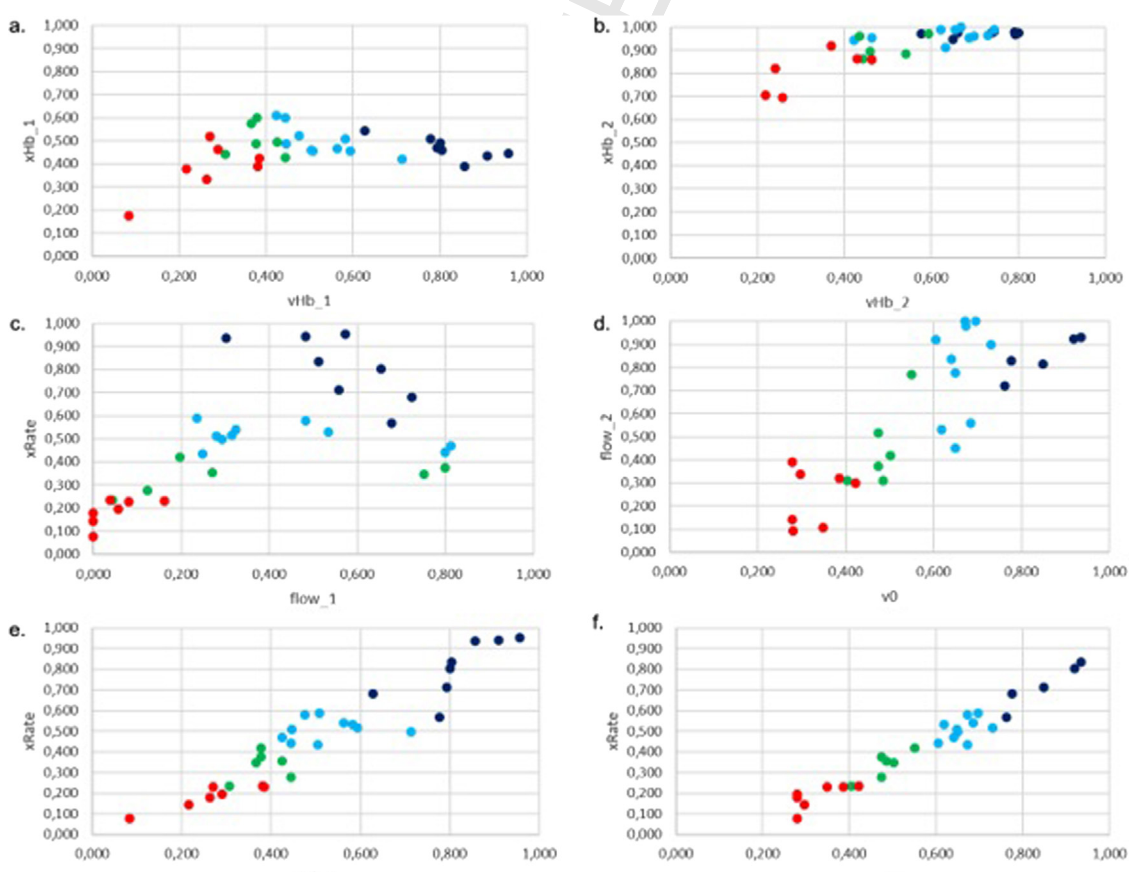

Fig. 3 – Secondary parameters for some examples of different clinically determined damage degrees: dark blue: 1st degree, turquoise: 2nd degree A, green: 2nd degree B, red: 3rd degree. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

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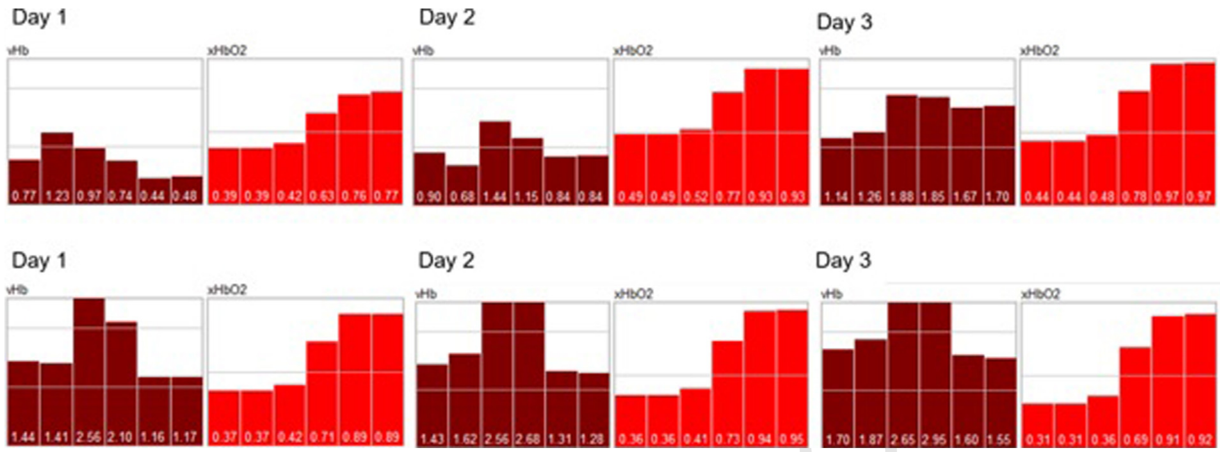

Fig. 4 – Perfusion parameter profiles of 1st degree/2nd degree A burn area (first row) and a 2nd degree B burn area (second row) for the first 3 days after burn injury (day 1).

from 1st degree wound areas, turquoise points from 2nd degree A, green points from 2nd degree B and red points from 3rd degree areas.

vHb_1 significantly decreases with the degree of damage, whereas xHb_1 shows no significant dependence (Fig. 3a), as well as xHb_2 (Fig. 3b). vHb_2 also decreases with the damage (Fig. 3b).

flow_1 and flow_2 show relative broad and varying distributions for 1st degree, 2nd degree A and B (Fig. 3c and d), whereas xRate and v0 exhibit compact distributions and a good separation of the damage degrees (Fig. 3e and f).

flow_1 corresponds to the superficial perfusion information that is available by visual inspection. There is not a clear separation of the burn classes.

It has to be noticed again, that the classification for 2nd degree A and B is based on (retrospective) clinical observations without objective verification.

3.1.2. *Perfusion profiles in the first three days*

The development of the microcirculation disorder in the first 3 day after burn is of great clinical interest. Often the damages increase from 2nd degree A to B. The challenge is to evaluate this dynamic process in terms of perfusion parameters and to develop algorithms for a reliable estimation of the resulting damage degree already in this period.

Generally, beside 3rd degree burns, the wounds show a strong and increasing hyperaemic reaction in the first 3 days, represented by increasing high vHb values and extreme $xHbO_2$_2 values (deep). The latter are induced by a much higher portion of arterial blood in the deeper layers (up to 10 times more than for normal perfusion).

2nd degree B burns exhibit initially distinct blood congestions in the layers 3 and 4, caused by the distortion of the capillary system (Fig. 4 second row). These congestions are less distinct in 1st degree I/2nd degree A burns (Fig. 4 first row). vHb-values are lower for these damage degrees.

3rd degree areas exhibit initially low and subsequently further decreasing values.

Concerning the secondary parameters, the progress of flow_1, flow_2, xRate and v0 seem to be characteristic (Fig. 7b and Fig. 11b):

- 1st degree/2nd degree A: v0 increases, flow_2 also, flow_1 increases or remains constant, xRate significantly increases;
- 2nd degree B: v0 increases, flow_2 increases to limited values, flow_1 decreases, xRate stagnates or increases slightly.

flow_1 and xRate represent the superficial perfusion, which shows no positive development for 2nd degree B wounds in the first three days. The xRate values indicate that there is a remaining oxygen consumption at superficial burns (1st degree/2nd degree A).

v0 and flow_2 represent the perfusion of the deeper layers, generally increasing for 2nd degree wounds, but with more limited values for 2nd degree B than for A.

3.2. *Examples*

In the following, two examples of 2nd degree wounds with initial difficult clinical assessments and analyzed.

3.2.1. *Example 1: female, age: 62, scald with hot water at the thigh*

Fig. 5 shows the RGB-images, calculated from the VIS-range of the remission spectra as objective colour images, and the secondary parameter images (vHb_1, $xHbO_2$_1, vHb_2, $xHbO_2$_2) of the selected burn area on the thigh for the first three days. The parameter values are colour-coded as depicted beside row 2.

The spatial inhomogeneity of the damage develops over the three days, clearly visible in the superficial layer parameters vHb_1 and $xHbO_2$_1. In the lower left part of the wound vHb_1 increases more than in the rest of the wound and $xHbO_2$_1 decreases in this region. In the deeper layers

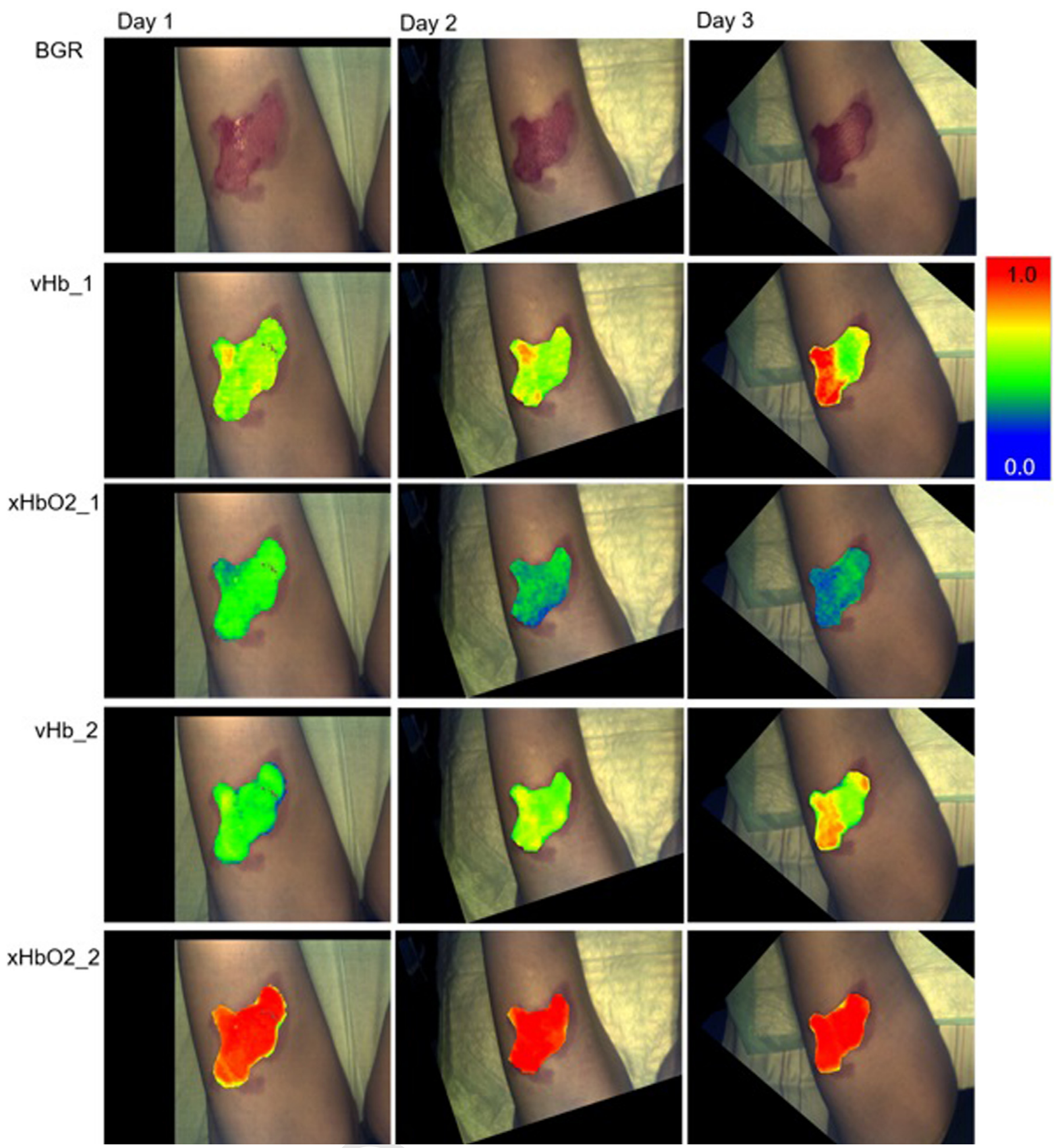

Fig. 5 – BGR-images, calculated from the remission spectra (first row) and colour-coded parameter images for the first three days after burn; the colour-scale is depicted beside row 2. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

vHb_2 also rises more in the lower left part, $xHbO_2$_2 shows extreme high values over the whole wound area. The damage of the upper right area of the wound seems to be more severe. For the whole wound area a severe damage of the superficial layers can be stated, taking into account the vHb profiles (Fig. 4 second row = profiles from the wound area in Fig. 5) showing a severe blood congestion (haematoma) caused by damage of the superficial layers (capillary system). But the perfusion quality of the deeper layers stands for some remaining healing potential.

Fig. 6 shows the colour-coded parameter images for flow_1, flow_2 and xRate. flow_1 values decrease and remain on a low level, flow_2 and xRate increase to relative high values. The upper right wound area shows lower resulting values than the lower left part.

In Fig. 7b the parameter values over the three days are shown for three selected test wound areas (Fig. 7a). Test area 1 and 3 represent the lower left part of the wound and test area 2 the upper right part.

The parameter values of the test areas depict quantitatively the impressions from the colour-coded parameter images (Fig. 6). In wound area 1 and 3 there is an increasing flow_2 and xRate, in area 2 a lesser increase of flow_2 and xRate. The flow_1 values remain small.

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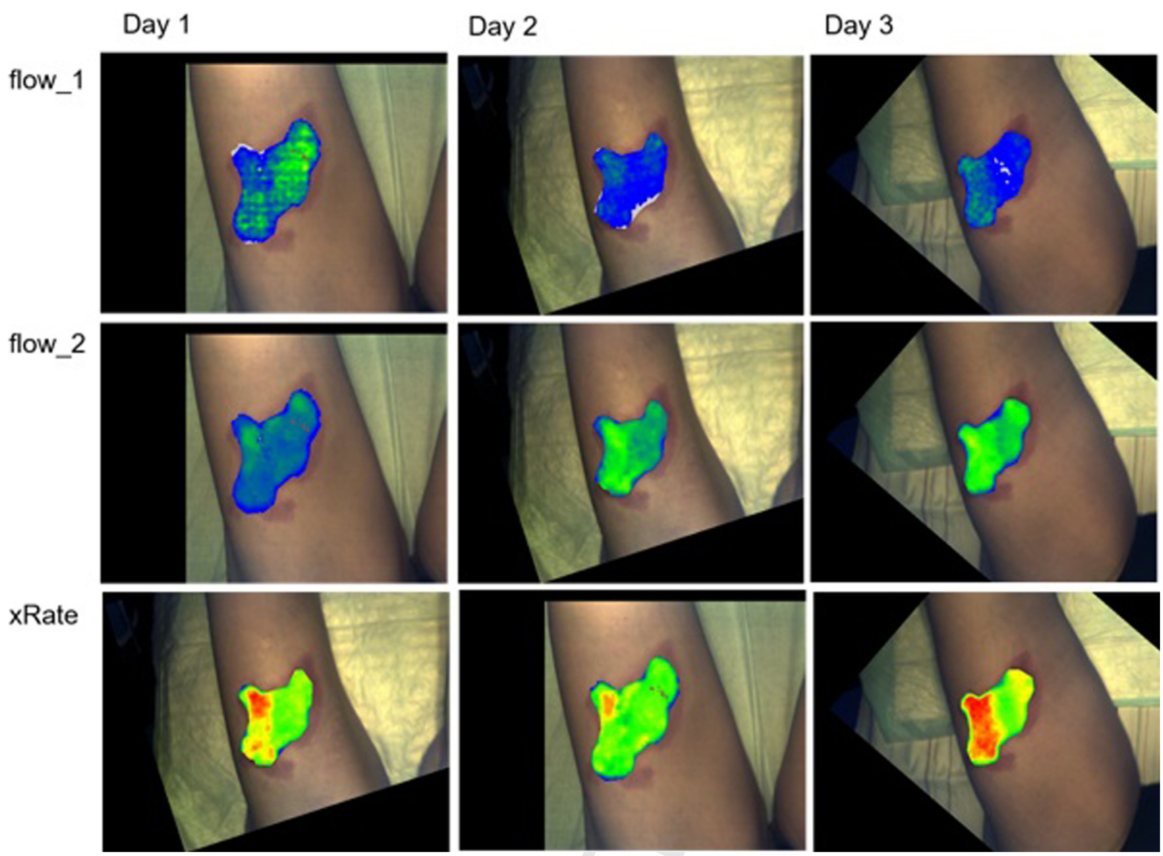

Fig. 6 – Colour-coded parameter images for the first 3 days after burn. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

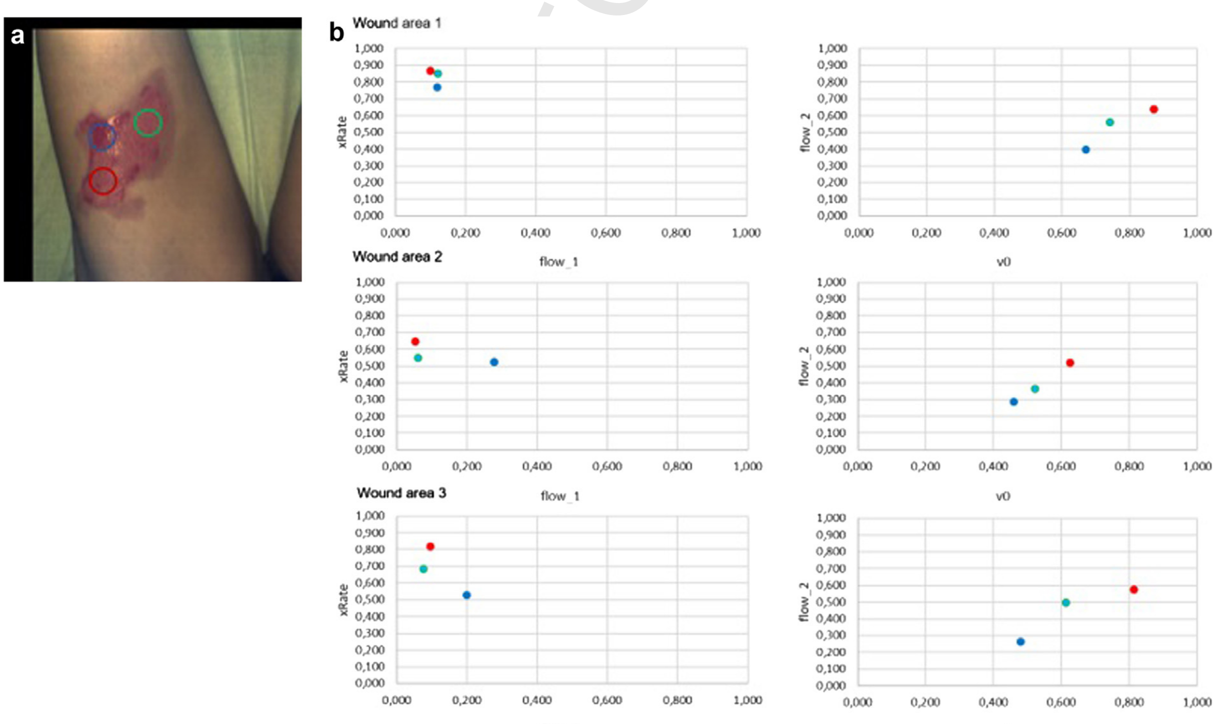

Fig. 7 – (a) Selected test areas: blue: area 1, green: area 2, red: area 3. (b) Parameter values for the test wound areas (Fig. 9a): blue: day 1, green: day 2, red: day 3. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

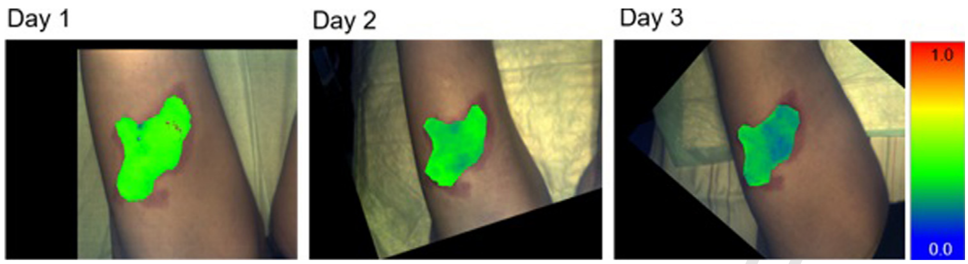

Fig. 8 – vH_2O parameter colour-coded. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Comparing the values with the final values of the classified burn degrees in Fig. 3, the flow_1 values are in the region of 2nd degree B, whereas xRate values are in the 1st degree region. v0 varies from 2nd degree A to 1st degree, whereas flow_2 values tend to 2nd degree A and 2nd degree B.

The xRate values in the first three days are outside of the distributions of Fig. 3. The calculation of the xRate parameter presumes a real flow, which is no longer valid with a severe damage. The congestion causes high vHb_1 and therefore also xRate values. Also, the flow_2 values in the first three days are below the final value distribution (Fig. 3). xRate and flow_2 values develop towards the final distribution during the next days.

Overall, this is a borderline situation between 2nd degree A and B with a more severe damage in test area 2. Leading are the decreasing low flow_1 values and the moderate flow_2 values in area 2 to classify this part of the wound rather as 2n degree B than A.

Fig. 8 shows the index parameter vH_2O. Initially, the values are slightly increased and decrease over the next three days.

3.2.2. *Example 2: male, age 23 years, scald with fat at hand*

The perfusion profiles from the wound area show that the hyperaemia increases over the three days, the congestion in layer 3 and 4 is not so distinct as in example 1 (Fig. 4 first row = profiles from the wound area in Fig. 9). Thereby the damage of the upper layers seems to be lesser than in example 1.

The parameter values of flow_1, flow_2 and xRate (Fig. 10) show a similar development than for example 1 (Fig. 6), but flow_1 remains nearly constant over the three days. Also, in this example, an increasing inhomogeneity of the damage is visible.

The parameter situation is similar to example 1, but flow_2 reaches higher values at day 3, flow_1 also remains at low values. Due to the high flow_2 values at day 3 and the slight increase of flow_1 (test area 1) the damage should be classified rather as 2nd degree A than B.

The vH_2O values are slightly lower than in example 1 (Fig. 12).

3.2.3. *Clinical estimations and results*

In example 1 the clinical estimation at day 1 was 2nd degree A, and was corrected to 2nd degree B at day 2 and 3; the wound was treated operatively. For the clinical estimation principally only the superficial features are available, mainly represented by flow_1. The relative normal xHb_1 values (red colouring, Fig. 5) and initially moderate flow_1 values lead to a 2nd degree A estimation. The strongly decreasing flow_1 values (Fig. 6) and also decreasing xHb_1 values (Fig. 5) result in the 2nd degree B correction.

In example 2 the flow_1 values are slightly higher than in example 1, and decrease only in the lower part of the wound. xHb_1 is slightly higher and remains constant. Because no changes are visible to the physician, the initial estimation 2nd degree A has not been changed and the wound has been treated conservatively.

To estimate the degree of damage the superficial perfusion quality represented by flow_1 and xRate, as well as the deeper perfusion, flow_2 and v0, have to be taken into account.

In both examples the deeper perfusion still has a good performance and flow_2 and v0 increase during the first three days, but in example 2 flow_2 achieves higher values.

Although the damage in example 1 seems to be more severe than in example 2, the parameter values indicate that a 2nd degree A is more adequate in both examples, with some spatial variations.

In general, a deep perfusion with positive development indicates a healing potential (→ 2nd degree A). At least this encourages a further conservative treatment and further measurements until the values show an ablating dynamic (→ 2nd degree B).

All these classifying assessments are actually based on our (still limited) experience with the interpretation of burn wounds based on HSI parameters and are not strictly validated.

4. Discussion

Hyperspectral imaging enables an unique information retrieval about the perfusion of skin and wounds. We present a new method, based on Hyperspectral imaging, for the comprehensive analysis of the perfusion situation of burn wounds and preliminary interpretation and classification results.

With the new HSI measurement and data processing 26 different parameters with good physiological interpretability describing the structure of burn wounds and the perfusion situation in detail are made available. With these parameters advanced insights in the perfusion of burn wounds are enabled. Furthermore, these parameters can be used as a highly specific basis for automated differentiating classifications of the degree of damage or healing potential.

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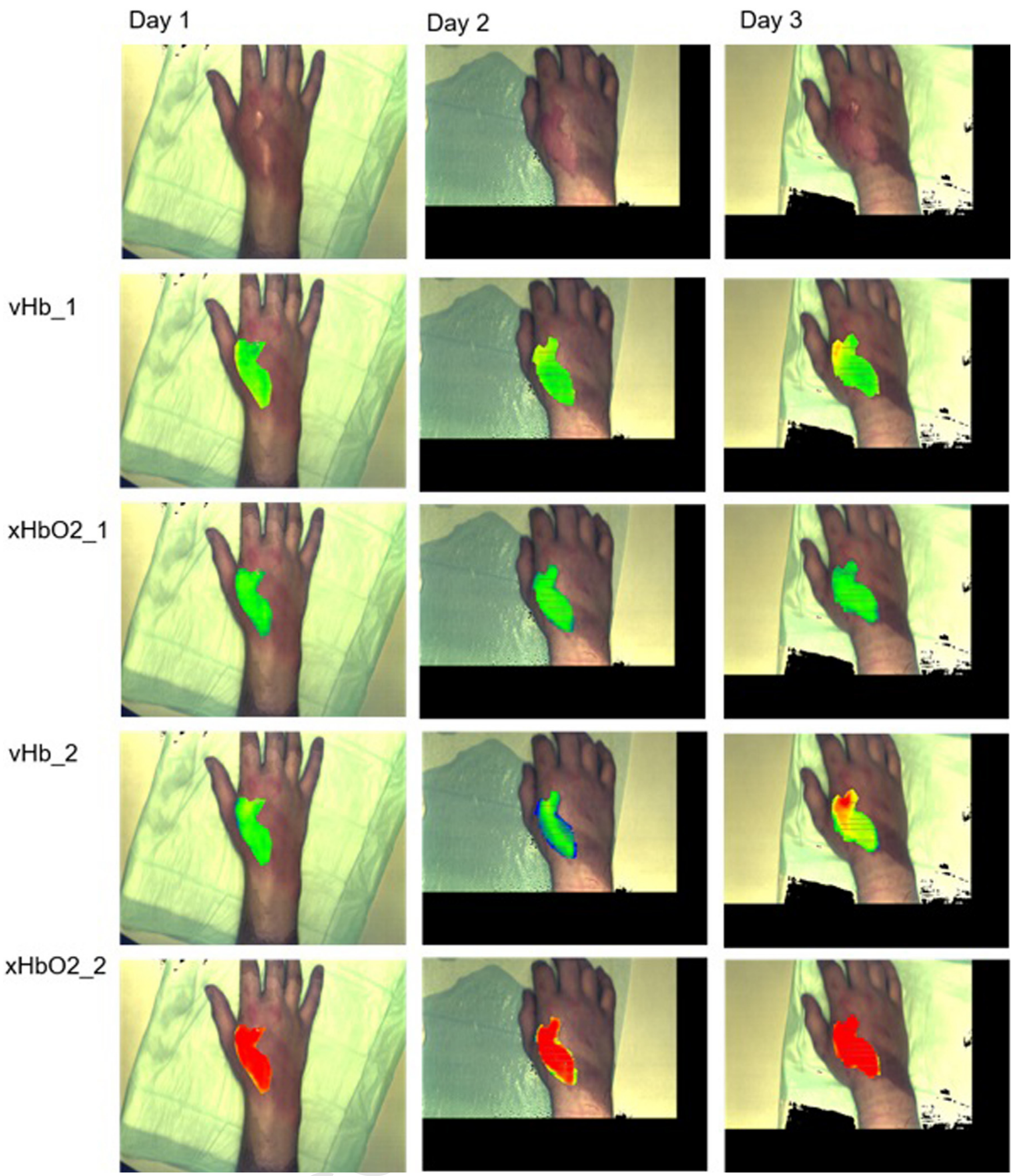

Fig. 9 – BGR-images, calculated from the remission spectra (first row) and colour-coded parameter images for the first 3 days after burn. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

The perfusion parameters are depicted as depth profiles providing information about the perfusion quality in the depth of the wound, but the profiles should not be confounded with a one-to-one mapping of the real layer structure of the wound tissue. However, the parameter profiles map the complex situation inside the wound for the first time.

We analyzed the primary perfusion parameters (perfusion depth profiles) and secondary deduced parameters partially presuming a blood-flow modelling through the different layers. The analysis of parameter value distributions from first measurement series over the first 3 days at adult patients shows significant dependences on the clinically determined degree of damage.

The analysis of the perfusion dynamics in the first three days especially for 2nd degree wounds shows that the developments of the parameter values for similar wounds in the borderline region 2nd degree A ↔ 2nd degree B are sufficient different to enable a very differentiated assessment of the damage or healing potential. For those wounds the value development up to the third day should be considered. 1st

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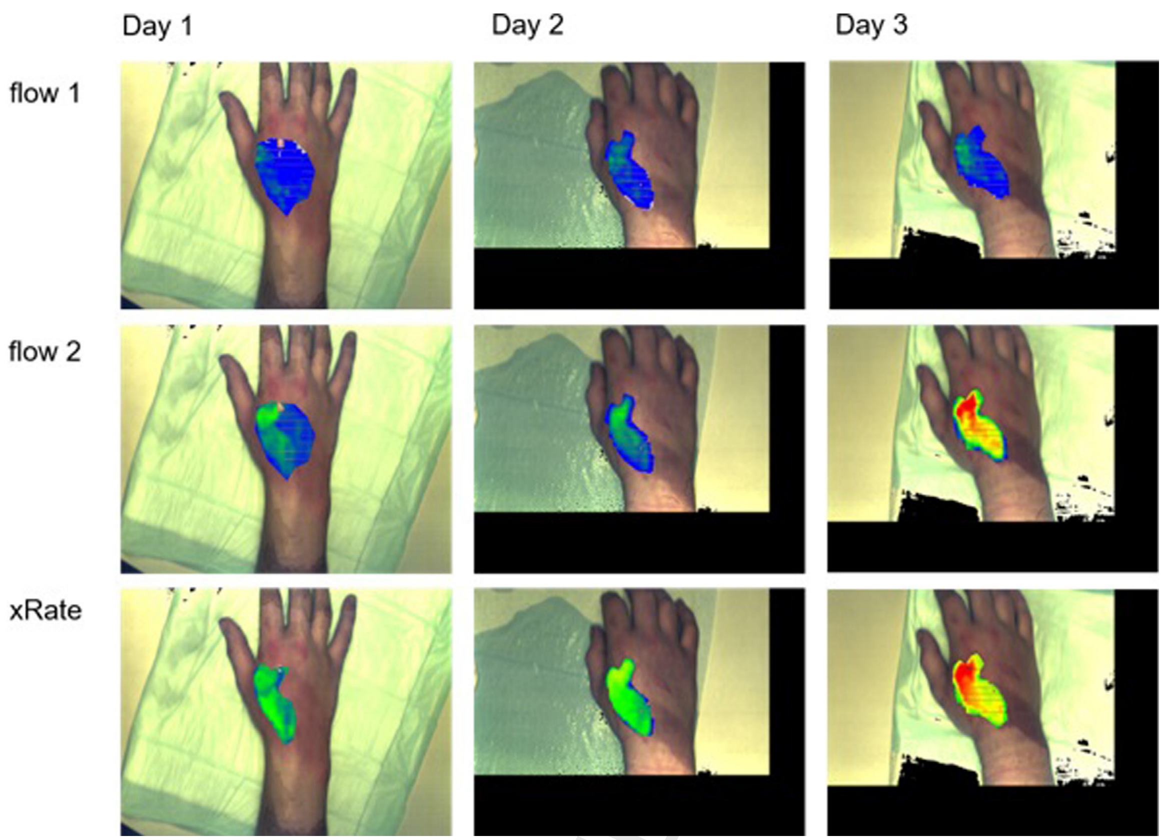

Fig. 10 – Colour-coded parameter images for the first 3 days after burn. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

degree and 3rd degree wounds can be reliably assessed at the first day.

The secondary parameters have been defined to obtain less parameters with high informative value. The interpretation of this parameters has to be adapted to the peculiarity of burn wounds. Formally these parameters are used and tested as "process parameters", efficiently describing the burn wound process and hence allowing for a quick overview and assessment.

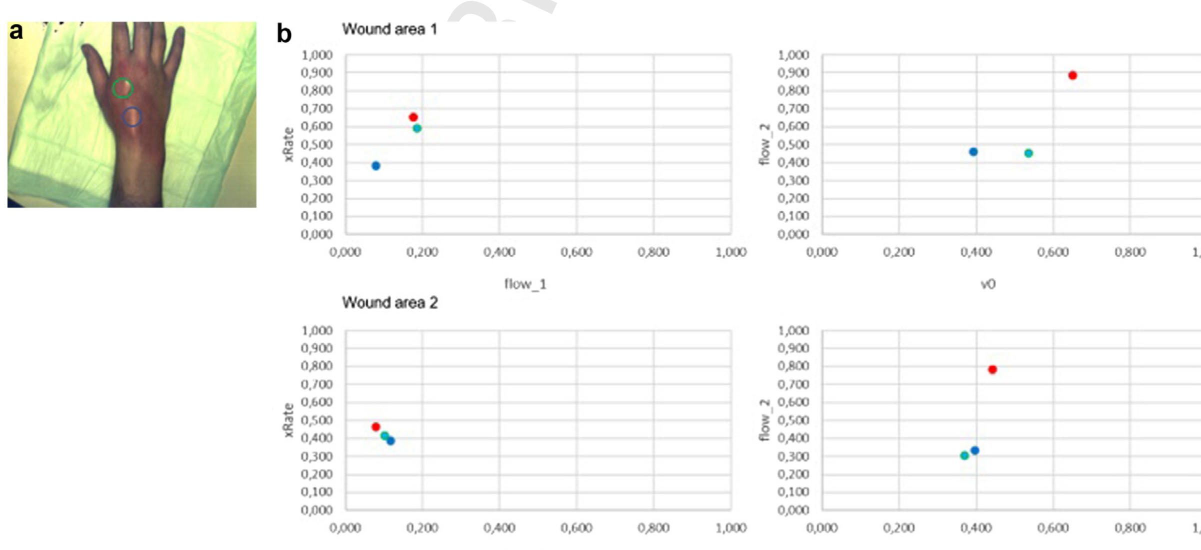

Fig. 11 – (a) Selected test areas: blue: area 1, green: area 2. (b) Parameter values for the test wound areas (Fig. 12a): blue: day 1, green: day 2, red: day 3. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

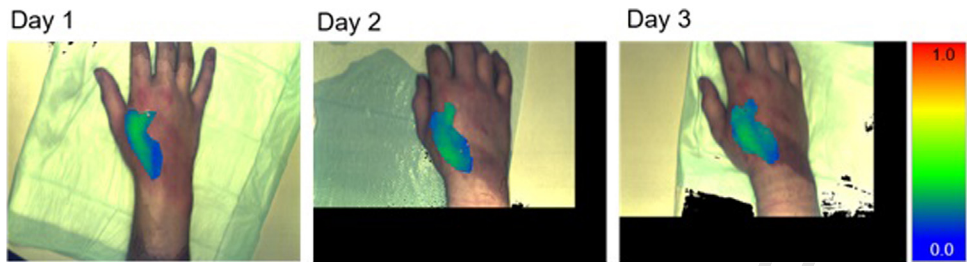

Fig. 12 – Parameter values vH_2O.

The parameter maps (Figs. 6 and 10) can be used as leading maps for an assessment of the skin damage. With some experience reliable assessments are already possible with these maps.

The initial values and the rate of change of the values especially for the flow_1, xRate, flow_2 and v0 parameters, as presented for test areas in Figs. 7b and 11b and discussed in Section 3.2.3, have to be evaluated in a validated automated classification procedure.

For 2nd degree burns the low flow_1 values represent the initial damage in the superficial layers. Critical for the assessment of the further perfusion development up to the resulting values (Fig. 3) are the details of the flow_1 in connection with the v0 and flow_2 development.

The differences of the parameter values between the first 3 days and the resulting values after more than 5 days indicate that the assessment of the degree of damage is very difficult in the first 3 days due to the distinctive wound dynamics of 2nd degree burns.

The first evaluations already confirm a good reproducibility of the parameter courses for similar degrees of damage. Nevertheless, in order to generate validated conclusions based on the parameters more extensive measurements have to be analyzed.

4.1. *Validation of the parameters and classifications*

A fundamental problem of the validation of the parameter-based assessment is the deficient reference classification in the region 2nd degree A ↔ 2nd degree B. The wound development allows a clinical confirmation for degree 2nd degree A but not for 2nd degree B, if it is treated operatively.

The availability of objective parameters, allowing for a better assessment of the deeper perfusion (not observable by visual inspection), often leads to a delayed decision between operative and conservative treatment. In this way the decision boundary based on the parameter can be successively determined with increasing precision.

4.2. *Comparison with other measurement techniques*

Laser Doppler Imaging (LDI) has been partially established as a decision supporting measuring method for the assessment of burn wounds with good results at the earliest at day 3 after burn [2–5]. The results are presented in terms of some healing potential classes (complementary to the degree of damage or burn depth).

LDI measures the speed distribution of the moving blood particles using the frequency shift of the back scattered light (Doppler effect). From this a ‘‘flow’’-parameter is been determined correlating with the averaged blood volume and the mean velocity of the blood particles in the captured measuring volume. This LD-flow parameter has been proved to be a meaningful measure for the mean perfusion quality in the measuring volume.

The informative value is been restricted mainly by the lack of a differentiation between superficial and deeper perfusion quality (could be principally extended by the use of 2 wavelength with different penetration depth) and no determination of oxygen saturation of haemoglobin.

A more differentiated analysis of the perfusion of the system ‘‘burn wound’’ requires a more detailed wound model and the possibility to determine the system parameters as performed with hyperspectral technology. This enables to analyze the perfusion dynamics in the first 3 days after burn and to develop a reliable early assessment procedure.

Another promising technology is Spatial Frequency Domain Imaging (SFDI) which has been only tested with animals up to now [21–23].

The main advantage of SFDI in comparison with HSI is, that the absorption and scattering parameters of the tissue can be determined separately. By determining scattering parameters, the depth and degree of damage can be estimated, because the denaturation of the collagen matrix is associated with a change of the scattering features.

But the determination of perfusion parameters is restrictive by the use of only a few different wavelengths. Due to the high spectral resolution of HSI, detailed features of the heterogenous remission spectra can be evaluated and the perfusion can be analyzed in more detail.

Overall, no other technology (fulfilling the requirements of clinical usability) provides more relevant parameters for system analysis as HSI.

4.3. *Advanced classification*

The decision between 2nd degree A and B seems to depend on the relation between the superficial (flow_1, xRate) and the deeper perfusion quality (flow_2, v0).

For a reliable assessment of the degree of damage all available parameters (including the structure parameters) have to be included and adequately weighted.

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For the development of a quantitative classification based on the parameters we defined a system of 4 classes (corresponding to the clinical degrees): class 1: self-healing (approx. 14 days); class 2_1: self-healing in approx. 21 days; class 2_2: not healing in 21 days, operative treatment recommended; class 3: no healing potential, operative treatment mandatory.

For the development and validation of an automated classification system, a database (basic knowledge data base for HSI measurements of burns) has been created and will be filled in actually performed standardized measuring series.

The development will be described in a subsequent publication.

Artificial Neuronal Networks or similar methods of machine learning has been tested to correlate spectral parameters gained from measurements with degrees of damage [14,27]. Despite the performance of machine learning methods, we are of the mind that parameters with physiological interpretability are more meaningful and easier to assess, even though they do not represent a strict mapping of the tissue system. Due to the consistency of the transformation the equivalence of model parameters a spectral information is given.

4.4. *Environment*

It is interesting to observe the perfusion values of the normal skin in the neighbourhood of the burn wound. These values are as a rule significantly lower than for normal perfused skin (without a burn wound) in the first 3 days indicating a broad regional reaction on the burn injury.

5. Conclusions

Critical 2nd degree burns are very difficult to estimate by the physician without deeper insight in the wound perfusion structure. The new HSI-method enables new objective and detailed insights into the burn wounds. The complexity even of the perfusion situation cannot be sufficiently determined by external inspection and by single averaging measurement parameters. Sufficient objective measuring parameters have to be available for a reliable assessment of the wound state.

The presented HSI-method enables even without the final classification process objective, detailed and quantified analysis and comparisons of wound reactions for instance to develop evidence-based optimized treatment strategies and methods.

Further research projects concern the reaction of the environmental perfusion of burn wounds and the perfusion of burn wounds at children. First measurements and evaluations exhibit significant different features of the wound perfusion at children (<5 years) compared with adults.

Disclosures

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Conflict of interest

The authors declare no conflicts of interests relevant to this article

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