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A compact hyperspectral camera for measurement of perfusion parameters in medicine

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Abstract: Worldwide, chronic wounds are still a major and increasing problem area in medicine with protracted suffering of patients and enormous costs. Beside conventional wound treatment, for instance kinds of oxygen therapy and cold plasma technology have been tested, providing an improvement in the perfusion of wounds and their healing potential, but these methods are unfortunately not sufficiently validated and accepted for clinical practice to date. Using hyperspectral imaging technology in the visible (VIS) and near infrared (NIR) region with high spectral and spatial resolution, perfusion parameters of tissue and wounds can be determined. We present a new compact hyperspectral camera which can be used in clinical practice. From hyperspectral data the hemoglobin oxygenation (StO₂), the relative concentration of hemoglobin [tissue hemoglobin index (THI)] and the so-called NIR-perfusion index can be determined. The first two parameters are calculated from the VIS-part of the spectrum and represent the perfusion of superficial tissue layers, whereas the NIR-perfusion index is calculated from the NIR-part representing the perfusion in deeper layers. First clinical measurements of transplanted flaps and chronic ulcer wounds show, that the perfusion level can be determined quantitatively allowing sensitive evaluation and monitoring for an optimization of the wound treatment planning and for validation of new treatment methods.

Keywords: free flap monitoring; hyperspectral imaging; perfusion imaging; tissue oxygenation; wound diagnostics.

Introduction

Chronic wounds are still a major and increasing problem area in medicine with protracted suffering of patients and enormous costs. There has been some improvement in the chronic wound treatment by involving different areas of expertise in the process of wound treatment (interdisciplinary strategy) and embedding wound management in a complex therapeutic attempt including a wide range of usual to unconventional treatments [1–5].

As a remaining critical central point in the dilemma of chronic wound therapy, a non-objective and often insufficient description of the wound state precludes a systematic and evidence-based planning and optimization of the treatment strategy if only different persons are involved.

The wound process in general cannot be objectively representable and traceable and accordingly the effect of different treatment methods cannot be objectively assessed. This results in a largely unsystematic and inefficient diagnostic and treatment process with prolonged suffering of the patients and also significantly compromising cost saving attempts in healthcare.

Actual wound diagnostics comprise
– visual assessment, which is subjective and depends on experience and acquires only superficial color- and shape related information of the wound, occasionally,
– transcutaneous oxygen monitoring (oxygen pressure TcPO₂) [6]: needs contact to the tissue, is time-consuming, measures only a local point and imprecisely (strong variations),
– tissue oximetry based on near infrared spectroscopy (NIRS) [7]: not contact-free, only does local measurements, limited information by using only multispectral information,
1. Laser-Doppler imaging (LDI) [8–10]: expensive, imprecise and cumbersome, influenced by all kind of moving objects in tissue (white blood cells)
2. ICG-NIR fluorescence imaging [11]: invasive, complicated, limited informative value, imprecise,
3. multispectral imaging [12, 13]: limited information content, expensive.

Fundamental for undisturbed wound healing is the adequate perfusion of the wound area and wound surroundings. An objective and comprehensive determination of perfusion parameters provides important information to the physician, which is not available with actual methods.

That means the determination of parameters allowing assessment of tissue oxygen supply, depending on the need, over the entire wound area and its surroundings with good spatial resolution to detect critical segments and trends. A second main task is to detect necrotic tissue in the wound area, which often cannot be identified visually.

Further requirements for usability in the clinical area are: contact-free and quick measurement without elaborate measurement conditions, burdens for the patients and additional load for the staff.

Hyperspectral imaging (imaging remission spectroscopy) in the visible (VIS) and NIR region basically has the potential to fulfill these demands [14–25].

We report about a new compact hyperspectral camera system designed for use in clinical practice. Actually the system has been used for perfusion monitoring of transplanted flaps for the coverage of severe non-healing wounds in plastic surgery, and also for perfusion measurements of chronic wounds and experimentally in other areas of application [26, 27].

Figure 1: The image of the object is projected to the slit plane and scanned by the movable spectrometer unit. (A) Principle of the optical spectroscopic system – pushbroom imager. (B) Hyperspectral camera with halogen lighting unit mounted on a medical cart.
under development, which will lead to a significantly reduced thermal irradiation of the object (a warming of the tissue caused by thermal irradiation may influence the superficial perfusion).

The camera is normally mounted on a moveable and flexible medical cart system (Figure 1B). For further technical details see also the article [28] in this issue.

**Calibration of the camera**

The calibration of the spectrographic system consists in the substantial elimination of all influences of system-related factors on spectra, so that only pure measured remission spectra from the object remain for evaluation.

For this,

1. optical bias and deformations of the image of the slit (y-axis) and the related spectra (x-axis) on the sensor are determined and corrected,
2. the position of the wavelengths on the λ-axis of the sensor are determined by imaging the light of a reference light source (krypton gas) with defined sharp spectral lines (calibration of the spectral coordinate),
3. the black level of the camera sensor is determined and subtracted from the measurement data,
4. the spectral response of the camera system is determined by measuring a white reference.

With 3. and 4., the calibrated measuring data follow as:

\[ \tilde{I}_x = \frac{I_x - I_b}{I_x'} - I_w \]

with \( I_x \) measured raw data (remission intensity); \( I_b \), black level, \( I_w \), white reference (depending on λ).

After this calibration and correction steps, the residual errors of optical distortions like keystone and smile are at a very low level and can be neglected in the parameter calculation.

**Calculation of perfusion parameters**

The measured remission spectra (Figure 2) are composed by contributions of different components of the tissue (skin, wound, secretions, biofilm). The related optical effects are scattering (fundamental for remission spectroscopy) and absorption with more or less characteristic spectral features of the different components. The task is to identify and quantify the actual components relevant for the clinical assessment. In perfused tissue the spectra are dominated by the absorption of hemoglobin, scattering is mainly caused by other components (like epidermis, dermis in normal skin, collagen, granulation tissue in wounds).

In a first approach hemoglobin-related parameters are calculated from the spectra, describing relevant perfusion qualities. This calculation is based on the model described in [29] with the main component hemoglobin, where the remitted intensity is described by

\[ I_x = I_0 e^{-\left(\frac{d}{\varepsilon}\right)} - \frac{d}{\varepsilon} \]

with \( I_0 \), illuminating intensity; \( \varepsilon(\lambda) \), background components; \( D(\lambda) \), "mean path length"; \( \sigma_{\text{h}} \), volume concentration of hemoglobin in the measuring volume; \( \varepsilon(\lambda) \), extinction coefficient of hemoglobin and \( x \), oxygenation; \( b(\lambda) \) is assumed to be linear: \( b(\lambda) = b_x + b_{\lambda} \lambda \) and \( D(\lambda) \) constant.

- **StO₂**: Oxygenation of the hemoglobin; to get a robust estimation of the oxygenation we chose an algorithm based on the second derivative of the absorption spectra of oxygenated (HbO₂) and reduced hemoglobin (Hb) eliminating constant and nearly linear contributions and using the spectral segments \( \Lambda_1: 570–590 \text{ nm} \) and \( \Lambda_2: 750–770 \text{ nm} \). With the approximation \( \varepsilon_{\text{h}}^o(\Lambda_1) = \varepsilon_{\text{h}}^r(\Lambda_2) = 0 \) follows:

\[ x = \frac{a_{12}}{a_{11}} \]

with \( a_{12} = \frac{\varepsilon_{\text{h}}^r(\Lambda_1)}{\varepsilon_{\text{h}}^o(\Lambda_2)} \) and \( a_{11} = \frac{\varepsilon_{\text{h}}^o(\Lambda_1)}{\varepsilon_{\text{h}}^r(\Lambda_2)} \), \( A = \ln \left( \frac{I_x}{I_w} \right) \) denoting the “absorbance”.

Considering the inhomogeneity of the measuring volume (different penetration depth in \( \Lambda_1 \) and \( \Lambda_2 \)) \( \varepsilon_{\text{h}}^o(\Lambda_1) \) and \( \varepsilon_{\text{h}}^r(\Lambda_2) \) have been replaced by variable scaling factors \( r_x \) and \( r_{\lambda} \) allowing for a calibration of calculated values to reference values:

\[ x = \frac{1/\tau_x A_o(\Lambda_1)}{1/\tau_x A_o(\Lambda_2) + 1/\tau_x A_o(\Lambda_1)} \]

- **THI**: Relative concentration of total hemoglobin (Hb + HbO₂) \( \sigma_{\text{h}} \) can be easily calculated from the same formulas using the

**Figure 2**: The measured remission spectra. Typical remission spectra (in the absorbance mode) of (A) diverse components of tissue and skin and (B) wounds in the VIS and NIR spectral range; the spectra are dominated by the hemoglobin absorption with its spectral characteristics in the regions 550–600 nm (double peak HbO₂, single peak Hb) and about 760 nm (peak Hb); from this the perfusion parameters can be estimated; a further relevant component, which is not recognizable by other methods, is necrotic tissue; this exhibits a characteristic peak about 650 nm.
same spectral regions. But we use an alternative calculation resulting from the model-based analysis of the dependence of the remitted intensities \( I(\lambda) \) from the parameters oxygenation and volume concentration hemoglobin. The parameters are principally of the form \( p = \frac{p_1 - p_2}{p_2 - p_1} \) with \( p_1 = -\ln \left( \frac{I_1(\lambda)}{I_1(\lambda)} \right) \) and \( p_2 \) for scaling to the range [0–1]. From the intensities the absorbances are calculated and averaged over a defined spectral range \( \Lambda = [\lambda_1, \lambda_2] \) resulting in \( \bar{A}(\Lambda) \). Then the parameter is calculated as
\[
p_\lambda = \frac{\bar{A}(\Lambda) - \bar{A}(\Lambda)}{\bar{A}(\Lambda) - \bar{A}(\Lambda)}
\]
For the calculation of THI the spectral ranges \( \Lambda_1 = [530–590] \) and \( \Lambda_2 = [785–825] \) are used. This parameter mainly depends on the \( \sigma \) in \( \Lambda_1 \); \( \Lambda_2 \) plays the role of a reference.

In the spectral region used for the calculation of \( \text{STO}_2 \) and THI the penetration depth of the illumination light is very low due to the high absorption of hemoglobin (<0.5 mm); therefore mainly superficial tissue layers contribute to this parameters.

- **NPI**: NIR perfusion index, calculated according to formular (3) from the NIR spectral region (700–900 nm) with \( \Lambda_1 = [655–735] \) and \( \Lambda_2 = [825–925] \). This parameter depends from \( \sigma \) as well as \( x \) in the form \( f(\sigma, x) \) representing a composite measure of perfusion. This composite parameter has proven to be more robust and sufficiently meaningful in comparison to a separated estimation of \( \sigma \) and \( x \) in this spectral region.

The penetration depth is approx. 3–5 mm, depending on the individual structure of the tissue; therefore also deeper tissue layers (up to approx. 5 mm) contribute more strongly to this parameter.

THI and NPI are index values, allowing differentiated relative comparison of the parameter values over the measured area, but the comparability of the absolute values between different localizations or persons is limited due to the variations of the individual skin or tissue. The calculation is based on a modeling of the interaction of light with the measured tissue, which is customary for tissue oximetry, but cannot implement the individual variations of tissue composition and structure [29].

For the parameters THI and NPI, already in this simple modeling, there are unknown factors, whereas the oxygenation calculation is valid if the modeling is valid.

For a detailed description and discussion of parameter calculations from the spectra, see also the article from [28] in this issue.

The control of the camera functions, the processing of the data, and the calculation of the parameters are performed by an original software including a data base specified for wound documentation (TIVITA™ Suite, Diaspective Vision GmbH, Am Salzhauff, Germany).

**Validation**

For the validation of the parameters calculated from the spectra, an internal study with voluntary healthy subjects has been performed. A so-called occlusion test comprises some phases with normal blood perfusion of an arm and with venous and arterial occlusion. The perfusion parameters are measured at the test hand and the other hand served as control (no intervention). The perfusion parameter values were averaged over a larger area of the hand. A conventional tissue oximetry sensor (MOXY, Fortiori Design) was applied at the test hand as a reference measurement system. This sensor measured parameters widely comparable with \( \text{STO}_2 \) and THI. These results underline very good accordance of the hyperspectral parameter values with the reference values. The time course of the parameter values over the different phases follows exactly the physiological expectations and shows very small variations of the values over short time segments with stable conditions, which proves the high stability of the test parameters.

**Clinical measurements**

Measurements with the camera system have been performed at different cooperating clinics. All ethical requirements are fulfilled, all measurements have been performed with declaration of consent of the patients.

The camera system as described above has been used in routine environments. Wounds and transplanted flaps have been measured at time of routine dressing changes with wound inspection (visual) and documentation (occasionally with a photo camera).

During routine wound visits, necrotic material and secretion materials were removed (more or less intense debridement) providing best conditions for hyperspectral imaging. Adjustment of the camera to the wound, execution of the measurement, and calculation of the parameters need not more than approx. 2 min.

**Results**

**Measurements at transplanted flaps for wound coverage**

Transplanted flaps are most vulnerable in the first 2–4 days after transplantation because the development of a sufficient perfusion (depending on the type of flap) in this period can be impaired by diverse factors. Significant perfusion deficits can easily lead to transplant loss with consecutive need for operative revision (i.e. new flap operation) which means a prolonged duration of stay and drawbacks for the wound healing and the patients.

Therefore in this critical period a frequent monitoring of the perfusion quality is crucial. This monitoring has up to now mainly been performed by visual inspection. This inspection is subjective, depends on experience, and perfusion deficits can only be detected if the deficient perfusion leads to visible changes (i.e. in color). Diverse methods have been tested but are not established in clinical practice [30–33]. Only tissue oximetry sensors (NIRS) are often used in US hospitals with good success.

Generally the knowledge of the perfusion dynamics in this early phase of a transplanted flap has been very limited up to now.

With the hyperspectral camera, measurements of transplanted flaps are performed in the first days after
transplantation, providing representative perfusion profiles with high spatial resolution (Figure 3).

The example in Figure 3 shows the extracted normalized RGB images, the parameters THI and StO$_2$ for the superficial and NIR for deeper perfusion (and additionally the tissue water index) measured at days 1, 3, 5, 7, 9, 11 and 13.

The detailed visualization of the distribution of the parameter values over the flap area in the course of the measurements enables a clinical monitoring analysis of spatial dynamics of perfusion.

At day 3 an area with higher THI and low StO$_2$ can be seen, representing a distorted perfusion (a slight kind of stasis), which becomes normalized in the following days.

An increased THI combined with a reduced StO$_2$ or NPI are an indication for a local venous occlusion. More detailed descriptions of clinical study are in preparation.

**Measurements at chronic wounds**

With the hyperspectral camera system the perfusion situation of chronic wounds can also be determined very accurately. Additionally necrotic tissue can be clearly differentiated, although this is not possible to visualize in many cases. The perfusion of this tissue is strongly degraded and the chemical conversion process leads to characteristic changes of the spectrum (especially about 640–650 nm as shown in Figure 2B).

The different types of necrotic tissue are derived from different parts of the same data set. The spectrum of necrotic tissue 1 generally shows a lower absorbance but the same spectral peak at 650 nm as in the dark necrotic tissue. This suggests that it is the same chemical process and therefore can also be called necrotic tissue.

Figure 4 shows a diabetic foot with necrotic tissue, depicted by the blue color in the StO$_2$ parameter images (very low oxygenation) and confirmed by the spectral characteristics.

**Hyperspectral imaging for evaluating wound therapeutics**

A sufficient perfusion is a fundamental factor for wound healing. As we know from tcPO$_2$ measurements of the feet in diabetic patients a persisting oxygenation lower than approx. 30% leads inevitably to the death of the tissue. The perfusion can be improved by different methods, but a lot of experimentally tested methods with noticeable effects are not sufficiently validated.

As a relatively new method “cold plasma” (CAP) has been applied and tested at chronic wounds [34, 35]. Regularly applied plasma treatment can enhance the wound healing significantly [36].

Figure 5 shows an example of the effect of the application of cold plasma at a chronic foot ulcer wound. A significant improvement of perfusion can be measured with the hyperspectral system. StO$_2$ and NIR perfusion clearly increased after CAP, also slightly the THI. This is visible
Figure 5: Diabetic foot wound treated with cold plasma; the \( \text{StO}_2 \) parameter false-color image depicts necrotic tissue; the pale area at the left side of the wound area cannot visually be identified as necrotic by the change of the false colors. Systematic studies yielding significant values for the improvements have not been performed until now.

Discussion

With the easy to use compact camera system, hyperspectral measurements of patients in the normal clinical environment are possible for the first time. Already through the first measurement phases a lot of new insights and findings about perfusion properties and dynamics in diverse clinical application areas have been arised, which have to be developed and interpreted in the next future.

In comparison to tissue oximetry based on NIRS spectroscopy and related techniques, especially the imaging measurement, which enables the analysis of the spatial characteristics of the perfusion and more reliable assessments of perfusion situations, constitutes a mainstay in wound diagnostics. In comparison to the laser-Doppler (or laser-speckle) imaging the determination of hemoglobin oxygenation is an important advantage. The
determination of three perfusion parameters with different complementary meanings allows for a more complete presentation of the complex perfusion situation.

The “flow”-parameter determined by laser-Doppler methods correlates directly with the movement of the blood particles (but also with diverse other movements, i.e. by leucocytes in the case of infection causing artifacts jeopardizing clinical interpretation). This “blood flow” is indirectly included in the parameters determined by the hyperspectral method: a good perfusion with high oxygenation implies a good flow, a disturbed flow causes a degraded oxygen supply (reduced oxygenation) of the tissue and a low THI (low influx of blood) or a high THI as an indicator of stasis (venous or arterial) in relative short times (a few seconds). The use of different parts of the spectrum for parameter calculation (VIS: \( \text{StO}_{2} \), THI, NIR: NPI) enables additional assessments in dependence on the depth of the tissue. This clearly recognizable differentiations and quantitative assessments are not possible based alone on the LDI-flow parameter.

In comparison to multispectral oximetry methods the use of the complete and highly resolved spectrum yields a significantly higher reliability and reproducibility of the parameter determination.

A limiting factor is still the modeling of the tissue and the measuring process itself, which is the basis for parameter calculation. The detailed individual three-dimensional structures of the tissue and the distributions of chemical components cannot be modeled with adequate exactness. Therefore the relative simple model used in practically all tissue oximetry devices yields only relative rough and averaged perfusion parameters.

The availability of high-resolution complete spectra and high-resolution images principally enables
- more differentiating analysis of the spectra including more components with the use of decomposition methods,
- the identification of relevant wound components like necrotic tissue, biofilms, foreign bodies
- the analysis of spatial structures by segmentation methods, classification of segments and class-specific parameter calculation form the spectra.

Those methods based on real measurement data are actually under development.

Crucial for the quality of the parameter determinations is the consideration of the measuring conditions to obtain reliable undistorted data. External light illuminating the measured area, which is not considered by the calibration process, can have significant influence on the parameter calculation and can lead to incorrect parameter values. Not every kind of external light source causes disturbances of the parameter calculation, this strongly depends on the spectrum of the light source.

The measurement guidelines require the prevention of strong external illumination of the measurement area. If the compliance of this guidelines cannot always be guaranteed in clinical practice, procedures have to be integrated in the data processing, which can detect disturbing external light contributions and inadequate distance to the target to avoid the output of false results.

Specific algorithms have been implemented to detect tissue from the known features of tissue spectra. With this step, also distortions leading from excessive ambient light and strongly corrupted spectra like specular reflections are detected. The respective spectra are marked as non-tissue and the parameters are not calculated so the pixel in the 2D images stay black. One of the next development steps will be the switch to a broadband LED-based lighting unit. This unit could be switched very fast so that the spectra of ambient light can be detected and corrected during the recording. Until this step is completed, the ambient light cannot be fully corrected.

**Clinical benefit**

The information provided with this new hyperspectral measuring system enables more objective and differentiated assessments of perfusion situations and dynamics.

This enables the externalization and quantification of wound describing parameters, the early detection of impairment of the healing process, the objective documentation of healing processes, an objective proof and monitoring of treatment effects and an evidence-based selection and comparison of treatment methods for quality assurance.

**Conclusion**

Actually the “TIVITA™ Tissue” hyperspectral camera is predominantly used in plastic surgery for monitoring of transplanted flaps and in wound management (chronic wounds). The perfusion of those flaps is critical in the first hours and days and needs monitoring providing clinically important parameters to detect healing impairment and impending transplant loss enabling early concerted reaction. This can be achieved with hyperspectral imaging adapted to the demands of clinical practice.

A second field of application is the objective proof of the effects on perfusion of new or alternative methods.
of treatments. Some promising methods provoking an improvement of the perfusion are not established because of a lack of evidence. In this way the systematic use of hyperspectral imaging for sensitive perfusion measurements may contribute to evidence based wound research and therewith to the development and optimization of new treatment tools.

Author Statement

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Conflict of interest: The hyperspectral camera prototype described in this publication was developed by Diaspective Vision GmbH. The first, second and third author are employees of this company. In the long term, Diaspective Vision has proprietary interest in the development of the camera system resulting in a product for routine clinical use. The clinical tests of the camera have been performed by clinicians (authors 4, 5 and 6). We certify that the clinical investigators and co-authors have no financial interests and financial arrangements with Diaspective Vision and have received no funding for the measurements and/or preparation of this manuscript. The cameras used during the measurements have been provided by Diaspective Vision.

Informed consent: All patients have signed an informed consent.

Ethical approval: Experimental hyperspectral measurements from patients for the evaluation of the new technology for perfusion measurements and wound description have obtained the ethics approval by the Ethics committee of the Ärztekammer Sachsen-Anhalt, Germany (35/17) and the Ethics Committee of the University of Greifswald (EUDAMED-No. CIV-17-02-018504). The study was conducted according to the Declaration of Helsinki.

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