Evaluation of hyperspectral imaging to quantify perfusion changes during the modified Allen test

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Abstract

Objectives: To evaluate the capability of hyperspectral imaging (HSI), a contactless and noninvasive technology, to monitor perfusion changes of the hand during a modified Allen test (MAT) and cuff occlusion test. Furthermore, the study aimed at obtaining objective perfusion parameters of the hand.

Methods: HSI of the hand was performed on 20 healthy volunteers with a commercially available HSI system during a MAT and a cuff occlusion test. Besides gathering red-green-blue (RGB) images, the perfusion parameters tissue hemoglobin index (THI), (superficial tissue) hemoglobin oxygenation (StO2), near-infrared perfusion (NIR), and tissue water index (TWI) were calculated for four different regions of interest on the hand. For the MAT, occlusion (OI; the ratio between the condition during occlusion and before occlusion) and reperfusion (RI; the ratio between the condition during occlusion and before occlusion) indices were calculated for each perfusion parameter. All data were correlated to the clinical findings.

Results: False-color images showed visible differences between the various perfusion conditions during the MAT and cuff occlusion test. THI, StO2, and NIR behaved as expected from physiology, while TWI did not in the context of this study. During rest, mean THI, StO2, and NIR of the hand were 34 ± 2, 72 ± 9, and 61 ± 6, respectively. The RI for THI showed a roundabout threefold increase after reperfusion of both radial and ulnar artery and was thus, distinctly pronounced when compared with StO2 and NIR (~1.25). The OI was lowest for THI when compared with StO2 and NIR.

Conclusions: HSI with its parameters THI, StO2, and NIR proved to be suitable to evaluate perfusion of the hand. By this, it could complement visual inspection during the MAT for evaluating the functionality of the superficial palmary arch before radial or ulnar artery harvest. The presented RI might deliver useful comparative values to detect pathological perfusion disorders at an early stage. As microcirculation monitoring is crucial for many medical issues, HSI shows potential to be used, besides further applications, in the monitoring of (free) flaps and transplants and microcirculation monitoring of critically ill patients.

Keywords
cuff occlusion test, hand perfusion, hemoglobin, hyperspectral imaging, modified Allen test, occlusion index, oxygenation, perfusion monitoring, reperfusion index, water content
INTRODUCTION

Hyperspectral imaging (HSI) has its origin in remote sensing and has been used in numerous nonmedical areas. In recent years, however, its application has been extended to medicine, where it offers great potential for noninvasive disease diagnosis and surgical guidance. This contact-less and noninvasive tool uses light from the visible and near-infrared spectrum for tissue illumination and the camera detects the diffuse remitted light. Different tissue components (e.g., hemoglobin, water) and their disease-related alterations affect the tissue’s scattering and absorption characteristics. Hence, it may become possible to identify and classify different biological tissues through the measured spectra. The application of HSI in medicine has proven to be useful in the characterization of wounds and the detection of cancerous tissue. Moreover, as it provides spectral information about hemoglobin, water, and oxygen supply, it has great potential also in perfusion monitoring. First studies showed its benefit in the assessment of peripheral artery disease and the evaluation of anastomoses and flaps. So far, however, HSI has neither gained widespread knowledge by doctors nor application outside of clinical studies. Reasons therefore might be systems being too big, too complicated, too expensive or only using a very limited number of spectral bands. This might change, however, due to rather innovative, commercially available HSI systems.

Simulating various perfusion conditions on the hand can be performed by different means. The Allen test, first described by Dr. Edgar V. Allen and later modified (modified Allen test [MAT]) is a routinely performed test to assess the performance of the superficial palmary arch before radial artery harvest, radial forearm free flaps and forearm artery cannulation. For this application, it is considered a fast, easy, and valid clinical tool, even though some authors criticize its sole use and claim the application of an additional objective test. Yet, an intact superficial palmary arch is also crucial before the less commonly performed ulnar forearm free flap is harvested. Another established approach to assess the microcirculation of the hand is to use a cuff occlusion test (COT) at the upper arm, which has been widely used as a comparative test for microcirculation monitoring before and has already been reported in combination with HSI on a few patients.

The purpose of this study was to both, qualitatively and quantitatively assess microcirculation of the hand in healthy volunteers during both a MAT and a COT at the upper arm using HSI. By this, it aimed at obtaining and establishing objective perfusion parameters for future comparisons with parameters obtained in patients, especially regarding the MAT. It was hypothesized that the perfusion parameters assessed by HSI correlate to the clinical findings.

MATERIAL AND METHODS

Equipment

By means of a CE-approved, commercially available hyperspectral camera system (TIVITA® Tissue System; Diaspective Vision GmbH) it is possible to acquire remission spectra in the range from 500 to 1000 nm for each pixel. The area of interest is illuminated with 6 halogen lamps positioned around the objective of the camera. The measurement creates a three-dimensional hyperspectral data cube with the spatial x, y-axis, and the spectral λ-axis with a spatial resolution of 640 × 480 pixels and a wavelength resolution of 5 nm. Besides a red-green-blue (RGB) image, the HSI system generates false-color coded images based on dedicated spectral features and thus provides information on the cutaneous and subcutaneous oxygenation pattern (StO2 and NIR perfusion index), the relative hemoglobin content in the tissue (tissue hemoglobin index [THI]) and the water content (tissue water index [TWI]). The perfusion parameters are calculated based on different equations and certain spectral bands, which are specific for each parameter. According to the considered spectral ranges and the corresponding penetration depths in human skin, the StO2 and NIR aim to assess the cutaneous hemoglobin oxygenation in superficial (<1 mm) and deeper tissue (<2 mm) layers.

Examinations

All clinical data were obtained and analyzed based on the approval by the ethics committee of the local medical faculty (IRB approval number 19-851) and in compliance with the Declaration of Helsinki. After written informed consent, 20 healthy volunteers underwent HSI of their nondominant hand (palm) during different test conditions as sketched in Figure 1. The nondominant hand was chosen as in clinical routine the radial or ulnar arteries are preferably harvested from the nondominant hand. The bars in Figure 1 indicate the moment of change regarding the perfusion status, for example, the start of occlusion or reperfusion. At the same time, they represent the time of shutter release—as the process of image acquisition endures approximately 10 s the image itself rises 10 s after the time indicated by the bars. Data assessment was performed in a darkened room to assure standardized light and temperature conditions. During measurement, patients lay in a supine position on a stretcher. For data acquisition, the HSI camera was placed with a distance of about 50 cm perpendicular to the area of interest focusing on the center of the palm of the hand. An HSI scan was performed every 30 s (timer-function of the system). After applying the cuff and a rest period of 5 min on the stretcher, patients first underwent a scan during rest. Subsequently, the cuff pressure was
raised to 80 mmHg for venous and to 200 mmHg for arterial occlusion. After a rest period of 5 min a MAT was performed. For this purpose, the radial artery or the ulnar artery were selectively released after both arteries were occluded for 30 s. In addition, the perfusion status of the hand was assessed by clinical standard observation with the naked eye and subjective categorization into color grades (rosy, blue, pale, cyanotic, hyperemic skin color) and on the RGB images. After completion of the data acquisition, all raw data were further analyzed.

### Data processing

For each of the four perfusion parameters, false-color images (FCI) were generated from the captured raw data according to a color scale (TIVITA Suite software, version 1.6.0.1; Diaspective Vision GmbH). The scale for each FCI ranged from 0 to 100, with 0 corresponding to blue and 100 corresponding to red color indicating minimum and maximum values. For perfusion assessment, StO2, THI, NIR, and TWI, were calculated at four different areas on the hand within a circular region of interest (ROI) as shown in Figure 2. The ROIs were manually selected, avoiding shaded areas. They were placed at the middle phalanx of the index finger and the auricular finger. The diameter of these ROIs was equivalent to the width of the respective middle phalanx. The remaining ROIs were placed at the thenar and hypothenar in a standardized procedure. In addition to the ROI sizes mentioned, each diameter was halved and quartered to investigate the influence of the diameter of the ROIs on the mean perfusion parameters. Furthermore, repeated HSI recordings were acquired from a small number of volunteers to investigate the influence of physiological fluctuations on each perfusion parameter.

To objectively analyze changes in perfusion during the MAT, an occlusion index (OI) and a reperfusion index (RI) were calculated for each perfusion parameter. The OI was defined as the quotient of the perfusion parameters during occlusion and the prior non-occlusion state (Equation 1). The RI was defined as the quotient of the perfusion parameters gathered during reperfusion and the preceding occlusion (Equation 2). The OI was calculated for the situation between rest and the following occlusion of both arteries and for the status of reperfusion of the radial artery and the following occlusion. The RI, on the other hand, was calculated separately for the release of the radial and ulnar arteries. For each perfusion parameter and each ROI mean values for both indices were calculated among all volunteers (see Equations 1 and 2, perfusion parameter [PP]).

\[
OI = \sum_{i=1}^{n} \frac{PP_{occlusion}^{(i)}}{PP_{non-occlusion}^{(i)}}, \quad (1)
\]

\[
RI = \sum_{i=1}^{n} \frac{PP_{reperfusion}^{(i)}}{PP_{occlusion}^{(i)}}, \quad (2)
\]

### Statistical analysis

Statistical analysis was performed with Matlab (version R2018b; MathWorks) and IBM SPSS Statistics (version 25; IBM). Average perfusion parameters and standard deviations for each ROI were calculated with Matlab. IBM SPSS Statistics was used to perform the Friedman test for testing statistically significant differences between different perfusion conditions of the MAT and COT. A value of \( p < 0.05 \) was considered significant.
RESULTS

HSI of the nondominant hand was performed during different conditions, as sketched in Figure 1, in 20 healthy volunteers, whose demographic and clinical data are summarized in Table 1. All volunteers tolerated the HSI examinations well. The examinations delivered FCIs and RGB images, which showed clear differences between the various perfusion states. During rest, StO2, NIR, and THI on the fingers showed higher values compared with the ROIs on the thenar and hypothenar (Table 2). The difference during rest was significant when comparing the ROI at the thenar with the one at the index finger for StO2, NIR, and THI. When comparing the ROI at the hypothenar and the one at the auricular finger a significant difference was evident only for THI. The determined values of NIR, StO2, and TWI at each ROI showed a standard deviation of approximately 10%, whereas THI shows the highest dispersion, especially on the thenar. Before defining the final size of the ROIs, the influence of the ROI diameter on perfusion parameters was evaluated. Here, no considerable influence was found in the context of this study. Furthermore, the repeated data acquisition showed no relevant changes in the calculated perfusion parameters.

COT

Figure 3 shows an example of a COT on the left hand in a volunteer, while Figure 4 shows the averaged perfusion

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**TABLE 1** Demographics and characteristics of healthy volunteers

|        |         |       |
|--------|---------|-------|
| Male   | 13 (65%)|       |
| Female | 7 (35%) |       |
| Age (years) | 38.1 (21.0–61.5) | |

Examined hand

|       |       |       |
|-------|-------|-------|
| Left  | 19 (95.0%) |       |
| Right | 1 (5.0%)  |       |

*Note:* Values are numbers of patients total and percent of the evaluated group. Age is given as median with range in parentheses.

**TABLE 2** Mean perfusion parameters during rest

| ROI                | NIR (± SD) | THI (± SD) | StO2 (± SD) | TWI (± SD) |
|--------------------|------------|------------|-------------|------------|
| Thenar (ROI T)     | 58 ± 5     | 21 ± 11    | 65 ± 8      | 44 ± 3     |
| Hypothenar (ROI H) | 59 ± 6     | 32 ± 12    | 72 ± 9      | 50 ± 5     |
| Index finger (ROI I)| 62 ± 8     | 46 ± 10    | 73 ± 11     | 45 ± 4     |
| Auricular finger (ROI A) | 62 ± 8 | 39 ± 11 | 72 ± 10 | 44 ± 5 |
| Mean (ROI T-A)     | 61 ± 6     | 34 ± 16    | 72 ± 9      | 46 ± 4     |

*Note:* Values represent mean and standard deviation calculated for each ROI during rest for the 20 volunteers. Last row gives a mean value from all ROIs for each perfusion parameter.

Abbreviations: NIR, near-infrared perfusion; ROI, regions of interest; StO2, hemoglobin oxygenation; THI, tissue hemoglobin index; TWI, tissue water index.
Figure 4. Averaged perfusion parameters calculated for each ROI (coded with color) during the cuff occlusion test among all volunteers. Error bars indicate the standard deviation for each averaged parameter. *Statistically significant differences ($p < 0.05$) between acquisition times for each perfusion parameter and each ROI. ROI, regions of interest.

Figure 5. Example of a modified Allen test performed on the right hand of a healthy volunteer, where each column represents a defined acquisition time. The first row shows the captured RGB image. In the following rows, the FCIs of each perfusion parameter are shown. The color bar on the right side depicts the numeric range from 0 to 100 for each perfusion parameter. FCI, false-color images; NIR, near-infrared perfusion; ROI, regions of interest; StO2, hemoglobin oxygenation; THI, tissue hemoglobin index; TWI, tissue water index.
parameters of the 20 volunteers for different times during the COT. Both, Figures 3 and 4 depict the first and last image/data set of both venous and arterial occlusion, next to those captured during rest and reperfusion. Clinically and on RGB images, volunteers showed a rosy skin color during rest. With venous occlusion only, the color of the skin changed to blue and with arterial occlusion to pale and in advanced stages to cyanotic. After release of occlusion, skin color changed within a few seconds to red and after approximately 1 min again to rosy. The extent and the promptness of color changes varied within patients. Generally, changes in the FCIs were clearly visible on the screen during the measurement. The longer the occlusion lasted, StO2 and NIR shifted continuously toward blue and lower absolute values indicating a decrease in tissue oxygenation both in superficial and deep layers due to venous occlusion with a reduced outflow of deoxygenated hemoglobin and additional arterial occlusion with missing inflow of oxygenated hemoglobin. After reperfusion, the color in the FCIs for both StO2 and NIR changed to a clear red of the palm and fingers and the absolute values for the two parameters increased, overshooting the mean values during rest. In contrast to that, THI changed from green-blue color during occlusion toward red indicating venous congestion. In a similar way, the calculated mean values increased. After reperfusion THI slightly decreased, however did not reach the rest state. The calculated mean values of the perfusion parameters show differences among the ROIs. These differences are most pronounced for the THI. Furthermore, when comparing the values of StO2, NIR, THI and TWI statistically significant differences are evident for some parameters and times (Figure 4, Table S1). Of note is the statistically significant difference for all parameters during arterial occlusion and reperfusion.

MAT

As depicted in Figure 5, during the MAT patients' skin color became pale during occlusion and red during reperfusion of both the radial and ulnar artery. Clinical findings correlated with RGB images. Visually all patients showed a normal MAT test with regular reperfusion. Color changes on the FCIs correlated to the changes in calculated mean values as shown in Figure 6, where the averaged perfusion parameters of the 20 healthy volunteers are illustrated. It can be observed that StO2 and NIR shifted toward blue during occlusion on FCIs, and changed to red, especially on the fingers after reperfusion indicating a missing and consecutive excessive inflow of oxygenated hemoglobin. Analogous to those visually detectable changes, both StO2 and NIR shifted to lower mean values during occlusion and significantly increased after reperfusion. THI changed to a deep blue during occlusion and to green after reperfusion. The color changes were also reflected in the calculated mean values, which showed a significant increase during reperfusion for both StO2 and NIR. THI showed a decrease, but did not reach the rest state. The color changes were most pronounced for the THI, indicating a significant decrease in tissue oxygenation during occlusion.

**FIGURE 6** Averaged perfusion parameters calculated for each ROI during the modified Allen test among all volunteers. Error bars indicate the standard deviation of each averaged parameter. Statistically significant differences ($p < 0.05$) between acquisition times for each perfusion parameter and each ROI. ROI, regions of interest.
A change to blue during occlusion indicates a missing inflow through the arteries but persistent outflow through the deep veins. Analogous to the visually detected changes also the mean values for THI clearly decreased during occlusion. In contrast, the TWI showed an increase during occlusion and a decrease during reperfusion. For the topmost majority of perfusion parameters at different ROIs, a statistically significant difference was found between occlusion and reperfusion (p < .05) (Figure 6, Table S2).

Table 3 gives the RI for each ROI regarding the release of the radial and ulnar artery, while Table 4 gives the mean OI calculated among the volunteers. Here, the mean values of the RIs show values bigger than 1 for NIR, StO2, and THI, indicating an increase in each perfusion parameter during reperfusion. In contrast, the TWI shows values smaller than 1. Although StO2, NIR, and TWI show a rather low standard deviation regarding both RI and OI, suggesting robust data, this does not hold true for the THI. The THI shows a threefold increase and the highest standard deviation after reperfusion, accentuated after reperfusion of the radial artery. The mean values of the OIs show values smaller than 1 for NIR, StO2, and THI, indicating a decrease for those perfusion parameters during reperfusion. In contrast, the TWI shows values bigger than 1.

### DISCUSSION

In the present study, the microcirculation of the hand was assessed during a MAT and a COT in 20 healthy volunteers by means of HSI. Quantitative data on perfusion parameters (StO2, THI, NIR, TWI) measured on the palm and fingers of healthy volunteers is reported.

Table 3

| NIR  | RR/Occlusion | RU/Occlusion | StO2  | RR/Occlusion | RU/Occlusion | THI   | RR/Occlusion | RU/Occlusion | TWI   | RR/Occlusion | RU/Occlusion |
|------|--------------|--------------|-------|--------------|--------------|-------|--------------|--------------|-------|--------------|--------------|
| ROI T| 1.14 ± 0.14  | 1.14 ± 0.11  | 1.30 ± 0.29 | 1.31 ± 0.22  | 4.10 ± 4.27  | 2.96 ± 2.99 | 0.92 ± 0.09  | 0.93 ± 0.05  | ROI H | 1.12 ± 0.12  | 1.14 ± 0.08  |
| ROI I| 1.25 ± 0.20  | 1.22 ± 0.10  | 1.26 ± 0.22 | 1.27 ± 0.14  | 2.62 ± 1.92  | 2.70 ± 2.20 | 0.87 ± 0.16  | 0.82 ± 0.10  | ROI A | 1.22 ± 0.19  | 1.25 ± 0.13  |
| ROI T| 1.25 ± 0.20  | 1.22 ± 0.10  | 1.26 ± 0.22 | 1.27 ± 0.14  | 2.62 ± 1.92  | 2.70 ± 2.20 | 0.87 ± 0.16  | 0.82 ± 0.10  | ROI A | 1.22 ± 0.19  | 1.25 ± 0.13  |

Table 4

| NIR  | Occlusion/Rest | Occlusion/RR | StO2  | Occlusion/Rest | Occlusion/RR | THI   | Occlusion/Rest | Occlusion/RR | TWI   | Occlusion/Rest | Occlusion/RR |
|------|----------------|-------------|-------|----------------|-------------|-------|----------------|-------------|-------|----------------|-------------|
| ROI T| 0.91 ± 0.12   | 0.87 ± 0.08 | 0.76 ± 0.21 | 0.75 ± 0.13  | 0.77 ± 0.65  | 0.50 ± 0.27 | 1.12 ± 0.11  | 1.08 ± 0.07  | ROI H | 0.94 ± 0.10   | 0.88 ± 0.05  |
| ROI I| 0.86 ± 0.22   | 0.79 ± 0.07 | 0.80 ± 0.17 | 0.77 ± 0.09  | 0.57 ± 0.35  | 0.45 ± 0.21 | 1.24 ± 0.16  | 1.23 ± 0.10  | ROI A | 0.87 ± 0.16   | 0.80 ± 0.08  |

Note: Calculated reperfusion indices at different ROIs for reperfusion of the radial artery and reperfusion of the ulnar artery after occlusion. Mean values ± standard deviation calculated among the volunteers are given.

Abbreviations: NIR, near-infrared perfusion; ROI, regions of interest; RR, reperfusion of radial artery; RU, reperfusion of ulnar artery; StO2, hemoglobin oxygenation; THI, tissue hemoglobin index; TWI, tissue water index.

**Table 3** Reperfusion indices

**Table 4** Occlusion indices

Note: Calculated occlusion indices at different ROIs for occlusion of both the radial and ulnar artery after rest (occlusion-rest) and for occlusion of both the radial and ulnar artery after reperfusion of the radial artery (occlusion-RR). Mean values ± standard deviation calculated among the volunteers are given.

Abbreviations: NIR, near-infrared perfusion; ROI, regions of interest; RR, reperfusion of radial artery; StO2, hemoglobin oxygenation; THI, tissue hemoglobin index; TWI, tissue water index.
however, NIRS is based on spectral bands in the near-infrared range and also depends on the evaluation algorithm used.51 On the other side, SpO2 is derived from light remitted from pulsing, arterial blood. Generally, the SpO2 is calculated by comparison of the remitted light from two light sources that emit at two different wavelengths.52,53 This approach allows to assess arterial oxygen saturation, however, does not deliver information about the tissue hemoglobin oxygenation.

The selected ROIs proofed to be suitable to assess the microcirculation of the hand. During both tests, parameters assessed at different ROIs showed changes in the same direction, however, showing evident differences in absolute values for different ROIs and time points. These differences were most prominent regarding the THI. Generally, perfusion parameters assessed at the fingers, seemed advantageous due to missing shaded areas, impeding the evaluation. Furthermore, pathological perfusion changes are expected to be clinically apparent at first at the fingers as they represent the boundary zone of the radial and ulnar artery. Parameters gathered from the thenar, showed the lowest absolute values during rest. Individual anatomy (fat and/or muscle content) and the fact, that the thenar does not have a plane surface might be responsible for that.

During the COT StO2, NIR and THI behaved as physiologically expected and thus compared favorably with previously published examples of COTs assessed with HSI.16,24,46,47 When comparing the perfusion parameters at rest and after reperfusion, the gathered values of StO2, NIR, and THI during reperfusion exceed those values at rest. This behavior is attributable to reactive hyperemia, which can be observed after an ischemic period in the limbs.54 As expected from a physiological point of view, same as in the present study (see Figure 4), all authors found an increase in tissue hemoglobin in venous occlusion and a decrease in tissue oxygenation.16,24,46,47 In none of the mentioned studies, absolute measures were given for the evaluated parameters. Yet, when looking at the parameter curves presented in a recently published study, our values of StO2, NIR, and THI during the COT seem similar.47 Even though not directly comparable, one publication presented perfusion parameters measured in the angiosome of the median palmar artery of healthy volunteers during rest.13 StO2 and NIR were 54.7 ± 2.6 and 60.3 ± 1.3, respectively. The mean values of the present work from all ROIs for StO2 and NIR were 72 ± 9 and 61 ± 6, respectively. The only larger difference was apparent for the THI averaging 14.8 ± 2.4 at the angiosome of the median palmar artery, compared with 34 ± 16 assessed for the palm of the hand. Patients with peripheral artery disease showed clearly lower values for StO2 and NIR, while THI was higher. This said, it is to be expected that patients needing radial artery harvest, show differences in perfusion parameters of the hand when compared with the herein investigated group of healthy volunteers, as they frequently suffer from vascular disease to a certain extent. A respective comparison with different study groups on a larger number of subjects could give weight to the findings of the present work.

Analogous to the changes during the COT also those during the MAT test behaved as expected by physiology. During occlusion of the radial and ulnar artery oxygenation parameters (StO2 and NIR) markedly decreased due to the missing supply with fresh oxygenated hemoglobin. In contrast to the venous occlusion during the COT, however, THI also decreased. The reason, therefore, is the missing arterial inflow in preserved outflow through the deep venous system. With exceptions related to a few ROIs, the changes in two consecutive scan periods of the MAT differed significantly for all perfusion parameters. As mentioned above, the fingers represent the boundary zones of the radial and ulnar arteries. Thus, the ROIs at the fingers are of special interest. Observations of perfusion parameters during the MAT using HSI have not been reported before. Although some authors consider the MAT alone, to be sufficient before radial artery harvest,35,40 others claim for more objective tests.36–38 As a standard procedure, in patients with a pathological MAT, Doppler and/or Duplex Ultrasound is considered to be the method of choice for further evaluation. In contrast to HSI, Doppler and/or duplex ultrasound allows to directly visualize both the radial and ulnar artery, possibly showing additional branches, calcifications and determine the peak systolic velocity.33,34,40 In clinical application computed tomography angiography could be useful to further diagnose and identify individual structural changes in cases of uncertainties using Doppler and/or duplex ultrasound. However, also pulse oximetry and digital plethysmography have been used before.55,56 Yet, for all mentioned methods clear cut-off values to differentiate between physiological and pathological hand perfusion are missing.37 This also holds true for the MAT test itself—time thresholds for reperfusion of 3–12 s have been reported by various authors, with 6 s as a cut-off showing the biggest accuracy.34,56,57 However, radial artery harvest has even been performed safely in patients with an abnormal MAT.34 As HSI showed clear changes in perfusion parameters during the MAT, it seems to be useful as an additional tool, besides the MAT, to assess the superficial palmary arch. Furthermore, HSI and Doppler and/or Duplex Ultrasound could ideally complement each other to further assess the perfusion of the hand in clinically pathologic MAT.

The herein calculated RIs regarding hemoglobin oxygenation (StO2 and NIR) and content (THI) might be valuable tools to distinguish between normal and pathological reperfusion and thus point out pathologic alterations of the superficial palmary arch, for example, caused by an incomplete superficial palmary arch or by arteriosclerosis. For the THI a wide distribution among the volunteers was found. Reasons might be a big interindividual variety even in
healthy subjects, eventually caused by anatomical variations such as differences in muscle mass, which contains myoglobin that shows similar absorption properties as hemoglobin. Furthermore, fat content in the skin, metabolic processes, or other unknown confounding factors in the calculation of the THI are considered. The OI might be used in detecting anatomic anomalies within the forearm arteries, when there is a missing or insufficient decrease in StO2, NIR, and especially THI. Furthermore, it could detect an insufficient occlusion of the arteries, a frequent reason for a false-negative MAT. Yet, a calculation of both the RI and the OI regarding only the ROIs at the fingers and StO2, NIR, and THI might be sufficient. As the method, so far, has only been applied in healthy volunteers the cut-off values are unclear and should in the future be compared with parameters raised in patients with consecutive successful radial or ulnar artery harvest. Furthermore, continuous measurement of those parameters would be interesting to see the time dependence of perfusion changes after release of either the ulnar or the radial artery. In the setup of a clinic frequently performing reconstructive head and neck surgery, the application of HSI seems of special interest, as the method could not only be used as an additional tool to assess the superficial palmary arch before radial forearm or ulnar forearm free flaps, but also for the postoperative monitoring of those flaps. Compared to ultrasound, the objective evaluation of blood and oxygen supply with HSI of the whole hand is of advantage. The ultrasound, on the other hand, allows for direct visualization of the arteries, of their diameter, arteriosclerotic alterations, and blood flow.

Tissue perfusion denominates the supply with both blood and oxygen. By providing information on both the oxygenation (StO2 and NIR) and the volume of the hemoglobin (THI) in tissue, it looks promising that by means of the TIVITA® Tissue System profound information on tissue perfusion could be given, allowing to draw conclusions on the reasons for a reduced oxygen supply of tissue. In contrast, pulse oximetry, for example, only gives information on oxygenation. TWI, within the study at hand, behaved contrary to our expectations. One factor might be the timely limited occlusion. As occlusion of the veins in the arm during the COT results in increased venous pressure, a shift in the osmotic gradient toward the tissue is expected, resulting in an increased TWI, which was not noticed. Also during the MAT, TWI did not behave as expected and increased during occlusion. This leads to the assumption that the TWI is either not suitable to evaluate the water content of the hand in the context of this study or the considered spectral bands used by the software (880–900 nm and 955–980 nm) are prone to external influences.

By giving profound information on tissue perfusion, HSI holds great potential in the postoperative surveillance of flaps and the early detection of the most common reasons for flap failure, thus arterial insufficiency or venous congestion. The radial forearm free flap is widely used for head and neck reconstructive surgery, while the use of an ulnar forearm free flap has only occasionally been reported. The proper function of the arterial and venous anastomosis after reconstruction is essential for the survival of free flaps. Although success rates are usually cited at 95% or higher, there is still a relevant number of flap failures. Postoperative clinical monitoring of flaps is the gold standard including assessment of flap color, rate of bleeding with scratch, and acoustic cutaneous Doppler. An early detection of perfusion changes is essential for flap salvage. Thus, an additional tool to objectively and early detect perfusion deterioration would be highly desirable. HSI has been successfully applied in preclinical models to assess anastomoses and flaps. Transferred to the clinic, HSI has been used in the monitoring of gastrointestinal anastomoses during open abdominal surgery and succeeded at monitoring physiological perfusion parameters. Furthermore, HSI was used in the monitoring of pedicled flaps. In a recent study the use of HSI in the intra- and postoperative monitoring of pedicled (n = 8) and free (n = 22) flaps in reconstructive head and neck surgery is described. HSI corresponded well to the clinical findings in two cases of venous congestion as well as in one case of arterial insufficiency, needing flap revision. Our personal unpublished experience with HSI in the monitoring of free flaps in reconstructive head and neck surgery indicates HSI to be effective in the monitoring of perfusion parameters. However, except for the anterior oral cavity, the feasibility to gather high-quality image sets is limited. A smaller system or an endoscopic version would be more convenient and thus highly desirable for flap monitoring in the oral cavity, pharynx, and larynx. Rigid and even to a greater degree, flexible endoscopic systems could, however, reach many more regions (e.g., the upper and lower aerodigestive tract) and thus address a whole range of additional clinical problems.

**CONCLUSION**

HSI proofed to be suitable to evaluate perfusion of the hand, by providing information on both oxygenation and volume of hemoglobin in tissue and thus allowing to give conclusions on reasons for impaired circulation. By this, it could complement visual inspection during a MAT for evaluating the performance of the superficial palmary arch before radial or ulnar artery harvest. Especially in clinically pathologic MAT, a comparison with the herein presented OI and RI might be useful to objectify clinical findings and detect pathological hand perfusion at an early stage. As microcirculation monitoring is crucial for many medical issues, HSI shows great potential to be used for a variety of clinical questionings, such as the monitoring of (free) flaps and transplants and microcirculation monitoring of critically ill patients.

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CONFLICT OF INTERESTS
The authors declare that there are no conflicts of interest.

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