Optical imaging and parametric characterization of frostbite changes in human hand tissues

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Frostbite is a condition in which body tissues freeze and cause damage to skin and soft tissues. The present work deals with the optical characterization of frostbite of the hands, which involves the skin and tissue structure beneath it and is carried out by laser reflectometry and Monte Carlo simulation. The grid of the dorsal side of the hand is developed on a computer monitor and the same is scanned point-to-point. Data obtained in the form of normalized back-scattered intensity (NBI), after interpolation and median filtering are displayed as colour-coded images. In controls the NBI is significantly higher at the abductor brevis muscle and minimum at the tendon of the flexor digitorum, pollicis longus and at the nails compared to that at the other regions. Due to frostbite the NBI values are lower at various locations in the fingers and dorsal sites in these subjects compared to those of the respective controls. The variation in NBI is maximum for the first probe compared to the other probes. The optical parameters, absorption and scattering coefficients, as determined by matching of the measured surface profile with that as obtained by Monte Carlo simulation of photon-scattering process, show an increase in absorption coefficient and decrease in scattering coefficient of the frostbite-affected tissues compared to those of control subjects.

Keywords: Back-scattered intensity, Frostbite, Monte Carlo simulation, multi-probe laser reflectometer, optical parameters.

FROSTBITE most likely occurs in body parts farthest from the heart, with their surface area exposed to low temperature. At or below −15°C (5°F), blood vessels close to the skin are constricted. This mechanism helps to preserve core body temperature, but during prolonged exposure this protective strategy can lead to reduced blood flow in some areas of the body to dangerously low levels. The combination of cold temperature and poor blood flow causes severe injury by freezing the tissue. The vascular phase (characterized by plasma leakage, stasis, coagulation and vasoospasm) occurs as ice crystals which continue to develop in the extracellular fluid. The final phase of direct freezing is the ischemic phase, resulting in tissue necrosis, thrombosis and autonomic dysfunction¹.

Frostbite most commonly affects the extremities. Although frostbite has been classified by degree², it may be more usefully classified as superficial or deep³. Superficial frostbite affects the skin and subcutaneous tissues, whereas deep frostbite affects bones, joints and tendons. The extent to which frostbite is affected can be ranked into four different degrees. The first-degree frostbite is similar to mild chilblain with hyperaemia and results in mild itching and oedema. Blistering and desquamation characterize the second degree or superficial frostbite. The skin could be white or blue and becomes hard and frozen, but the tissues underneath are still undamaged. Third degree or deep frostbite is associated with necrosis and de-colouration of skin and subcutaneous tissue with ulceration. The damage also extends beyond the skin, and can destroy underlying soft tissue, muscle, bone and blood vessels. Fourth-degree frostbite includes destruction of connective tissues and bone, with gangrene and eventual loss of tissue. The long-term consequences are devastating, including amputation, chronic pain and osteoarthritis¹.

Frostbite causes injury to the tissue by direct ice-crystal formation at the cellular level with its dehydration and micro-vascular occlusion. Muscle that initially appears viable on reperfusion, may subsequently become necrotic because of microcirculatory collapse. Since muscle is a sensitive tissue in frostbite injury, technetium-99m limb scintigraphy is used to assess tissue viability in experimental animals⁴. The scintigraphic findings of hypoperfusion and hyperperfusion co-related well with the pathological findings⁴. Applications of other techniques such as ultrasonic scanning with high-frequency transducer, computed tomography and magnetic resonance imaging could also provide valuable data on clinical status of affected tissues, which could prove to be effective in the recovery of frost-affected subjects⁵. In contrast, optical techniques are not only economically viable, but are sensitive to detect the close-to-skin as well as deeper tissue changes⁶,⁷. The photon transport in a tissue medium is based on spatial variation of refractive indices in the medium, thus leading to their interaction with the tissue at sub-cellular level. The change

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in refractive indices is quite early and significant when compared to the change in attenuation coefficient (X-ray) or in acoustic impedance (ultrasound), at any given stage of a disease process. This makes optical imaging techniques ideally suited for early detection of abnormalities. Optical imaging techniques use non-ionic light radiations, which could further be applied to continuously monitor the physiological/pathological variations in the human body and could yield information regarding the metabolic state of tissues with high sensitivity.

Light scattering depends on the size, morphology and structure of the components in the tissues (e.g. lipid membrane, collagen fibres and nuclei). Variations in these components due to disease affect the scattering properties, thus providing a means for diagnostic purpose. By analysing the back-scattered light fraction after undergoing absorption and scattering within the tissues, information on variation in internal composition of the tissues can be obtained. Optical parameters such as absorption coefficient (\(\mu_a\)), scattering coefficient (\(\mu_s\)) and anisotropy parameter (\(g\)) further complement the characterization of various types of biological tissues. These parameters determine the photon flux distribution within the tissues and are obtained by direct\(^{13}\) or indirect\(^{14}\) methods. As there are tissue structural changes in frostbite of the hands, the objective of the present work is to identify these changes through multilayer imaging and to characterize these in terms of their optical parameters in comparison with that of control subjects.

**Materials and methods**

**Multi-probe laser reflectometer**

Schematic diagram of the multi-probe reflectometer for the measurement of spatial variation of surface reflectance from various layers of tissue is shown in Figure 1. Laser light from a diode laser of power 3 mW, operating at 670 nm, was transmitted onto the tissue surface by an input fibre of active diameter 1 mm and length 1000 mm. The back-scattered radiation from the tissue surface was collected by three output fibres of the same diameter and length, arranged in parallel in the measurement probe. The probe was always held perpendicular to the tissue surface, which ensured maximum penetration and back-scattered intensity compared with that of other angles of incidence. The back-scattered light signals collected by the output fibres were digitized and interfaced to a computer for further processing. Finally, the measured intensity values were converted into the corresponding normalized back-scattered intensity (NBI), given in terms of the percentage of incident intensity. Further details of this technique are given elsewhere\(^{10}\).

**Data acquisition**

Prior to data collection from each tissue the reflectometer reading was adjusted to zero by placing the probe on the surface of black rubber. The body fat of each subject was measured using bioelectrical impedance method\(^{15}\) and the results were also cross-checked by measuring with body fat calipers (Accumax calipers). In this method a low non-invasive electric current was passed through the body and the resistance was measured. Thereafter, using data on the person’s weight, height, age and sex, by measuring the total body water and fat-free mass, the body fat percentage was calculated. To determine the subcutaneous fat layer thickness of the body, fat calipers were used to test the skin fold by pinching of the skin precisely using calipers at several standardized points on the body. These measurements were converted to an estimated body fat percentage using the equation:

\[
\text{Body fat} = (0.1051 \times \text{Sum of skin folds measured at triceps, subcapular, supraspinale, abdominal, thigh, calf}) + 2.585.
\]

The body fat measurement was made using two methods on the subject and the difference in the measurement using these methods should not exceed more than 5%. Out of five healthy male subjects with light-brown skin complexion in the age group of 25–40 years and with body weight in the range 55–90 kg, two subjects as control 1 and control 2 with fat content matching (within ±2%) with that of frostbite subject 1 (11.9%) and subject 2 (23.6%) respectively, were selected.

Prior to reflectance measurement, all the subjects were familiarized with the procedure and the hair on the dorsal side of the palm region was completely removed by shaving the region and the surface was cleaned. Thereafter, each subject was asked to relax for 5 min in an air-conditioned room maintained at temperature of 25 ± 1°C. The skin temperature at the measurement site was 35.0 ± 0.5°C.

To acquire reflectance data, the grid and the outline of the dorsal region were developed and displayed on a
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computer monitor as shown in Figure 2. The measurement probe was moved at various locations of the dorsal side of the palm manually, in increments of 5 mm. The maximum reflectance at each location corresponding to vertical position of the probe was recorded. Scanning was performed starting from the left of the wrist base and was carried out in increments of 5 mm, moving towards its right side. Once the end of the grid was reached, the probe was moved one step of 5 mm away from the base and scanning was carried out till the end of the grid was reached. This procedure continued till the complete dorsal side of the palm region was scanned. The reflectance data at each point from each fibre represented an average of 100 samples, which averaged out the effect of pulsation due to blood flow. The complete data acquisition on the palm region took approximately 1 h.

The data acquired using the above method were in the form of locations of observation points (x-y coordinates) and their back-scattered intensity values. After converting these into corresponding NBI values, the surface profiles were plotted. With an exponential fit, the NBI values at intermediate locations were obtained.

Data processing

The acquired NBI data on the surface along the depth at selected locations of observation points were not smooth due to the presence of spurious effects. These datapoints were smoothed by neighbourhood averaging. The set of NBI values was at discrete locations on the surface. From these data, by an interpolation procedure a regular pattern that completely covers the entire surface was obtained.

Median filtering

The interpolated matrix was not suitable for display because of the associated noise during data acquisition and interpolation procedures, which appears as discrete or isolated strong pixel variations that are not spatially correlated with the image. This was corrected by median filtering of the image. This procedure involves a nonlinear filter, and does not introduce any new datapoint. Typically, these operations are concerned with nearest neighbours only, so that the new value of an element depends on nine previous values, i.e. the element itself and its eight nearest neighbours. For this, the input data value was replaced by the median value, that is

\[ v(m, n) = \text{median}\{v(m - k, n - l), (k, l) \in W\}, \]

where \( W \) is a suitably chosen window of size \( 3 \times 3 \). By moving the window point by point, the median value at the centre was calculated. Using this procedure the median filtered data, free from noise, at all grid points were obtained.

Display

The NBI variation data, represented as an image matrix, were mapped into 27 different colours. Scaling was done according to the observed maximum and minimum values and was equally divided into 27 divisions. For good contrast these colour-coded images with a resolution of 640 x 480 pixels were displayed on the outline of the dorsal side. For this, the NBI data as measured at various locations by first, second and third fibres were separately interpolated, median-filtered and displayed using the procedure mentioned above. These images show the variation of NBI as received from various depths on the tissue surface.

Optical parameters

The Monte Carlo procedure was applied to simulate light transport by sending photons or photon packages on a random walk through a virtual tissue sample. The path of every photon package was simulated until it emerged or was absorbed. The model assumes a non-deterministic, stochastic nature of light scattering and absorption of individual photons. For determination of optical parameters at each location on the hand, the measured surface back-scattered profile, using the Monte Carlo procedure was simulated.

In the simulation procedure, the photon beam enters the tissue surface orthogonally. The tissues are assumed to have semi-infinite extensions in depth and width. The base coordinate of the tissue and the centre of the beam coincide at coordinate \((0,0,0)\). Determination of optical properties by curve-fitting method was based on the inverse problem. This was achieved by matching the measured surface profiles of tissues with the simulated profiles. For determination of absorption and scattering
coefficients and anisotropy parameter, the simulation procedure in two steps was carried out. In the first step, the tissue medium was considered as isotropic ($g = 0$) and corresponding absorption ($\mu_a$) and reduced scattering coefficients ($\mu_s'$) were determined. For this condition the reduced scattering coefficient was the same as the scattering coefficient ($\mu_s$). The diffuse reflectance profiles were computed for various combinations of $\mu_s'$ and $\mu_a$, and the best matched profile with the measured reflectance profile, by iterative procedure using chi-square test ($\chi^2_{0.95}$ and r.m.s. error 0.006), was obtained. In the second step, the anisotropic nature of the tissues ($g \neq 0$) was considered. For the purpose of simulation the value of $\mu_s$ as determined above was kept constant, and the diffuse reflectance profiles for various combinations of $g$ and $\mu_s'$ were computed. By obtaining the best fit of these profiles ($\chi^2_{0.95}$ and r.m.s. error 0.002), the corresponding optical parameters $\mu_s$ and $g$ were obtained. The flow chart of the entire process is shown in Figure 3, and the programing was carried out in C language to run on the Windows platforms. Further details of the algorithm for determination of back-scattered surface profile are given elsewhere.\(^\text{15}\)

Regional variations of NBI

The NBI and the optical parameters, as determined by the above procedure, of the human dorsal side of the palm showed point-to-point variations. For comparison, the dorsal side region was divided into five different sub-regions (Figure 4). The thumb was divided into three regions, with nails, phalanx bone and metacarpal bone as regions 1, 2 and 3 respectively. The index or forefinger was divided into four regions, with nail, middle phalanx, proximal phalanx and metacarpal bone as regions 1, 2, 3 and 4 respectively. The same was repeated for the middle, ring and small finger. Region 5 starting from the base of the wrist consists of carpal bones and ulnar artery entering the palm and flexor retinaculum muscles. For each region, the mean values of NBI and optical parameters were calculated. Statistical analysis was carried out by comparing with region 5 of control 1 (with fat content similar to subject 1) and frostbite subjects with Student’s $t$-test.

Results

Based on the NBI data of control 1 (fat content 11.9%) as obtained using the three fibres, the images were reconstructed (Figure 5). Selecting the NBI interval as $6.1 \times 10^{-3}$ between successive colours for the 2 mm detector probe, Figure 5a shows the distribution of NBI data over the dorsal side of the palm surface. Variation in NBI shows the changes in tissue structure over various locations. Keeping the NBI interval as $3.5 \times 10^{-3}$ and $1.43 \times 10^{-3}$ between successive colours for the 4 and 6 mm detector probes, the distribution of NBI data is shown in Figure 5b and c respectively. Based on the surface profiles of NBI from each point, the optical parameters, absorption coefficient, scattering coefficient and anisotropy parameters were obtained.

In control 1 NBI was significantly higher at the abductor brevis muscle for 2, 4 and 6 mm detector probes, contributed by the high scattering coefficient and low absorp-
From NBI variations of the 2 and 4 mm detector probes, the ulnar artery, deep branch of ulnar, superficial branch of ulnar, digital artery, radialis indicis artery and princeps pollicis were also observed to be associated with low absorption coefficient and high scattering coefficients. As the 6 mm detector probe collects scattered radiation from the deeper layers, contributions of the bones to lower values of NBI clearly reproduce the structures of the carpal, metacarpal, proximal, middle phalanx and distal bones. Intermediate values of NBI were observed in the muscles placed between the metacarpal bones associated with intermediate values of absorption and scattering coefficients.

Higher values of NBI, observed in the first, second, third and fourth palmar interosseus muscles, are attributed to their higher scattering coefficient and low absorption coefficient. Intermediate values of NBI were observed in the dorsal interosseus, first muscle in the medial side of the thumb, second muscle in the palmar side of the index finger, third muscle in the lateral side of the fourth digit, fourth muscle in the lateral side of the fifth digit, and these regions also showed intermediate values of optical properties. The nails, tendon of flexor digitorum and pollicis longus for the three detector probes had low values of NBI associated with high absorption and low scattering coefficients.

Comparative analysis of NBI of the frostbite and control subjects is shown in Figure 6. The photograph of the frostbite affected hand of subject 1 (body fat 11.9%) is shown in Figure 6a. On physical examination of the subject affected with frostbite, the phalanx regions were red in colour. Variations in NBI of this for the 2 mm detector probe are shown in Figure 6b, and those of control subject is shown in Figure 6c. The photograph of frostbite-affected subject 2 (body fat 23.6%) is shown in Figure 7a. On physical examination of the subject, it was observed that the nails, phalax and metacarpal regions were black in colour. Variations in NBI obtained from subject 2 for the 2 mm detector probe are shown in Figure 7b, and those of control subject are shown in Figure 7c.

Comparison of regional variation of NBI for control and frostbite-affected subjects for the 2 mm detector probe is given in Table 1. The comparison was carried out by measuring the probability of a finding occurring (i.e. rejecting the null hypothesis) by chance alone, given that the null hypothesis is actually true. By convention, a P-value <0.05 is often considered significant. Region 1 (nails) had no significant variations for both the subjects. In region 2, subject 1 had a significantly low NBI ($P < 0.026$) compared to control 1. For subject 2, this region was associated with significant variation ($P < 0.0318$) of NBI. In region 3, subject 1 had no significant variations in NBI, whereas subject 2 had significant variations ($P < 0.0318$) in NBI in this region compared to control 1. No significant variation in regions 4 and 5 was observed, as the tissues in these regions were not affected by frostbite.

The regional variation of NBI for the five regions of the control and frostbite-affected subjects for the 4 mm detector probe is given in Table 2. Regions 1–5 show no significant variation for subject 1 compared to that of control 1. For subject 2, significant variation ($P < 0.0153$) in NBI was observed at region 2, whereas the other regions showed no significant variation compared to that of control 1. This indicates that the tissue injury in region 2 is deeper in subject 2 compared to subject 1. In regions 3–5, subject 1 affected by frostbite does not show any significant variations for the 4 mm detector probe, whereas subject 2 showed significant variation ($P < 0.0153$) in region 3 and there was no significant variation in regions 4 and 5.

Table 3 shows a comparison of the absorptions coefficients ($\mu_a$) of control 1 and frostbite-affected subjects. In region 1, there was no significant variations, whereas in region 2 the absorption coefficient varied from significant in subject 1 ($P < 0.032$) to highly significant in subject 2 ($P < 0.0058$). In region 3, no significant change was observed in subject 1, whereas a significant change was observed in subject 2. In regions 4 and 5, no significant change in the NBI was observed compared to that of control 1.

Variation in scattering coefficients ($\mu_s$) of the control and frostbite-affected subjects is shown in Table 4. In region 1, there was no significant variations in the scattering coefficients for both control 1 and frostbite-affected subjects. In region 2, the scattering coefficient decreased significantly in subject 1 ($P < 0.021$) and subject 2.
(P < 0.0421). In region 3, a significant decrease was observed only for subject 2. No significant variation in scattering coefficient in regions 4 and 5 compared to that of control 1 was observed. This shows that scattering properties are not affected in these regions. Table 5 shows the variation in anisotropy parameter (g) obtained for control 1 and frostbite-affected subjects. In contrast to other parameters, no significant change in this parameter was observed at all the regions.

**Discussion**

The use of laser radiation for imaging and characterization of biological tissues offers promise for the development of safe, non-invasive and inexpensive clinical imaging modalities with diagnostic ability. The surface profile of NBI in combination with similar profiles as obtained by Monte Carlo simulation provides the procedure to obtain the optical parameters of tissues. By simulation of the
Table 2. NBI variations of human palm regions for normal and frostbite-affected subjects as determined by the second probe placed at 4 mm

<table>
<thead>
<tr>
<th>Region</th>
<th>Normal</th>
<th>Subject 1</th>
<th>Subject 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.01089 ± 0.0034*</td>
<td>0.01078 ± 0.0022</td>
<td>0.00842 ± 0.0027</td>
</tr>
<tr>
<td>2</td>
<td>0.02882 ± 0.004</td>
<td>0.02745 ± 0.0025</td>
<td>0.01453 ± 0.0035**</td>
</tr>
<tr>
<td>3</td>
<td>0.02153 ± 0.0017</td>
<td>0.02018 ± 0.0034</td>
<td>0.01731 ± 0.0022**</td>
</tr>
<tr>
<td>4</td>
<td>0.01955 ± 0.0023</td>
<td>0.01843 ± 0.0019</td>
<td>0.01863 ± 0.0019</td>
</tr>
<tr>
<td>5</td>
<td>0.03152 ± 0.0019</td>
<td>0.03063 ± 0.0027</td>
<td>0.03084 ± 0.0016</td>
</tr>
</tbody>
</table>

*Mean ± SD; **P < 0.0153.

Table 3. Absorption coefficient of dorsal region of hand of control 1 and frostbite-affected subjects

<table>
<thead>
<tr>
<th>Region</th>
<th>Normal subject</th>
<th>Subject 1</th>
<th>Subject 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.215 ± 0.019*</td>
<td>2.228 ± 0.021</td>
<td>2.312 ± 0.021</td>
</tr>
<tr>
<td>2</td>
<td>2.384 ± 0.022</td>
<td>2.473 ± 0.014**</td>
<td>2.582 ± 0.014***</td>
</tr>
<tr>
<td>3</td>
<td>2.467 ± 0.028</td>
<td>2.618 ± 0.023</td>
<td>2.523 ± 0.026***</td>
</tr>
<tr>
<td>4</td>
<td>2.589 ± 0.021</td>
<td>2.623 ± 0.022</td>
<td>2.498 ± 0.014</td>
</tr>
<tr>
<td>5</td>
<td>2.153 ± 0.014</td>
<td>2.178 ± 0.018</td>
<td>2.178 ± 0.023</td>
</tr>
</tbody>
</table>

*Mean ± SD; **P < 0.032; ***P < 0.0058.

Table 4. Scattering coefficient of dorsal region of human hand of control 1 and frostbite-affected subjects

<table>
<thead>
<tr>
<th>Region</th>
<th>Normal subject</th>
<th>Subject 1</th>
<th>Subject 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>183.22 ± 0.32*</td>
<td>182.78 ± 0.22</td>
<td>182.11 ± 0.22</td>
</tr>
<tr>
<td>2</td>
<td>186.75 ± 0.17</td>
<td>183.76 ± 0.24**</td>
<td>180.13 ± 0.14***</td>
</tr>
<tr>
<td>3</td>
<td>188.63 ± 0.24</td>
<td>186.34 ± 0.14</td>
<td>179.93 ± 0.14***</td>
</tr>
<tr>
<td>4</td>
<td>189.74 ± 0.28</td>
<td>189.12 ± 0.17</td>
<td>188.12 ± 0.17</td>
</tr>
<tr>
<td>5</td>
<td>191.69 ± 0.19</td>
<td>191.18 ± 0.12</td>
<td>191.22 ± 0.13</td>
</tr>
</tbody>
</table>

*Mean ± SD; **P < 0.021; ***P < 0.0421.

Skin and subcutaneous tissues are maintained at a constant temperature (98.6°F) by the circulating blood. During frostbite, less warm blood reaches the skin and body parts such as fingers, toes, ears and nose cool more rapidly, leading to cellular injury and vascular impairment. Due to cold injury the skin properties changes and depth of damage increases depending upon the levels of cold injury. The cold-affected skin and tissues beneath show a decrease in NBI as indicated by three detector probes compared to the control subjects. The optical parameters show increased absorption and decreased scattering coefficients for the frostbite-affected subjects. The changes are further enhanced with discoloration of the skin. However, the nail region is not affected by frostbite as shown by insignificant variation of NBI over this region.

The multi-probe laser reflectometer receives photons emerging from various depths of the tissues. The depth and degree of light penetration through the tissue is a function of wavelength, intensity of light and optical photon scattering process using these optical parameters, further details of photon scattering within the biological medium essentially required for photodynamic therapy and for analysis of tissue structural details, could be obtained. Based on the variation of NBI and optical parameters, the compositional variation in tissues could be determined. Various images of NBI and optical properties obtained were compared with the anatomical structure of the human hand, and the results are in agreement with the anatomical details.

The present study shows that NBI profiles vary over various regions of the dorsal side of the palm. Scattering from the muscles primarily depends on their fibre orientation and perfusion of oxygenated blood. Localization of blood vessels is primarily contributed by the smooth muscle composition of these vessels, as observed by first and second fibres. Variation of pigmentation, presence of veins and alteration in collagen tissues could also be contributory factors in the changes in NBI.
Table 5. Anisotropy parameter of dorsal region of human hand of control 1 and frostbite-affected subjects

<table>
<thead>
<tr>
<th>Region</th>
<th>Normal subject</th>
<th>Subject 1</th>
<th>Subject 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.936 ± 0.001*</td>
<td>0.932 ± 0.001</td>
<td>0.938 ± 0.002</td>
</tr>
<tr>
<td>2</td>
<td>0.953 ± 0.003</td>
<td>0.948 ± 0.001</td>
<td>0.942 ± 0.001</td>
</tr>
<tr>
<td>3</td>
<td>0.962 ± 0.002</td>
<td>0.962 ± 0.002</td>
<td>0.957 ± 0.002</td>
</tr>
<tr>
<td>4</td>
<td>0.974 ± 0.002</td>
<td>0.975 ± 0.001</td>
<td>0.964 ± 0.002</td>
</tr>
<tr>
<td>5</td>
<td>0.982 ± 0.001</td>
<td>0.981 ± 0.003</td>
<td>0.982 ± 0.001</td>
</tr>
</tbody>
</table>

*Mean ± SD.

properties of the tissue. The depth of penetration in the 630–640 nm visible red region is between 8 and 10 mm, while in the infrared region it is between 30 and 40 mm. Based on this it is possible to find out the extent of damage to the tissues at various depths and frostbite levels can be graded accordingly. For subject 1, the decrease in NBI was observed by the first probe, but not the second and third probes, which signifies that frostbite for this subject had affected the skin and adjacent tissues. Pigmentation in the skin in subject 1 was not affected, whereas it was affected in subject 2. The decrease in NBI corresponds to increased absorption coefficient and decreased scattering coefficient. The anisotropy parameter was not affected in this process and corresponded to forward dominate scattering. The level of frostbite in subject 1 could be classified as grade one³.

For subject 2, the decrease in NBI was observed in the first and second detector probes in regions 2 and 3, but not in the third detector probe, which signifies that frostbite had affected the skin and subcutaneous tissues, but not deeper layers of tissues and bones. Based on this the level of frostbite in subject 2 could be classified as grade two³.

In conclusion, laser reflectance imaging is a simple, non-invasive technique which provides details of tissue compositional variation, and could easily be used for the detection of frostbite and its classification level. Thereafter, measures could be taken to rehabilitate patients within a short span of time.


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