Optical characterization and imaging of biological tissues

R. Srinivasan, D. Kumar and Megha Singh*
Biomedical Engineering Division, Indian Institute of Technology, Chennai 600 036, India

The biological tissues exhibit their distinct optical characteristics. Their point-to-point compositional variation could either be determined by the optical parameters or reconstruction of the reflectance or tomographic images. The optical parameters, absorption and reduced scattering coefficients of goat tissues and human post-mastectomy breast specimen are determined by matching of their reflectance profile, as measured by multi-probe laser reflectometer, with that obtained by Monte Carlo simulation of optical scattering. The reflectance image of the mastectomy sample is reconstructed by measurement of reflectance at its various locations. The image thus obtained shows point-to-point variation in tissue composition. By multi-slice tomographic system the size and shape of the inclusions of different optical properties in a phantom made of goat fat are determined. By these procedures the structural variation in healthy and diseased tissues are determined and their relevance to early detection of tumour in tissues is discussed.

Laser radiation possesses nearly single frequency, high degree of temporal and spatial coherence, enormous intensity and highly plane-polarized characteristics. When a laser beam is incident on a thick tissue surface such as the human arm, due to mismatch in refractive index at the air–tissue interface, a part of this is backscattered, whereas the remaining part is absorbed in the tissues. Due to high scattering and low absorption, the penetration of light within the 'therapeutic window' (600–1300 nm) is more, which also leads to high backscattering from the surface after deep penetration. The spatial distribution of the backscattered and transmitted components contains information on the metabolic, physiologic or possibly structural status of tissues. Optical reflectance imaging of tissues, a novel concept in diagnostic medicine, is applied to determine the variation in internal structure of tissues. The images of tissues as obtained on the surface of the human hand and forearm, reconstructed by diffuse backscattered radiation, show the compositional variation in tissues below the skin, whereas the contribution due to skin pigmentation at all locations in healthy skin tissues remains constant. These images are in qualitative agreement with the perfusion images as obtained by laser Doppler flow meter.

The variability of scattering in biological tissues is attributed to the absorption ($\mu_a$) and reduced scattering ($\mu_s'$) coefficients. In contrast to their direct measurement by double integration spheres on tissue slices, these parameters for intact organs and phantoms are generally determined by indirect techniques, which involve the experimental measurement of spatial variation of reflectance on the tissue surface and matching of this curve by iterative procedure either based on optical diffusion theory or Monte Carlo procedure. Recently, by measuring the surface reflectance profiles and matching them with those obtained by Monte Carlo simulation, we have determined the variability of optical parameters at various regions of the human forearm and their images are reconstructed. Such a procedure could further be applied to characterize healthy and diseased tissues.

The transmitted component, after passing through thinner, complex tissues (finger) and thicker, soft tissues (breast, neonatal head, testis, etc.), also contains information on selective absorption by the internal tissue constituents. The cigar-shaped scattered volume within a tissue in a given span of time, contains all possible paths.

*For correspondence. (e-mail: msingh@iitm.ac.in)
of photons. Through the development of short laser pulses and fast detecting system, it has been possible to detect the partially coherent photons and suppress photons that have experienced excessive scattering. This effect has further been minimized by techniques such as time-resolved profile, modified tomographic reconstruction, and by reducing the effect of background heterogeneity.

The detection of tumor in human breast is one of the major objectives of the optical techniques. The various developments of optical tomography include its applications to tissue-equivalent phantoms. For direct applications to detect breast tumors, the techniques based on optical tomography (OT) and frequency domain optical mammography (FDOM) are developed. In OT, the breast is placed between the laser source and detectors for tomogram reconstruction, whereas in FDOM the breast is sandwiched between two optical plates and the transmitted laser light is scanned by a set of photo detectors. As the latter technique presents the three-dimensional breast structure in two dimensions, the actual shape and size of the inclusions could not be well determined. Recently, using three-dimensional OT of tissue phantoms we have shown that these details could be obtained from the sequence of tomograms of the phantoms. But inclusion of optical fibres either to deliver laser light from the laser source to optical head or to collect transmitted intensity data leads to loss of power or intensity, which is also affected by the optical characteristics of fibres.

The incidence of breast cancer is on the increase internationally. The procedures applied for early detection of tumour and routine examination of breast may save many precious lives. Hence the objectives of the present work are not only to provide the procedure to characterize the biological tissues in terms of their optical parameters, but also to present our techniques for reflectance imaging of tissue abnormalities below the tissue surface and tomographic imaging of compositional variation in breast phantoms and biological tissues.

Materials and methods

Multi-slice optical tomography system

The schematic of the laser multi-slice tomography system is shown in Figure 1. This primarily consists of four sub-systems: laser system, optoelectronic system, mechanical system, and data acquisition and image reconstruction system.

The laser system consists of a laser diode (Sanyo, DL7032), driven by the laser diode driver LCD500EC (Thorlabs Inc.), operating at 830 nm with maximum power 100 mW. The laser beam is collimated by diffraction limited aspheric collimation optics, encased in the collimation tube (LT220P-B). This laser beam is expanded into a thin sheath of thickness 1.0 mm by a rod lens system (BK7, 5D-8L, Natsume Optical Co.).

The opto-electronic system consists of an array of 16 photodiodes (BPW34, Siemens) which detect the laser radiation after passing through tissue or tissue-phantom. These photodiodes are of high-speed and low-noise characteristics with good sensitivity (0.9) at 830 nm. The laser source with lens system was placed on one arm of the mechanical scanner assembly, while the photo-detector array was mounted in front of it in a fan-beam configuration. Both the laser system and detector array were placed horizontally on the circumference of a circle of radius 80.0 mm. The angle subtended by the fan-beam configuration was 60° and the angle between two adjacent detectors was 4°. The linear distance between two successive photodiodes on the array ring was 10.42 mm. The current outputs from the photodiodes were converted into their corresponding voltages by a current-to-voltage converter.

The mechanical system consisted of three stepper motors, SM1, SM2 and SM3, to carry out upward and downward, rotational and pitch movements of scanning assembly, respectively. The movement of the gears G1 and G2 was controlled by SM3, which varied the separation between laser diode and photodiode array. The stepper motor motion was controlled by a three-axis motion controller card (PCL201, DMS) which generated the control sequence. These control signals were amplified by a stepper motor driver card (PCL782, DMS), designed to obtain multiple trans-axial projections at a given height to construct the tomogram. For tomographic reconstruction, the breast phantom was mounted in the phantom holder and placed at the centre of the laser–photodiode assembly after adjusting the pitch by SM3 and height by SM1.

The scanner head was moved by SM2 to take hundred projections (step angle 3.6°) at a given height of the phantom. The same procedure was repeated to obtain projections at various heights. The entire mechanical system was fabricated in steel and painted black to prevent any stray reflections of laser light.

The data, in the form of projections, were acquired by a 16-channel, 16-bit resolution, high-speed data acquisition card (PCL-816) and stored in the computer for further processing. Each projection consisted of 16 samples. The projection data of 100 projections at a given height were in the form of an array of 100 × 16. The stored data were preprocessed to remove any noise in the dataset and then interpolated to 64 datapoints from 16 measured outputs of photodiodes. The cross-section of the object was obtained by convolution–back projection algorithm.

Prior to tomogram reconstruction of an object, a blank run (without breast phantom) to capture the dark current in the photodiodes in dark environment was conducted. The dataset was subtracted from the input array of 100 × 16 phantom data matrix (background subtraction). The noise in the subtracted dataset was median-filtered (3 × 3 window) and interpolated to 100 × 64 data set. Each pro-
projection \( R_{\theta}(\gamma') \) was sampled with a sampling interval of angle \( \theta \). If \( n \) denotes integer values and \( \theta \), represents the angles at which projections are taken, then \( R_{\theta}(n\theta) \) denotes the fan-beam projection. For each projection, the modified projection \( R_{\theta}(n\theta) \) was obtained as

\[
R_{\theta}(n\theta) = R_{\theta}(n\theta) * D * \cos(n\theta),
\]

where \( D \) is the distance of the source from the origin \( O \). \( n = 0 \) corresponds to the ray passing through the centre of the object.

Filtered projection was obtained by convoluting each modified projection \( R_{\theta}(n\theta) \) with the impulse response of a filter function \( g(n\theta) \),

\[
Q_{\theta}(n\theta) = R_{\theta}(n\theta) * g(n\theta).
\]

Finally, the reconstructed image was obtained by

\[
f(x, y) = \Delta \beta \sum_{\gamma}^M \left( \frac{1}{L}(x, y, \beta, \gamma) \times Q_{\beta}(\gamma') \right),
\]

where \( \gamma' \) is angle of the fan-beam ray that passes through the point \((x, y)\) and \( \Delta \beta = 2\pi/M \); \( M \) is the total number of angles; \( L \) the distance from the source S to that point \((x, y)\).

When the computed value of \( \gamma' \) did not correspond to one of the \((n\theta)\) for which \( Q_{\theta} \) is known, an interpolation (linear) was performed. The reconstructed images were displayed with 256 grey levels. Further details of tomogram reconstruction are given elsewhere. The raw image is generally associated with noise and poor contrast. Therefore, details of the image were not clearly delineated. To overcome this, the images obtained by the present technique were processed by histogram equalization, smoothing, image segmentation, edge detection and contour extraction. Further details of the implementation of these procedures are given elsewhere.

**Multi-probe laser reflectometer**

The schematic of the multi-probe laser reflectometer for measurement of spatial variation of reflectance on the surface of tissues is shown in Figure 2. The laser light beam from a compact CW semiconductor laser of power 3 mW (LDM 135, Imatronic), wavelength 670 nm and
beam diameter 0.1 cm, was guided by the input fibre of core diameter 0.1 cm and length 100 cm onto the tissue surface. The backscattered light from the tissue surface was collected by three output fibres of the same length and core diameter (E 89328-A WVM VW-1, HP). These fibres were arranged in a straight line at 0.2, 0.4 and 0.6 cm (centre-to-centre) from the input fibre and were placed in a cylindrical, stainless-steel tube containing PVC block of diameter 2.0 cm and length 5.0 cm. To avoid contact of the fibres with tissues and to maintain a separation of 0.1 cm from the tissue surface, this probe was further fitted with an aluminum ring at its front end. This addition at the front end leads to the acceptance angle of 22°, resulting in the collection of diffuse backscattered components by the fibres, with a minor contribution from the nearby region due to overlapping of radiations at various locations. The probe was always held perpendicular to the tissue surface as this ensured the maximum penetration and backscattered intensity compared to that of other incidence angles. Intensity losses were minimized by carefully polishing the end faces of the fibres. Further details of this procedure are given elsewhere 12,32.

Preparation of biological tissue samples and their phantom

Freshly excised fat, liver and spleen tissues of goat were procured from a commercial butcher and cleaned thoroughly to remove dirt, if any. Thereafter, to remove traces of blood, these were soaked in physiological saline for 30 min. Prior to measuring their reflectance, the saline and moisture from their surfaces were mopped with blotting paper. These tissues were used within 1 h after collection.

The post-mastectomy breast specimen was collected from one of the local hospitals. The histopathology report of this breast specimen revealed that it was affected by intraductal carcinoma. The entire surface of the specimen was thoroughly mopped with blotting paper soaked in physiological saline, before collecting the reflectance data from its base.

For phantom preparation of biological tissues, a hollow, conical glass model of 80 mm height and 80 mm base diameter was filled with goat fat. From the base of this fat-filled model, the goat liver sample of diameter 8 mm and spleen of diameter 15 mm up to a height of 6.5 cm, located at different positions were introduced (Figure 3).

Measurement of surface reflectance profiles of biological tissues

The surface reflectance profiles of tissues were measured by holding the probe perpendicular to the tissue surface. An absorbing boundary condition (no light reflected back from the tissue surface), by covering the surface of each organ with an exposed black X-ray film except the area covered by the fibre probe, was obtained10. Reflectance measurements were the same after removal of part of the lower and side portions of the tissues, which confirmed the semi-infinite extensions in depth and width of the medium, as required for modelling. Measurements were performed at room temperature and were repeated at four nearby locations of each tissue. The output data obtained by three photodiodes were normalized with respect to incident intensity, measured by holding the source fibre directly on the photo-detector, which was also measured by laser power meter (Edmund Scientific, USA). The backscattered intensity measured by each photodiode from the surface of healthy tissue of the mastectomy sample was converted to corresponding power by a calibration chart. Finally these values of backscattered intensity were normalized with respect to incident intensity as measured by the power meter. For reconstruction of surface backscattered profile, this normalized backscattered intensity (NBI) was plotted against radial distance of the detector from the beam entry point. Prior to data collection from

![Figure 2](image_url)  
**Figure 2.** Schematic of multi-probe laser reflectometer.

![Figure 3](image_url)  
**Figure 3.** Breast phantom made of fat with inclusions of spleen (a) and liver (b).
each tissue, the reflectometer reading was adjusted to zero by placing the probe on the surface of black rubber. The reflectance data from tissues were collected in dark environment at around 20°C. Further details of this measurement technique are given elsewhere\(^2\).

**Monte Carlo simulation of the NBI profile**

For determination of optical parameters at each location of the forearm, the measured surface backscattered profile by Monte Carlo procedure was simulated\(^3\). This simulation is a stochastic procedure based on the following assumptions: (i) the photons incident on the tissue are neutral particles, (ii) the tissue is a homogeneous medium, (iii) beam of photons is monochromatic.

The incident photon-beam profile with each photon of unit intensity \((WT_1)\) is considered. The simulated propagation path of a photon, after incident normally on the tissue surface at \((0, 0, 0)\) is shown in Figure 4. Thereafter, it moves along the direction of incidence without deflection and reaches the new position 2 from position 1 with absorbed dose \(D_1 = \mu_s/\mu_t\).

Due to tissue–photon interaction, the attenuated intensity is given by

\[
\Delta Q = (WT_1)(\mu_s/\mu_t),
\]

where \(\mu_s\) is the absorption coefficient \((\text{cm}^{-1})\), \(\mu_t\) is the total attenuation coefficient \((\text{cm}^{-1})\)

\[
= (\mu'_s + \mu_s),
\]

\(\mu'_s\) is the reduced scattering coefficient.

The corresponding path length \(l\) in the tissue medium is given by

\[
l = -(\ln R)/\mu_s,
\]

where \(R\) is a random number between 0 and 1.

Thereafter, the new photon intensity \(WT_2\) is calculated by

\[
WT_2 = WT_1 (\mu'_s/\mu_s).
\]

After position 2, the photon is scattered and deflected. The deflection angle \(\theta\) is calculated by

\[
m = \frac{1}{2g} \left[ 1 + g^2 - \left( \frac{(1 - g^2)}{(1 + g^2 + 2g\xi)} \right)^2 \right] \text{ for } g \neq 0.
\]

where \(g = \cos\theta\) and \(\xi\) is a random number between 0 and 1.

For \(g = 0\) (isotropic conditions), the instantaneous value of \(m\) \((=\cos\theta)\) is

\[
m = (2\xi - 1).
\]

The selection of azimuthal angle \(\psi\) is given by

\[
\psi = 2\pi\gamma,
\]

where \(\gamma\) is a random number between 0 and 1.

The propagation of photon in the tissue medium continues till photon intensity reduces either to 5% of the incident intensity or the same photon is emitted from the tissue surface by backscattering. Thereafter, the next photon enters the tissue and repeats the same process described earlier. By this process, tracking of all the photons is carried out. The emitted fractions of photons corresponding to radial positions similar to those of the measurement probe are represented as NBI, given as percentage fraction of backscattered intensity of photons over the surface with respect to the incident \((10^5)\) photons. The placement of input and output fibres in the measurement probe at a distance 0.1 cm from the tissue surface was further accounted for in the simulation by keeping the photon collection regions of size same as that of diode, away from the surface by the same distance. The simulation program was written in C language.

**Determination of optical parameters**

The optical parameters of healthy section of the mastectomy specimen were determined by matching of the measured NBI surface profile with that as simulated by Monte Carlo procedure. For isotropic scattering, the absorption and reduced scattering coefficients were deter-
determined. This condition implies that the reduced scattering coefficient ($\mu_r$) was the same as the scattering coefficient ($\mu_s$). The simulated profiles were calculated for various combinations of $\mu_r$ and $\mu_s$ and the best-matched profile with the measured surface profile (with chi-value = 0.99) was selected (Figure 5). The optical parameters of the tissue were the same as those used for Monte Carlo simulation. Further details of this procedure are given elsewhere\textsuperscript{13}.

**Reflectance imaging of human mastectomy specimen**

To acquire data from a mastectomy specimen along with their spatial location, a $16 \times 16$ grid overlapping the outline of the breast (base diameter 80.0 mm) was developed and displayed on the monitor (Figure 6). While the cursor was moved across the breast outline, the scanning probe was placed gently on the corresponding location and the data were collected. By this procedure, the reflectance data over the entire breast surface along with spatial coordinates were collected. The data at each point represent an average of 100 samples. Prior to data collection from each tissue, the reflectometer reading was adjusted to zero by placing the probe on the surface of black rubber.

Data acquired by this procedure were converted to NBI. Since scanning was done manually, all measurement points do not match with regular grid points. Regularization of the data was done by interpolation procedure using Pythagorean equation and a regular grid of $16 \times 16$ equally spaced points was constructed. If the NBI value directly coincided with the corresponding regular grid points, the value was directly assigned to that point; otherwise interpolation was carried out. To remove any specular noise associated with the data, the interpolated image matrix was filtered by a $3 \times 3$ median filter. Finally, the filtered matrix data, depending on their values, were displayed as a pseudo colour-coded image. Further details of reflectance image reconstruction are given elsewhere\textsuperscript{8}.

**Results**

The optical parameters of different tissues, as calculated by the above procedure, are shown in Table 1. The reduced scattering coefficient is the maximum for goat fat and minimum for normal area of the cancerous breast tissues. The reduced scattering coefficient of cancerous area is less than that of liver. The absorption coefficient is maximum for goat spleen and minimum for normal area of cancerous breast. The absorption coefficient of cancerous area is more than that of goat fat. These variations are primarily attributed to the compositional changes in tissues.

Figure 7 shows the reflectance image obtained at the surface of the post-mastectomy breast sample along with its NBI variation. Change in tissue composition at various locations in the cancerous and other regions is clearly

**Figure 6.** Outline of base of the breast superposed on $16 \times 16$ grid for reflectance imaging of mastectomy specimen.

**Table 1.** Scattering and absorption parameters of normal and cancerous breast tissues at laser wavelength of 830 nm

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Reduced scattering coefficient (cm$^{-1}$)</th>
<th>Absorption coefficient (cm$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Goat fat</td>
<td>32.0</td>
<td>0.5</td>
</tr>
<tr>
<td>Goat liver</td>
<td>12.6</td>
<td>1.3</td>
</tr>
<tr>
<td>Goat spleen</td>
<td>14.0</td>
<td>4.0</td>
</tr>
<tr>
<td>Normal area of cancerous</td>
<td>8.5</td>
<td>0.34</td>
</tr>
<tr>
<td>breast cut surface</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cancerous area cut surface</td>
<td>10</td>
<td>0.7</td>
</tr>
</tbody>
</table>
observed. Formation of the core region due to its lower reflectance compared to that of the surrounding tumour area is identified. Other regions, which are marginally affected, are also identified by variation in reflectance.

Tomographic slices of the biological tissue phantom are shown in Figure 8. Due to high absorption coefficients of liver and spleen compared to that of fat, these inclusions could be identified in slices up to a diameter of 5.0 cm. In the slice of diameter 7.0 cm, due to increased scattering, the inclusions are not well identified. Similarly, their scans show better clarity at lesser thickness, whereas the broadening of peaks due to enhanced scattering is observed at larger thickness. The change in intensity produced by inclusion of spleen is more than that of liver tissues.

The inner structural details of goat kidney, as obtained by the present technique at sub-, trans- and supra-pelvic levels, are shown in Figure 9. In each of these scans structural variation is associated with poor resolution, attributed to increased scattering due to complex tissue structure. For localization of inner structure, the contouring of these images is carried out. By this process, details of the cortex and medulla regions are observed. The internal structure at the trans-pelvic level is more complex compared to that at other levels.

Figure 7. Reconstructed reflectance image as measured by first fibre at the base of the mastectomy breast sample, showing variation in tissue composition. The central region is normal, whereas the tissues shown in darker shades are malignant tissues. Reflectance of malignant tissues is lesser than that of healthy tissues. Variation in composition of malignancy is further identified.

Figure 8. Images of fat phantom with inclusions of spleen and liver tissues obtained at various slice diameters, showing the prominence of the inclusions.
Discussion

The present tomographic system consists of laser source and photodiodes separated by a fixed distance and the conical phantom is placed at the centre. This separation decreases with change in position of the scanning system as this is moved towards the apex of the cone. The incidence of light on the phantom is through the sheath of light and the trans-illuminated component is received after a fixed separation. Due to this increase in source–detector separation, the contribution due to scattering at the detector is decreased.

In addition to this the arrangement of photodiodes with window of size 0.25 cm × 0.25 cm, makes it possible not only to receive the straight propagating photons but also the less scattered ones. The transmitted data by each photodiode are collected for a duration of 30 μs and finally these are averaged over 100 observations. This procedure further averages out any variation in the output signals of photodiodes.

The transillumination tomographic reconstruction procedure has greater detection efficiency compared to that of transillumination. This procedure is based on the fan-beam configuration, which is efficient and less time-consuming compared to that of parallel-beam configuration.

Further processing of the tomographic images by other procedures helps in viewing the various details within the tomograms.

The steady-state, spatially resolved, diffuse reflectance profile is characteristic of a tissue structure and its metabolic state. By analysing this reflectance profile, one can get the structural and functional details of the tissue across the penetration depth of the photons. The influence of any optical inhomogeneity (structural or rapid metabolic changes) in the tissue layers could easily be detected by this technique.

The present study shows that backscattering from the biological tissues depends on their composition and blood volume. The distinct variation in composition that depends on their functional aspects, is a contributory factor to their reflectance, which is significantly different from that of skin colour. The arrangement of tissues within the organ and the orientation of fibres in the muscle are the major contributory factors in the variability of the surface profile of the backscattered radiation. Placement of the optical fibres away from the beam entry point minimizes contributions from the top layers of the tissues.

Determination of the optical parameters is an integral part of tissue characterization. Optical parameters, which depend on the variation in tissue composition, are deter-
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determined by the best-fit of experimental and theoretical curves with chi-value 0.99. These parameters further depend on the measurement techniques and conditions, sample preparations and accuracy used during simulation and curve-fitting procedure. Theoretically, Monte Carlo simulation did not always provide a good description of the reflectance close to the source (< 0.2 cm)\(^1\). Therefore, for this curve-fitting procedure the first measurement is taken by placing the fibre 0.2 cm away from the input fibre.

The absorption and reduced scattering coefficients as measured by the present procedure are in agreement with those reported by other procedures\(^2\). Fat scattering is more compared to other tissues. For spleen, the high absorption and low scattering coefficients are attributed to its structure and blood contents\(^3\).

The reflectance imaging system is based on multi-probe detection of the backscattered component. By these measurements the spatial profile of the backscattered radiation is constructed. As these radiations emerge from various depths, their variability provides information on the tissue compositional changes in the medium\(^4\). Clinically, if the tumour is at a fairly advanced stage, the reflectance image reconstructed by this procedure clearly shows the details of the tumour. The resolution of the images obtained for tissues below the skin can further be improved by changing the scale, to provide details of pathological changes in the tissues\(^5\).

For imaging of fresh tissues, the kidney, which exhibits large tissue compositional variation from its medulla to cortex, is used. Tomographic slices of the kidney are obtained at various levels. For better visualization the contours are extracted, showing the variability in tissue structure. Although structural details of the kidney are not well visualized, this work still highlights the possibility of its application to monitor changes in the thick, soft tissues such as human breast.

In conclusion, the principal motive behind the development of optical tomography, which is cost-effective compared to time-resolved and frequency-domain techniques, is to provide a safe and effective method of detecting and specifying diseases in the breast and other soft-tissue structures. Applications of this system to tissue-phantoms and biological tissues show that the present technique could be employed to detect structural changes.

This technique is sensitive to detect changes in absorption coefficient and the minimum size of the inclusion detected in a 50 mm diameter slice is 3.0 mm. Due to its simplicity and versatility, this may prove to be an ideal system for structural analysis of soft tissues. Optical parameters quantify the tissue changes, whereas reflectance imaging could further provide data on variability of tissue composition below the skin over an entire organ.


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