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ORIGINAL ARTICLE

In vivo study of age-related changes in the optical properties of the skin

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Abstract The optical properties of the skin (absorption coefficient, scattering coefficient, refractive index) may serve to characterize the skin and are important for correct light dosimetry in many optical diagnostic procedures and laser treatments especially photodynamic therapy and laser therapy. We determined in vivo the optical properties of tissues near the wrist, elbow and knee in subjects of different ages using diffuse reflectance spectroscopy, having in view the establishment of laser system types for the laser treatment of posttraumatic lesions in subjects of different ages. Diffuse reflection of light from biological tissue is due to the variation in refractive index of tissular and cellular components and the surrounding medium and depends on the wavelength of the incident optical radiation. The diffuse reflectance spectrum of the tissues tested showed two maxima localized at $\lambda_{M1} \approx 610$ nm and $\lambda_{M2} \approx 675$ nm. Laser systems which emit radiation at these wavelengths are not efficient for the treatment of joints, regardless of the subject's age. The deep tissues have a strong absorption in the range 630–700 nm, which indicates that for treating posttraumatic lesions we can use laser systems such as the He–Ne laser, the GaAlAs laser, and the InGaAlAs laser. Using Kramers-Kronig analysis of the diffuse reflectance spectra, the optical parameters $n(\omega)$ and $k(\omega)$ were determined. The age-dependent changes in these optical parameters of tissue must be taken into consideration and the use of laser treatments or optical diagnosis methods must be based on a knowledge of these properties and of the optical radiation parameters.

Keywords Skin · Optical properties · Age · Diffuse reflectance · Kramers-Kronig analysis · Wrist · Elbow · Knee

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Introduction

The optical properties of biological media can give useful and critical information about chromophores, and the physiological functions and structure of different biological media. Any change in the structure or chemical composition of biological tissues caused by aging or some diseases leads to a change in their optical properties and therefore in the optical parameters describing them. Determination of the optical properties of biological tissues is of particular interest because of the potential for using light as a noninvasive diagnostic and therapeutic tool in various biomedical applications [1].

The optical properties of biological tissues are described by several optical parameters, such as the absorption coefficient (μ_a), the scattering coefficient (μ_s), the anisotropy factor (g), the total attenuation coefficient (μ_t) and the mean free path (mfp). These optical parameters cannot be measured directly, but they can be determined from absorbance, transmittance or diffuse reflectance measurements of optical radiation, based on models of the propagation of optical radiation through biological media [2–10]. The diffuse reflectance of light by biological tissues is determined by the absorption and scattering properties of the tissues, which are related to their chemical structure and composition. The main absorbers of the skin are blood [11], keratin [12] and melanosomes [13]. The scattering particles in the skin consist of lipids and proteins (keratin, melanin, collagen, elastic fibers) in the fluids and between skin cells [14]. The lipid particles are found in the cell membrane, in the stratum corneum and in the intracellular particles. Stronger absorption leads to weaker diffuse reflectance and greater scattering leads to wider diffuse reflectance.

In this study we determined based on in vivo measurements of diffuse reflectance the absorption coefficient and refractive index of the tissues near the wrist, elbow and knee in subjects of different ages, aiming to establish the best laser system for laser therapy of posttraumatic lesions. The diffuse reflectance spectra acquired in the VIS domain were analyzed using Kramers-Kronig analysis in order to determine the absorption coefficient and refractive index of the tissues, which play important roles in the choice of the optimal laser irradiation parameters for laser therapy of posttraumatic lesions. The studies were conducted in different age groups of subjects.

Materials and methods

Human subjects

The study included clinically healthy human subjects aged between 16 and 72 years. Several inclusion and exclusion criteria were used for the selection of the subjects. Each subject provided written informed consent to participate to the study. The subjects, were divided into three experimental groups: group A (7 subjects, 16–18 years), group B (12 subjects, 18–50 years) and group C (10 subjects, 50–72 years).

Determination of optical parameters of the skin

For the study of the optical properties of the skin, diffuse reflectance spectra were acquired at the wrist, elbow and knee. We chose identical points near the joints, i.e. the head of the ulna for the wrist, the olecranon for the elbow, and the tip of the patella for the knee. At the chosen points the skin is close to the bone (except the bursae over the olecranon and patella). Optical reflectance spectra in the wavelength range 500–1,100 nm were acquired using an AvaSpec spectrophotometer (Avantes). This portable spectrophotometer is equipped with a tungsten halogen lamp, CCD detector array (2,048 pixels) and a reflection probe type FCR-7IR200-2 with one illuminating fiber in the center surrounded by six fibers, which collect the light reflected from the sample. The optical properties of the skin (refractive index and extinction coefficient) were determined indirectly using the Kramers-Kronig analysis of the diffuse reflectance spectra.

Kramers-Kronig relations mathematically decompose the complex reflectance spectra $R(\omega)$ into two different spectra [15, 16]:

- Refractive index spectrum $n(\omega)$, i.e. the real part of the complex refractive index:

$$n(\omega) = \frac{1 - R(\omega)}{1 + R(\omega) - 2\sqrt{R(\omega)}\cos\varphi(\omega)} \tag{1}$$

- Extinction coefficient spectrum $k(\omega)$, i.e. the imaginary part of the complex refractive index:

$$k(\omega) = \frac{-2\sqrt{R(\omega)}\cos\varphi(\omega)}{1 + R(\omega) - 2\sqrt{R(\omega)}\cos\varphi(\omega)} \tag{2}$$

where:

– $R(\omega)$ represents the reflectance spectra on the complete spectral domain of (0–∞).
– $\varphi(\omega)$ is the phase function defined thus:

$$\varphi(\omega) = \frac{1}{2\pi}\int_0^{\infty} \ln\left|\frac{\omega' - \omega}{\omega' + \omega}\right| \frac{d\ln R(\omega')}{d\omega'} d\omega' \tag{3}$$

The next steps in determining the refractive index $n(\omega)$ and extinction coefficient $k(\omega)$ of the skin using this method were as follows:

- Acquisition of the reflectance spectra of the skin in the spectral domain $\lambda \in$ 400–1,000 nm (equivalent to $\omega \in$ 10,000–25,000 cm^{-1}).
- Extrapolation of the measured data in the spectral domain of $\omega_m = 0$ cm^{-1} and $\omega_M \rightarrow \infty$ cm^{-1}, aiming to obtain the reflectance spectra on the complete spectral domain of 0-∞ cm^{-1} using as extrapolation function the power function, which describes the optical behavior in these ranges.
- Determination of the optical constants $n(\omega)$ and $k(\omega)$ from $R(\omega)$.

Results and discussion

Representative diffuse reflectance spectra from the wrist, elbow and knee in the different age groups of subjects are shown in Fig. 1.

All of the tissues tested had two main reflectance maxima (at $\lambda_{1\text{-max}} \approx 610$ nm, and $\lambda_{2\text{-max}} \approx 675$ nm) and a reflectance minimum at $\lambda_{min} \approx 630$ nm. The maximal values of diffuse reflectance, depending on age, were in the range 33,059–64,601% for the elbow, 13,830–76,988% for the knee, and 46,128–61,389% for the wrist. The maximal

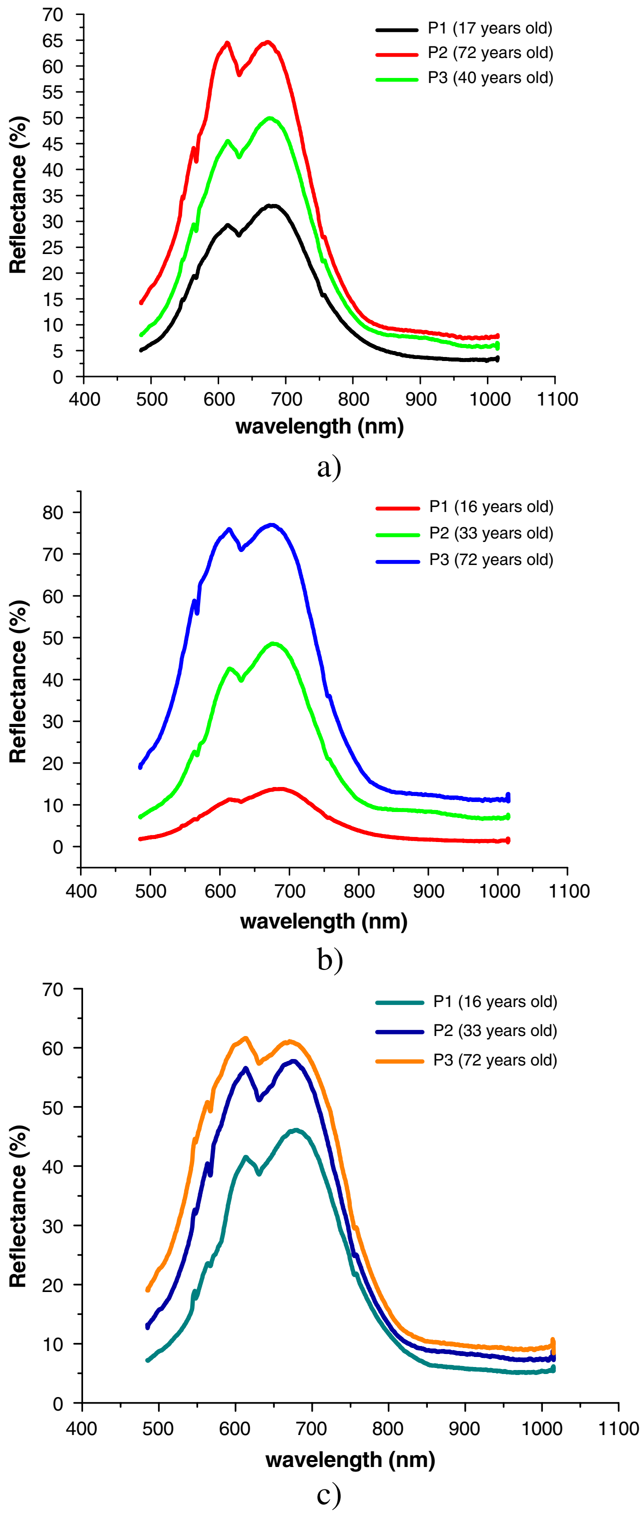

Fig. 1 Diffuse reflectance spectra for (**a**) the elbow, (**b**) the knee and (**c**) the wrist

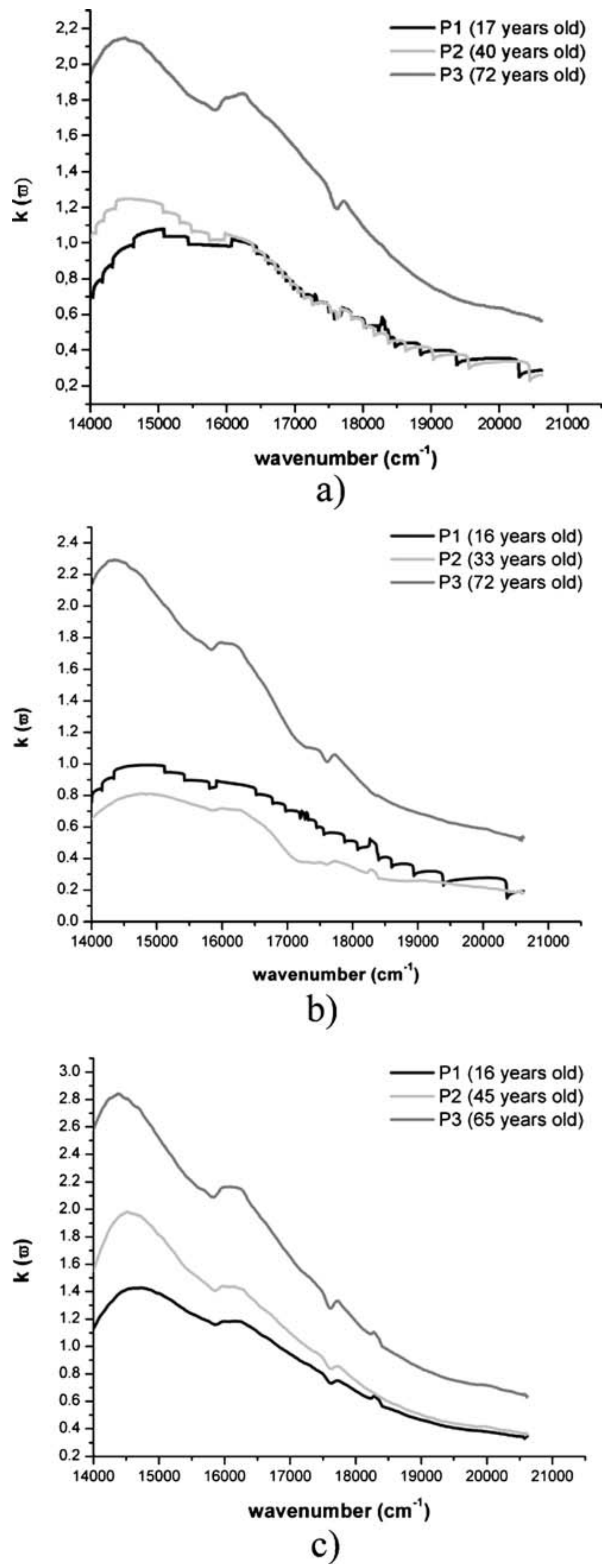

Fig. 2 Calculated absorption spectra for (**a**) the elbow, (**b**) the knee, and (**c**) the wrist

values increased with age. It is important to emphasize that the general pattern of the spectra remained the same irrespective of age, probably because the chromophores are the same; only their concentrations and the tissue

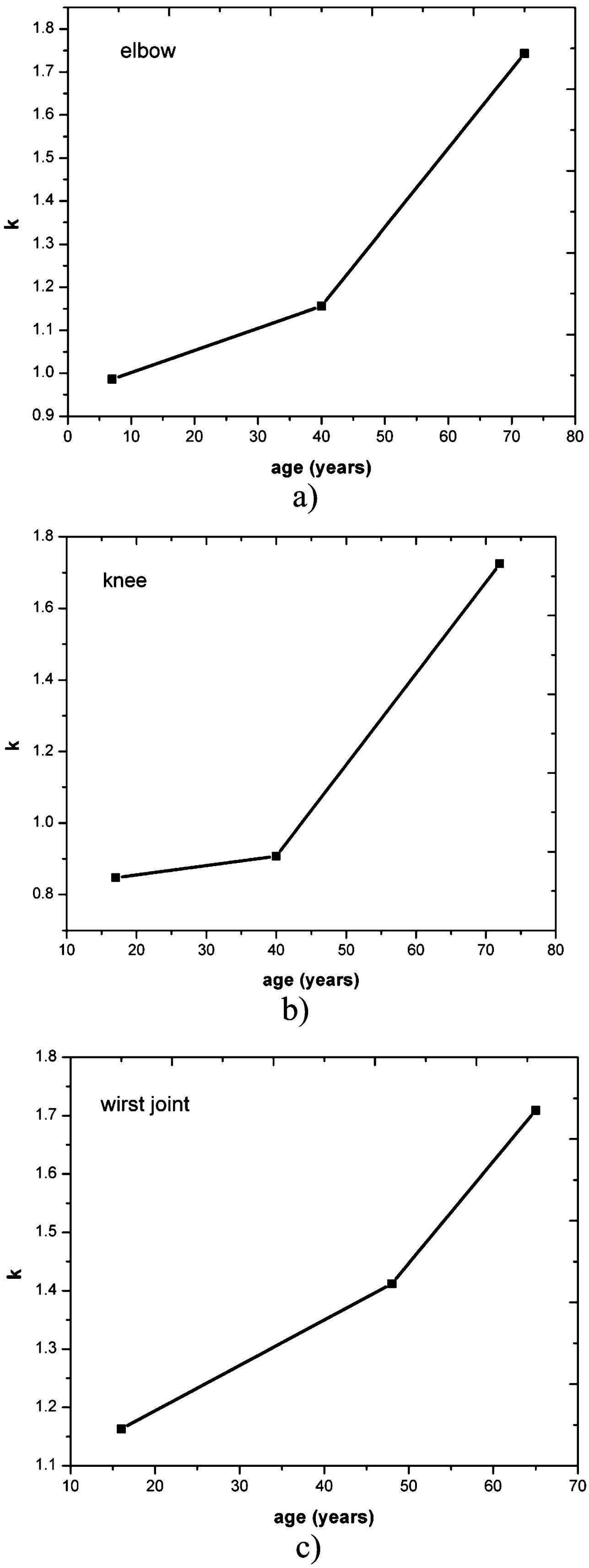

Fig. 3 Variations in calculated extinction coefficient with age (λ = 632 nm) for (**a**) the elbow, (**b**) the knee, and (**c**) the wrist

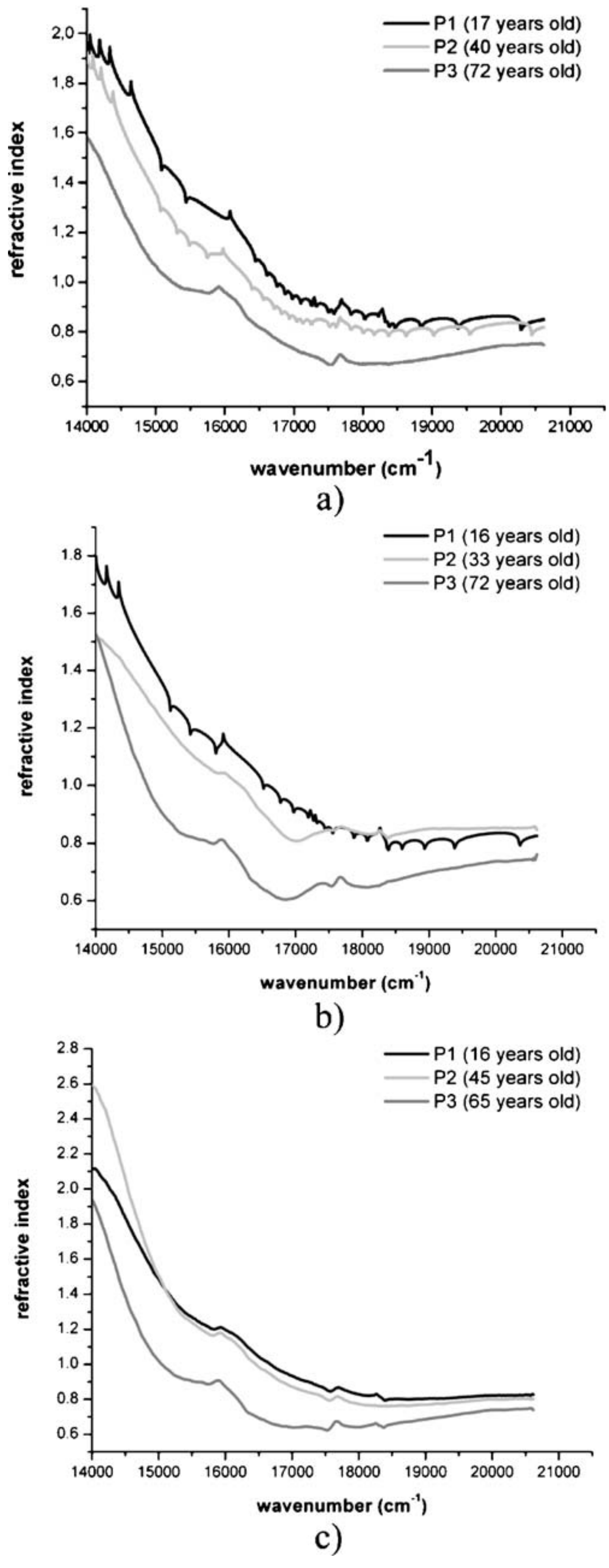

Fig. 4 Spectral distribution of refractive index n(ω) for subjects of different ages for (**a**) the elbow, (**b**) the knee, and (**c**) the wrist

architecture are prone to age-related changes. By placing the probe over bony prominences near the joints (head of the ulna, olecranon and patella), the diffuse reflection of light takes place at the structural limits of the various tissues (i.e. the basal membrane, the surface of the bone). Thinning of the skin with age, together with the flattening of the dermoepidermal junction could explain the greater values of maximal reflectance obtained in older subjects.

The Kramers-Kronig analysis of the reflectance data led to the calculation of extinction coefficient spectra (Fig. 2) and of the variation in refractive indices with wavenumber (ω) (Fig. 4) for the wrist, elbow and knee in subjects of different ages.

Figure 2 shows that the biological tissues tested had a greater absorption in the spectral domain 600–700 nm and a lower absorption in the spectral domain 500–600 nm. Taking this into account, laser radiation in the wavelength range 600–700 nm (radiation emitted by laser systems such as the He–Ne laser, the InGaAlP laser, and the GaAlAs laser) is heavily absorbed by the tissues and has a low tissue penetration depth and can only be used in the treatment of superficial posttraumatic lesions (wounds, contusions, hematomas, etc). Radiation with λ in the range 500–600 nm can penetrate more deeply into tissue. Laser systems with emission in this wavelength range can be used for the treatment of deep lesions, such as sprains, strains or ligament rupture.

In older subjects light absorption was dominant, while in younger subjects light scattering was dominant (Fig. 3).

Absorption of light by different tissues is due to known chromophores (hemoglobin, melanin) and the increased absorption observed in older subject could be explained by the hyperkeratosis over bony prominences (such as the areas we tested) associated with older age. Therefore, for laser therapy of superficial posttraumatic lesions, the irradiation parameters (laser power and exposure time) should be set at lower values for older individuals than for younger individuals.

The variation in refractive index with wavenumber (ω) for the different tissues studied was dependent on the age of the subject (Fig. 4). For all types of tissues studied, the refractive index increased with age and the propagation speed of optical radiation through the tested tissues decreased due to multiple scatterings.

For wavelengths in the visible domain corresponding to laser systems often used for the treatment of less deep lesions of the joints, the refractive index and extinction coefficient values are presented in Table 1.

Conclusion

Knowledge of the optical properties of biological media and of the optical parameters defining these media is essential for optimizing the use of optical diagnostic and therapeutic methods. Variations in the optical properties of tissue with age must be taken into consideration in certain situations and the use of any optical therapeutic or diagnostic method must be based on knowledge of these properties and optical radiation parameters. Optical parameters can be calculated using theoretical methods (models for transport of radiation through biological media) or can be determined using experimental methods (mainly spectroscopic methods).

In this study, diffuse reflectance spectroscopy was used to determine in vivo the optical properties of periarticular tissues having in view the establishment of the laser systems types for therapy of posttraumatic lesions in subjects of different ages. Using Kramers-Kronig analysis of the diffuse reflectance spectra of tissues near the wrist, elbow and knee, the optical parameters $n(\omega)$ and $k(\omega)$ were determined. Based on these parameters the laser system types in the VIS domain should be used only for superficial lesions due to their lower penetration in the tissues tested.

The results showed that the Kramers–Kronig analysis of reflectance spectra could be used as a method to calculate the extinction coefficient and refractive index of biological media from measurement of the diffuse reflectance, and this method could be used as a diagnostic method based in the evaluation of changes in optical parameters induced by different factors, including age. The results also showed constant alterations of the optical parameters of the tissues studied with age, even in different but similar (anatomically) body regions. Although these alterations were expected, the results confirm the method as being adequate for in vivo measurements of optical parameters of tissues.

Although there was no consistent correlation between the optical measurements and age, the findings should be taken into account when setting the parameters of optical diagnostic or treatment devices according to patient age.

Table 1 Refractive index and extinction coefficient values of periarticular tissues at different wavelengths

λ (nm)	Wrist (subject age 17years)		Knee (subject age 33years)		Elbow (subject age 65years)	
	n	k	n	k	n	k
632	1.2011	1.1607	1.0435	0.7081	0.9611	1.7437
635	1.2133	1.1847	1.0533	0.7105	0.9556	1.7620
670	1.5254	1.3986	1,2525	0.8091	1.0884	2.0432

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