

Exploring the Relationship between Hypodermis Thickness and Diffuse Reflectance Spectra in Different Age and Gender Groups

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Abstract. This study explores the use of spatially-resolved diffuse reflectance spectroscopy for non-invasive method for measuring subcutaneous fat layer thickness. We investigated the relationship between spectroscopic measurements and hypodermis thickness, particularly focusing on lipid absorption amplitudes at varying source-detector distances. Our results reveal that lipid absorption amplitude is directly proportional to hypodermis thickness for layers between 1 and 4 mm at a source-detector fiber distance of 14 mm. The lipid absorption amplitude plateaued beyond a thickness of 4 mm, limiting sensitivity for the thicker hypodermis layer. An exponential function was revealed to describe lipid absorption amplitude dependence on the fat thickness across different source-detector distances. Physiological factors, namely sex, was found to significantly influence absorption amplitudes, while age-related differences were not identified. A predictive model was developed, yielding moderate accuracy (RMSE = 1.56 mm) in estimating hypodermis thickness, which is limited by the reference method. The study concludes that spatially-resolved diffuse reflectance spectroscopy is a promising method for assessing subcutaneous fat thickness, benefiting from its non-invasive nature and immediate results. However, accuracy is influenced by physiological characteristics, including sex and age, thus requiring personalized calibration. Future research should aim to refine predictive models and assess the technique's applicability across various body sites and a wider range of hypodermis thicknesses. © 2024 Journal of Biomedical Photonics & Engineering.

Keywords: spatially-resolved diffuse reflectance spectroscopy; hypodermis thickness; lipid absorption amplitude; physiological parameters; non-invasive assessment technique.

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1 Introduction

Measuring the thickness of the subcutaneous adipose layer is a critical aspect of medical practice and research

related to human health [1]. The subcutaneous fat layer serves as a key element in regulating body temperature, protecting organs from injury, and acting as a significant source of energy [1, 2]. The thickness of subcutaneous fat

can vary considerably among individuals and is influenced by a multitude of factors, including genetics, lifestyle, diet, and level of physical activity [3]. This variability underscores the importance of accurate assessment techniques for the subcutaneous adipose layer, which have implications for diagnosing and managing various health conditions.

Various approaches have been developed and are currently used in practice to assess the thickness of subcutaneous fat. Among the most common methods are those based on ultrasonography (US) [4–7], magnetic resonance imaging (MRI) [8–11], various types of biopsy [12], and a range of optical methods [13–15]. High-frequency ultrasound [4] is widely used to determine the thickness of the skin layers, underlying subcutaneous fat and muscles [5–7]. However, accurate and reproducible results require a high level of skill from the person interpreting US images. The MRI has also gained widespread acceptance as a comprehensive method for examining the human body. The resolution of modern equipment is about 0.1 mm [8], which allows MRI to be successfully applied in studying the structure of the skin [9–11]. However, the main disadvantages of this method are its relatively high cost and equipment size, which hinders its widespread use in clinical settings. Biopsy is currently considered the “gold standard” for analyzing skin structure in medicine. Its main drawback lies in the invasiveness of the method and the need for time-consuming laboratory investigations. Moreover, there are indications that biopsy-based measurements may have significant discrepancies compared to *in vivo* methods due to the loss of elasticity of the hypodermis after excision and prolonged storage of the skin sample, as well as potential distortion of the sample during procedures conducted for laboratory studies.

Diffuse reflectance spectroscopy (DRS) is often used to analyze the composition [16, 17] and thickness of biological tissues [18, 19] due to its relative simplicity, non-invasiveness and high measurement speed. In particular, the use of DRS may offer a promising solution to the problem of determining the thickness of the subcutaneous layer [13–15]. The main absorber in the dermis in the near-infrared (NIR) range is water, while lipids are the absorbers in the subcutaneous fat. Consequently, due to the different absorption spectra shapes of these layers (Fig. 1 (C)), depth scanning of the reflected signal would allow for the determination of hypodermis thickness (Fig. 1 (E)). Choi et al. (2008) [13] developed a non-invasive system for measuring the thickness of subcutaneous fat layer using DRS in the spectral range from 1000 to 1700 nm. Their results showed a high coefficient of determination ($R^2 = 0.9954$) between the measured and actual thickness of the subcutaneous fat layer in *ex vivo* tissue samples. In a study [14], the optical response around 930 nm, associated with lipid absorption, was investigated and compared with ultrasound images, MRI, and caliper measurements for assessing subcutaneous fat thickness.

The results showed that optical methods can be an effective way to assess the thickness of subcutaneous fat with an average determination error of 1.2 and 0.8 mm, when using caliper and ultrasonic examination as the reference method respectively. The use of ultrasound as a reference method for measuring subcutaneous fat thickness has shown a high coefficient of determination when compared with DRS data, achieving an R^2 value of 0.9. This indicates a strong correlation and potential for accurate assessment of body tissue characteristics, such as the thickness of the subcutaneous fat layer. In study [15], the authors proposed the application of diffuse optical spectroscopy with temporal resolution within the same spectral range to evaluate body tissue composition with similar accuracy. However, a limitation of these studies is the small sample size of volunteers, which restricts the ability to discern correlations between the optical response and physiological attributes, including age and gender of the participants. This underscores the need for further research with larger and more diverse populations to better understand the implications of optical measurements in the context of varying human physiological characteristics.

In this study, we assessed the thickness of the subcutaneous fat layer using spatially-resolved DRS on a group of 247 volunteers. The caliper measurement method was used for comparison in the creation of a method for determining the thickness of the hypodermis based on DRS data. The research included an analysis of the relationship between lipid absorption amplitude and the thickness of the subcutaneous fat layer, as determined by both DRS and caliper measurements. The impact of varying distances between the light source and the detector on this correlation was also explored. Additionally, machine learning methods were utilized to evaluate the impact of physiological parameters, such as gender and age, on the optical response of the skin. Statistically significant differences in relationships for subjects of different genders were identified, demonstrating the influence of these parameters on the accuracy of the predictive model. Furthermore, data from DRS were used to solve the inverse problem of estimating the thickness of the subcutaneous fat layer, achieving a determination accuracy within an error margin of 1.5 mm.

2 Materials and Methods

2.1 Cohort Description

A total of 247 subjects (97 males and 150 females) participated in a simultaneous caliper skin fold readings and near-infrared DRS study (Fig. 1 (C), (E), (F)). The age of the participant was 25 ± 13 years spanning from 4 to 71 (Fig. 1(D)). The skin of the inner side of the forearm at a distance of approximately 5-10 cm from the elbow without visible vessels was used as a measurement site.

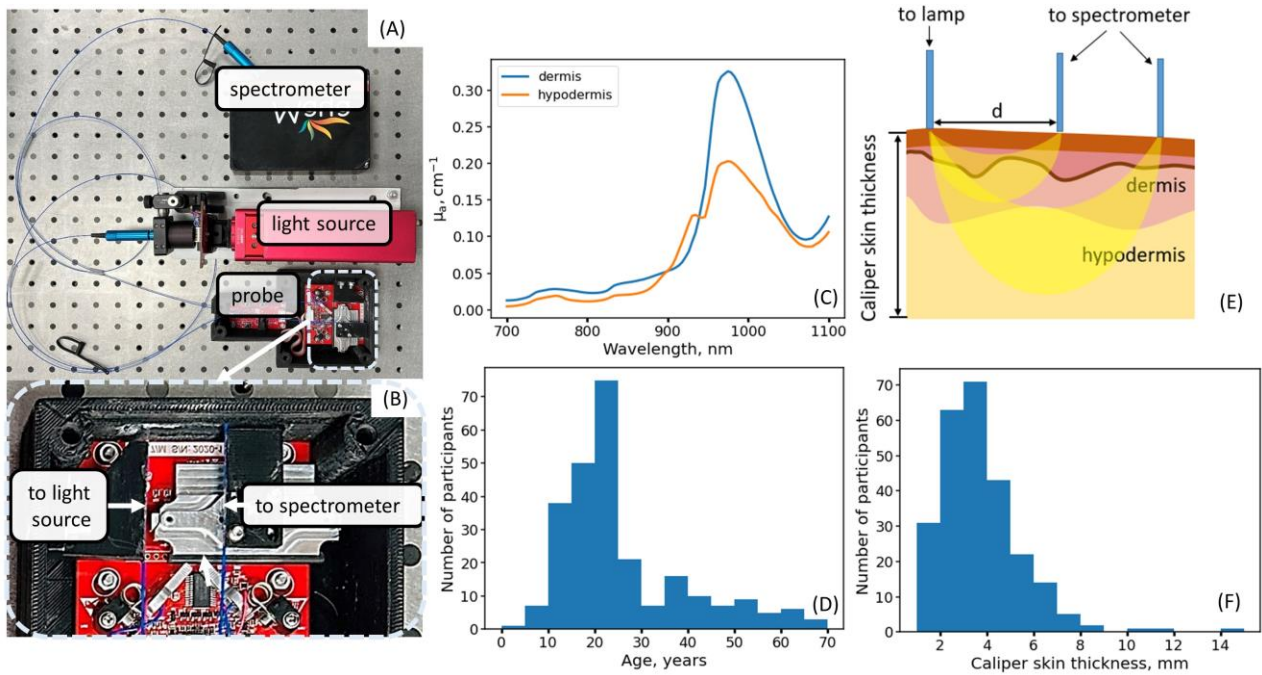


Fig. 1 (A) Photo of the experimental setup implementing diffuse reflectance spectroscopy. (B) Photo of the fiber optic probe used for measuring diffuse reflectance spectra at various distances between the source and detector. (C) Absorption spectra of the dermis and hypodermis. (D) Age distribution histogram of participants in the experiment. (E) Illustration of photon trajectories emitted from the source fiber, reaching the detector fiber at various distances between the source and detector. (F) Caliper skin thickness distribution histogram of participants in the experiment.

2.2 Diffuse Reflectance Spectra Measurements

The device used to measure spatially resolved diffuse reflectance spectra is described in detail in Ref. [20]. Briefly, the measurement component of the device featured a probe composed of two optical fibers, each with a core diameter of 550 μm . This probe was designed to detect diffuse reflection signals from the skin, functioning at various distances between the source and detector across a spectral range of 800–1100 nm (Fig. 1 (A), (B)). This probe allows the measurement of diffuse reflectance spectra at distances between the source and detector fibers, denoted as d , ranging from 0 to 14 mm with a step of 1 mm. A halogen lamp, SLS201 (Thorlabs, USA), was used as the light source and a Maya Pro 2000 spectrometer (Ocean Optics, USA) was used as the detector.

Diffuse reflectance spectra were calculated as follows:

$$R(\lambda) = \frac{I_{\text{skin}}(\lambda) - I_{\text{dark}}(\lambda)}{I_{\text{ref}}(\lambda) - I_{\text{dark}}(\lambda)}, \quad (1)$$

where $I_{\text{skin}}(\lambda)$ is the measured intensity of the reflected signal from the skin, $I_{\text{dark}}(\lambda)$ represents background signal, and $I_{\text{ref}}(\lambda)$ is the measured intensity from the standard ($R = 99\%$, LabSphere, USA).

We calculated the effective optical density as follows [10]:

$$OD(\lambda) = -\log_{10}(R(\lambda)). \quad (2)$$

The absorption amplitude of lipid, denoted as Lipid Index (LI), was calculated from the effective optical density spectra using the following Eq. (3):

$$LI = OD(930 \text{ nm}) - OD(860 \text{ nm}). \quad (3)$$

The absorption amplitude of water, denoted as Water Index (WI), was calculated from the effective optical density spectra using the following Eq. (4):

$$WI = OD(970 \text{ nm}) - OD(860 \text{ nm}) \quad (4)$$

The possibility of assessing lipid and water content by these ratios was discussed in detail in our previous studies [20, 21].

In this study, to assess changes in parameters characteristic of different groups, the model proposed in Ref. [14] was used as one of the options. For this the dependency of the lipid absorption amplitude LI in the measured diffuse reflectance spectrum on the thickness of the hypodermis $d_{\text{hypodermis}}$, as measured using a caliper, was approximated for each distance between the source and detector fibers using the following exponential relationship:

$$LI = A(1 - e^{-B \cdot d_{\text{hypodermis}}}), \quad (5)$$

where $A > 0$ and $B > 0$.

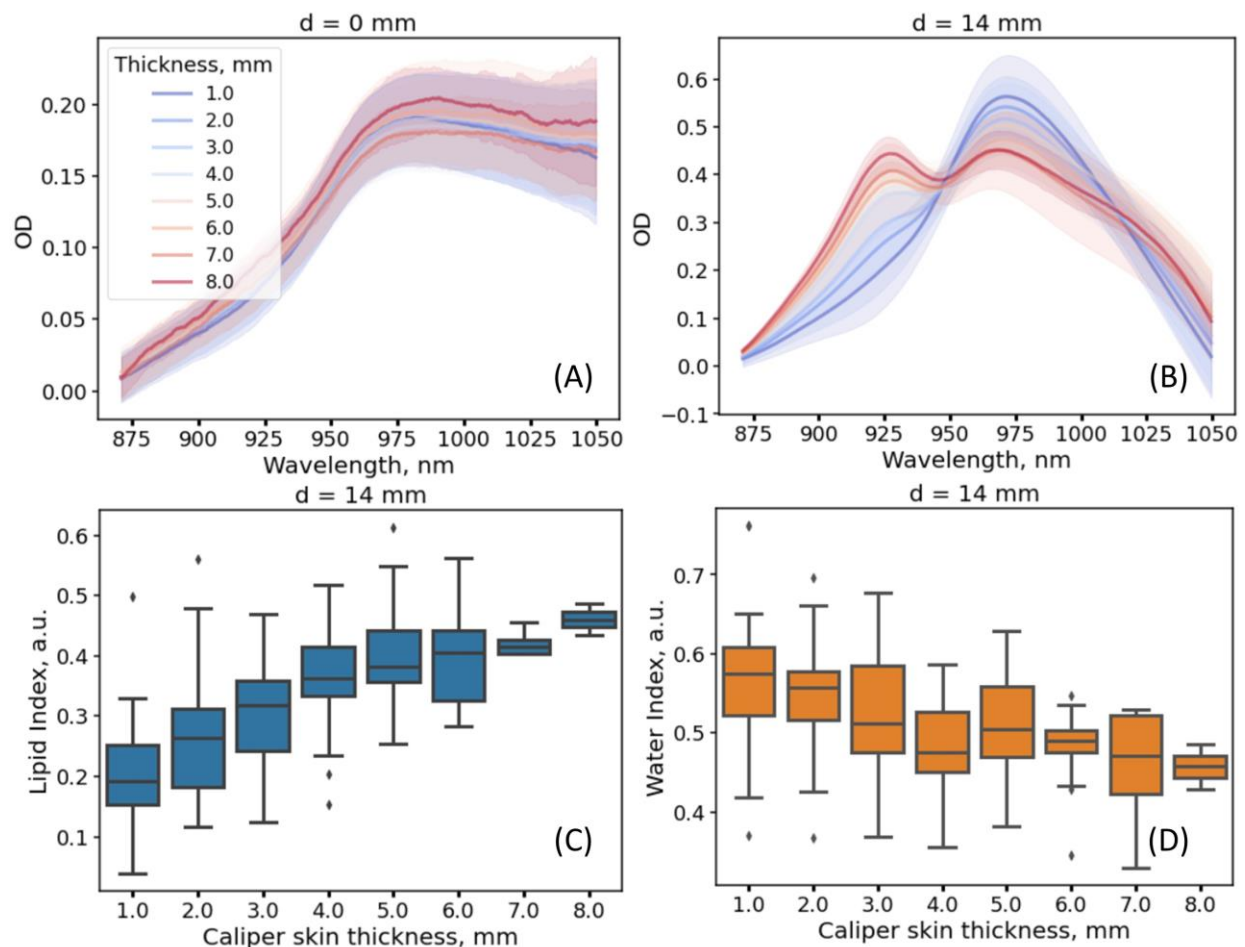


Fig. 2 Diffuse reflectance spectra measured at distances between the source and detector of $d = 0$ mm (A) and $d = 14$ mm (B) on the arms of volunteers with varying thicknesses of subcutaneous fat layer. (C) Dependence of lipid absorption amplitude, estimated from diffuse reflectance spectra at a 14 mm distance, on skin thickness measured by caliper (Eq. (3)). (D) Dependence of water absorption amplitude (Eq. (4)), estimated from diffuse reflectance spectra at a 14 mm distance, on skin thickness measured by caliper.

2.3 Assessment of the Significance of Physiological Parameters

As a second approach to assess changes in response, machine learning models were developed to determine the LI for various sets of physiological features, including gender and age. These models were subsequently compared. The entire sample was divided into a training set (80% of dataset), used for model training and validation, and a test set (20% of dataset), used to assess the quality of the resulting models with features such as gender and age. This division was made once. The relationship between the LI and the thickness of the hypodermis is nonlinear (refer to Eq. (5)). Therefore, to construct a baseline model from hypodermis thickness, linear regression with polynomial transformation of this parameter was employed. The optimal polynomial degree was determined through cross-validation. Cross-validation was done on the training set by randomly dividing the dataset into 5 folds. Prior to training the models, all features were standardized using the following algorithm: the median value of each feature

was subtracted and then divided by the interquartile range of that feature. Gender was encoded as a binary numerical feature: 0 for female and 1 for male. The quality of the resulting machine learning models was evaluated by the coefficient of determination (R^2) and the root mean square error (RMSE), estimated on the test set.

3 Results and Discussion

Fig. 2 (A) and (B) depict the diffuse reflectance spectra measured at distances of 0 and 14 mm between the source and detector on volunteers with varying thicknesses of the subcutaneous fat layer on the arm. The spectra exhibit a local absorption maximum for water around 970 nm and for lipids around 930 nm.

The spectra measured at a small distance ($d = 0$ mm, Fig. 2(A)), showed only slight variations for different thicknesses of the subcutaneous fat layer. At greater distances between the source and detector, a significant increase in lipid absorption amplitude and a decrease in water absorption amplitude were observed (Fig. 2(B)).

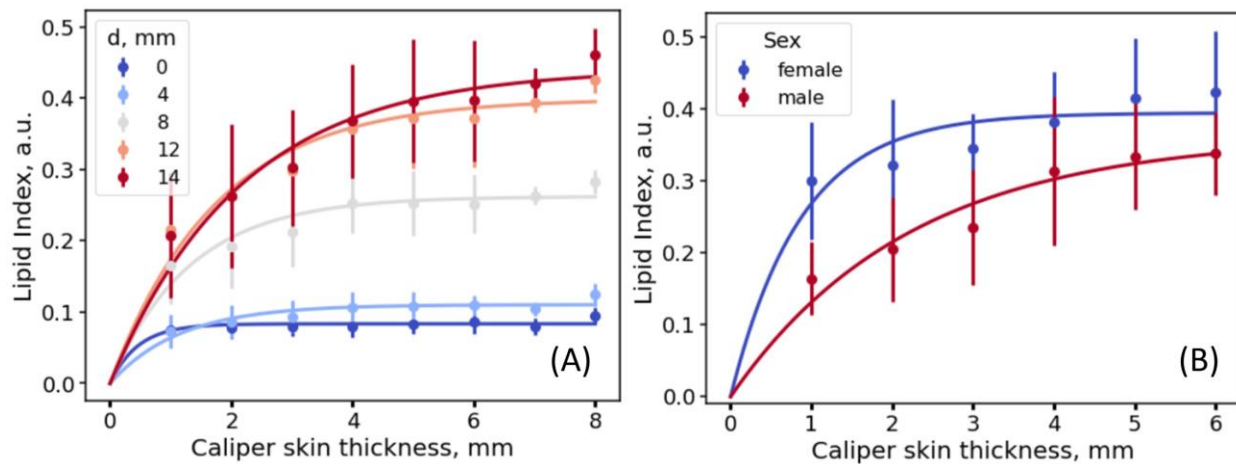


Fig. 3 (A) Dependence of lipid absorption amplitude (Lipid Index), estimated from diffuse reflectance spectra at various distances between the source and detector, on hypodermis thickness. Different distances between the source and detector are color-coded. (B) Dependence of lipid absorption amplitude, estimated from diffuse reflectance spectra at a distance of 14 mm between the source and detector, on hypodermis thickness. The gender of volunteers participating in the study is color-coded. On all panels, points indicate the mean value of Lipid Index and corresponding standard deviation for groups with fixed hypodermis thickness. Solid curves represent data approximation according to Eq. (5).

A direct proportionality between the measured lipid absorption amplitude and the thickness of the hypodermis, as measured by calipers, within a thickness range of 1 to 4 mm was observed (Fig. 1 (C)). Negative correlation was also observed between the water absorption amplitude and the thickness of the hypodermis (Fig. 2 (D)) within the same range of thicknesses. However, for thicknesses greater than 4 mm, the amplitudes of water and lipid absorption did not change significantly (Fig. 2 (C) and (D)).

Utilizing the spectra measured at various distances between the source and detector, we constructed relationships between the LI and the thickness of the hypodermis, as measured by calipers (Fig. 3 (A)). It is evident that the greater the distance between the source and detector, the larger the difference between the maximum and minimum values of the LI for the given caliper measurements.

The relationships presented in Fig. 3 were approximated by an exponential function (Eq. (4)). It was found that the maximum amplitude value $A = 0.44 \pm 0.02$ occurs at the greatest distance between the source and detector. The amplitude value A may differ from the value presented in Ref. [14], as it is contingent upon the method of assessing the contribution of lipids to the reflected signal, the distance between the source and detector fibers, and the diameters of the optical fibers.

As can be seen from Fig. 3 (A), for LI values averaged over groups with the same skin thickness, the dependence on skin thickness can be considered exponential. However, the standard deviation for each value of hypodermal thickness was high, which may be due to physiological parameters such as age and gender. To compare the observed contribution of lipids to the skin diffuse reflectance for different sexes, similar dependences and approximations were obtained using data divided into subsamples by sex (Fig. 3 (B)).

Statistically significant differences were observed between these relationships for individuals of different sexes: $A = 0.39 \pm 0.02$ for females, $A = 0.36 \pm 0.03$ for males.

The difference in dependencies presented in Fig. 3 (B) is most likely due to the fact that the thickness of the dermis for women is on average less than that for men, and when measuring fold thickness using a caliper, the sum of the thicknesses of the dermis and hypodermis is measured. Thus, with equal caliper readings, the thickness of the hypodermis in women is higher, which is reflected in the DRS response and higher values of the calculated LI .

Next, using the obtained data, an inverse problem was solved. That is, using DRS data, the thickness of the hypodermis measured by calipers was determined. From the relationship presented in Fig. 3 (A) and approximation in Eq. (4), the hypodermis thickness in the range from 1 to 4 mm was expressed from the lipid absorption amplitude LI . As a result, the predicted model determines the thickness of the hypodermis with an accuracy of $R^2 = 0.24$ and $RMSE = 1.56$ mm. The obtained error is comparable to results achieved in work [14].

Due to the limited number of samples for some skin thicknesses, it was difficult to further divide the sample into groups by age to compare the parameters of the approximating dependencies for different age groups. Therefore, we assessed the influence of age on the lipid absorption amplitude using machine learning approach. To this end, models were constructed to determine the LI for different sets of physiological features, as described in Table 1. Linear regression with polynomial transformation was used as the base model for dependency on hypodermis thickness. Cross-validation revealed that the optimal degree of polynomial transformation is 3.

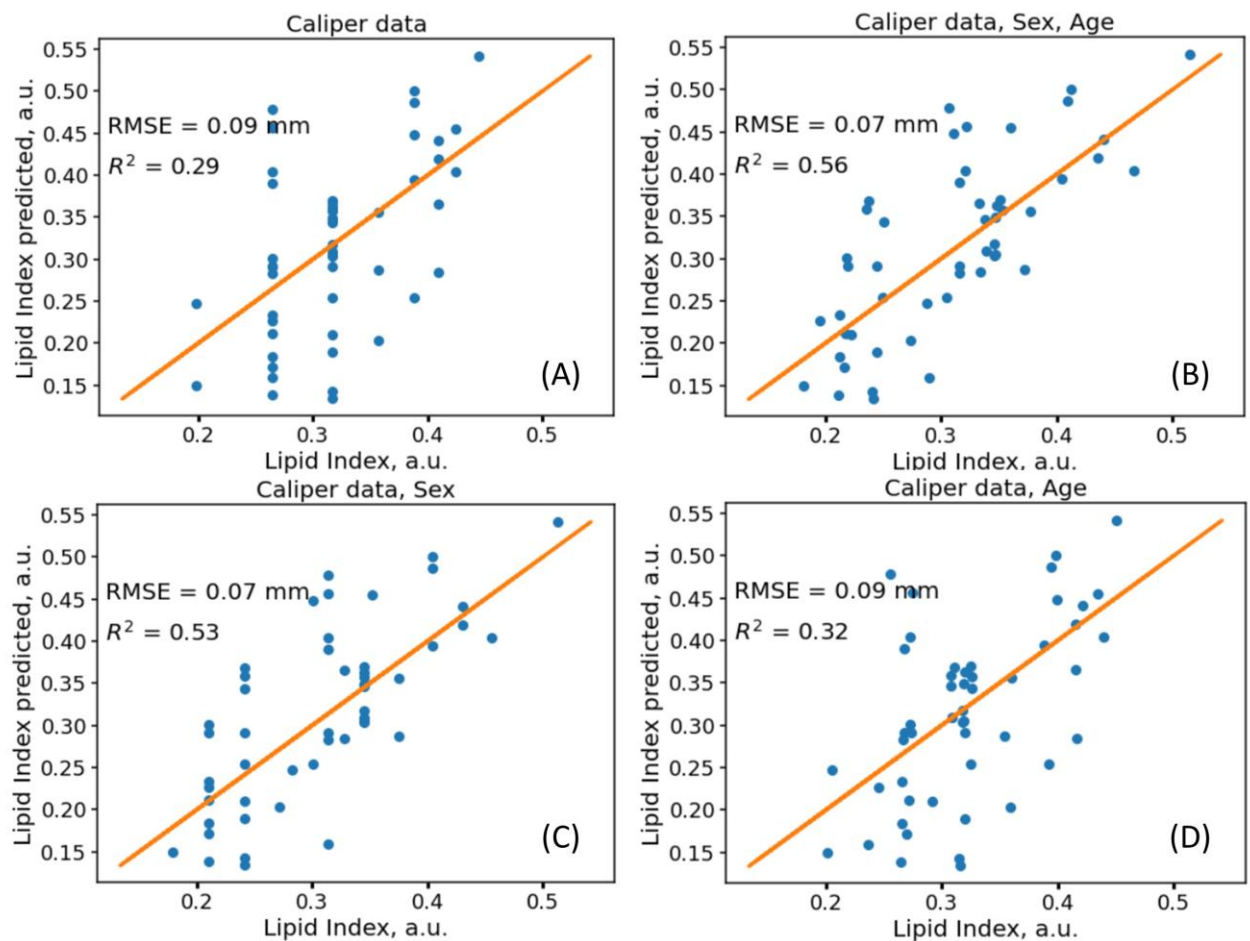


Fig. 4 Dependence of predicted Lipid Index values on measured cases when various feature sets were used: (A) caliper data, (B) caliper data, gender, age, (C) caliper data and gender, (D) caliper data and age.

Table 1 Feature sets for estimating water absorption amplitude using machine learning methods and corresponding coefficients of determination and standard deviation for the obtained models in determining lipid absorption amplitude *LI*.

№	Feature sets	R^2	RMSE
1	Caliper data, Sex, Age	0.56	0.07
2	Caliper data, Sex	0.53	0.07
3	Caliper data, Age	0.32	0.09
4	Caliper data	0.29	0.09

It was found that adding a binary feature defining the sex of the volunteer significantly increased the prediction accuracy on the test sample ($R^2 = 0.53$, $RMSE = 0.07$, Fig. 4 (C)), compared to the base model considering only caliper data ($R^2 = 0.29$, $RMSE = 0.09$, Fig. 4 (A)). This result is consistent with results obtained using the exponential approximation. Further addition of a feature defining the volunteer's age lead to slightly improved accuracy of the model ($R^2 = 0.53$, $RMSE = 0.07$, Fig. 4 (B)), however, this increase was not statistically significant. Similarly, no improvement in RMSE metrics was observed when comparing the base model with the model based on caliper data and age (Fig. 4 (A, D), Table 1, № 3,4).

Thus, both methods indicate the influence of sex on the dependence of the *LI* on the thickness of the hypodermis. In the sample considered, there was no significant effect of age on the shape of this dependence.

4 Conclusion

This study successfully demonstrated the application of spatially-resolved DRS as a non-invasive method for measuring the thickness of the subcutaneous fat layer. Our findings indicate that, while small distances between the source and detector do not provide significant variations in spectroscopic measurements, increasing this distance reveals a clear relationship between lipid and

water absorption amplitudes and the thickness of the hypodermis. The lipid absorption amplitude showed a direct proportionality to the hypodermis thickness for layers between 1 to 4 mm, as corroborated by traditional caliper measurements. Conversely, the water absorption amplitude exhibited negative correlation within the same thickness range. However, beyond a 4 mm thickness, these relationships plateaued, suggesting a limit to the method's sensitivity for thicker fat layers for a given source-detector distance. By establishing a *LI* and relating it to caliper measurements across different source-detector distances, we derived an exponential function that describes this relationship, with the function's maximum amplitude occurring at the largest distance. Notably, sex was identified as significant physiological factor influencing the absorption amplitudes, highlighting the importance of considering physiological factors in optical measurements. The predictive model developed from our data was able to estimate hypodermis thickness with moderate accuracy (RMSE = 1.56 mm), which is limited by the reference method. In conclusion, spatially-resolved DRS offers a promising alternative for assessing subcutaneous fat layer thickness. The method has a number of advantages, such as non-invasiveness, the ability to obtain immediate results, and, most importantly, the molecular specificity of the signal, allowing for a more detailed analysis of tissue composition. Unlike calipers, which do not account for the thickness of the dermis, our approach

enables the analysis of a broader spectrum of tissue characteristics. In certain situations, measuring with calipers can be difficult due to curved surfaces, such as when measuring facial skin. In these cases, optical methods may be more appropriate. This makes our method particularly valuable for determining the characteristics of subcutaneous layers of bio-tissues and body composition. However, its accuracy is influenced by physiological characteristics, underscoring the need for personalized adjustment when using this technique in clinical or research settings. Future work should focus on refining the predictive models and exploring the utility of this method for different body sites and a broader range of hypodermis thicknesses.

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Disclosures

The authors state no conflict of interest.

References

1. P. Trayhurn, J. H. Beattie, "[Physiological role of adipose tissue: white adipose tissue as an endocrine and secretory organ](#)," *Proceedings of the Nutrition Society* 60(3), 329–339 (2001).
2. E. D. Rosen, B. M. Spiegelman, "[Adipocytes as regulators of energy balance and glucose homeostasis](#)," *Nature* 444(7121), 847–853 (2006).
3. C. E. Elks, M. Den Hoed, J. H. Zhao, S. J. Sharp, N. J. Wareham, R. J. F. Loos, and K. K. Ong, "[Variability in the heritability of body mass index: a systematic review and meta-regression](#)," *Frontiers in Endocrinology* 3, 29 (2012).
4. H. Alexander, A. D. Miller, "[Determining skin thickness with pulsed ultra sound](#)," *Journal of Investigative Dermatology* 72(1), 17–19 (1979).
5. L. O. Olsen, H. Takiwaki, and J. Serup, "[High-frequency ultrasound characterization of normal skin. Skin thickness and echographic density of 22 anatomical sites](#)," *Skin Research and Technology* 1(2), 74–80 (1995).
6. C. Eisenbeiss, J. Welzel, W. Eichler, and K. Klotz, "[Influence of body water distribution on skin thickness: measurements using high-frequency ultrasound](#)," *British Journal of Dermatology* 144(5), 947–951 (2001).
7. K. Hoffmann, M. Stücker, T. Dirschka, S. Goertz, S. El-Gammal, K. Dirting, A. Hoffmann, and P. Altmeyer, "[Twenty MHz B-scan sonography for visualization and skin thickness measurement of human skin](#)," *Journal of the European Academy of Dermatology and Venereology* 3(3), 302–313 (1994).
8. J. Stefanowska, D. Zakowiecki, and K. Cal, "[Magnetic resonance imaging of the skin](#)," *Journal of the European Academy of Dermatology and Venereology* 24(8), 875–880 (2010).
9. S. Aubry, C. Casile, P. Humbert, J. Jehl, C. Vidal, and B. Kastler, "[Feasibility study of 3-T MR imaging of the skin](#)," *European Radiology* 19, 1595–1603 (2009).
10. J. Bittoun, H. Saint-Jalmes, B. G. Querleux, L. Darrasse, O. Jolivet, I. Idy-Peretti, M. Wartski, S. B. Richard, and J. L. Leveque, "[In vivo high-resolution MR imaging of the skin in a whole-body system at 1.5 T](#)," *Radiology* 176(2), 457–460 (1990).
11. I. Idy-Peretti, J. Bittoun, F. A. Alliot, R. V. Cluzan, S. B. Richard, and B. G. Querleux, "[Lymphedematous skin and subcutis: in vivo high resolution magnetic resonance imaging evaluation](#)," *Journal of Investigative Dermatology* 110(5), 782–787 (1998).
12. C. Y. Tan, B. Statham, R. Marks, and P. A. Payne, "[Skin thickness measurement by pulsed ultrasound; its reproducibility, validation and variability](#)," *British Journal of Dermatology* 106(6), 657–667 (1982).

13. Y. S. Choi, H. K. Hong, B. J. Kim, M. N. Kim, and H. D. Park, “[Development of a non-invasive measurement system to the thickness of the subcutaneous adipose tissue layer](#),” *Experimental Dermatology* 17(6), 537–541 (2008).
14. D. Geraskin, H. Boeth, and M. Kohl-Bareis, “[Optical measurement of adipose tissue thickness and comparison with ultrasound, magnetic resonance imaging, and callipers](#),” *Journal of Biomedical Optics* 14(4), 044017 (2009).
15. R. V. Warren, R. Bar-Yoseph, B. Hill, D. Reilly, A. Chiu, S. Radom-Aizik, D. M. Cooper, and B. J. Tromberg, “[Diffuse optical spectroscopic method for tissue and body composition assessment](#),” *Journal of Biomedical Optics* 27(6), 065002 (2022).
16. S. Inga, Z. Aleksejs, Z. Ilona, K. Janis, and S. Janis, “[Comparison of a near-infrared reflectance spectroscopy system and skin conductance measurements for in vivo estimation of skin hydration: a clinical study](#),” *Journal of Biomedical Photonics & Engineering* 3(1), 010310 (2017).
17. I. Fredriksson, M. Larsson, and T. Strömberg, “[Machine learning for direct oxygen saturation and hemoglobin concentration assessment using diffuse reflectance spectroscopy](#),” *Journal of Biomedical Optics* 25(11), 112905 (2020).
18. F. Geldof, B. Dashtbozorg, B. H. Hendriks, H. J. Sterenborg, and T. J. Ruers, “[Layer thickness prediction and tissue classification in two-layered tissue structures using diffuse reflectance spectroscopy](#),” *Scientific Reports* 12(1), 1698 (2022).
19. E. E. Nikonova, G. S. Budylin, A. S. Kochmareva, A. Y. Turkina, P. S. Timashev, and E. A. Shirshin, “[Dental pulp location and dentin thickness assessment in situ with diffuse reflectance spectroscopy](#),” *Journal of Biomedical Photonics & Engineering* 8(4), 040507 (2022).
20. D. A. Davydov, G. S. Budylin, A. V. Baev, D. V. Vaypan, E. M. Seredenina, S. T. Matskeplishvili, S. A. Evlashin, A. A. Kamalov, and E. A. Shirshin, “[Monitoring the skin structure during edema in vivo with spatially resolved diffuse reflectance spectroscopy](#),” *Journal of Biomedical Optics* 28(5), 057002 (2023).
21. G. S. Budylin, D. A. Davydov, N. V. Zlobina, A. V. Baev, V. G. Artyushenko, B. P. Yakimov, and E. A. Shirshin, “[In vivo sensing of cutaneous edema: A comparative study of diffuse reflectance, Raman spectroscopy and multispectral imaging](#),” *Journal of Biophotonics* 15(1), e202100268 (2022).