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Hyperspectral image-based analysis of thermal damage in living liver undergoing laser ablation

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ABSTRACT

Laser ablation (LA) is a minimally invasive procedure based on light/tissue interaction aimed to induce a controlled tumor necrosis by increasing tissue temperature. Given the relationship between tissue damage and produced heat, LA needs a fine control of evolving thermal effects in order to evaluate and control procedure outcome. This study relies on biomedical optics principles for non-invasive diagnostic tools development, and presents a contactless approach based on hyperspectral imaging (HSI) to monitor thermal damage during *in vivo* porcine LA. By collecting relative pixel-by-pixel reflectance/absorbance of a wide range spectrum (500-1000nm), HSI can track molecular structure modifications caused by the thermoablative procedure. Indeed, these modifications alter tissue light scattering and absorption. In order to investigate tissue spectrum change by increasing temperature, HSI was collected at fixed maximum temperatures (37, 60, 70, 80, 90, 100, 110 °C) and immediately after LA (1, 2, 3, 4, and 5 minutes). Tissue spectral response for two tests was analyzed also relying on the ablated area considered. Regions of Interest of different dimensions (16, 77, and 170 pixels) were placed in the images after applying a motion correction. Obtained spectra show noticeable variations once a specific temperature threshold has been reached (80-100 °C). Specifically, the measured absorbance variation for selected wavelengths (630, 760, 960nm, for methemoglobin, deoxyhemoglobin, and water respectively) confirms tissue optical behavior dependence with its thermal state. This preliminary investigation discloses the potential of HSI measurement to characterize LA damage, encouraging future studies to standardize this novel technique.

Keywords: hyperspectral imaging, thermal effects, laser ablation, tissue optical response, tissue chromophores, temperature monitoring

1. INTRODUCTION

1.1 Thermal effects control during thermal ablation

Thermal ablation refers to the destruction of tissue with elevated temperatures, hyperthermia, or depressed temperatures, hypothermia [1]. The main objective of a thermal ablation procedure is to act on tumor tissue, minimizing damage on surrounding healthy regions. The liver is a common site of both primary tumors and of metastases from different organs of the gastrointestinal tract, given the large blood inflow coming from the portal venous system. Traditionally, surgery associated or not to chemotherapy represented the main therapeutic option. However, nowadays thermal procedures [2] are gaining importance. In particular, laser ablation (LA) is gaining acceptance as a potential alternative treatment for liver neoplastic diseases [3]. LA is a minimally invasive thermal therapy in which laser light is guided through a small fiber to irradiate the target. Irradiated light is converted to absorbed energy, thereby producing a thermal effect for the tissue undergoing the procedure. Tissue damage strictly depends on temperatures reached locally and application time. Indeed, irreversible cell damage occurs at temperatures around 43-45° C only after a prolonged exposure time (30 and 60 minutes) [4]. When temperatures overcome the threshold of 40° C for a prolonged time, modifications in cellular membrane and cytoskeleton occur. In these conditions, changes in enzyme complexes for DNA synthesis and repair are observed [5]. Conversely, temperatures above 60° C are extremely cytotoxic. Complete necrosis occurs almost instantaneously at this temperature because of the inactivation of vital enzymes leading to protein denaturation and consequently to coagulative necrosis [6]. Consequently, continuous control of a thermal ablation process is necessary to achieve a complete and optimal tumor ablation. Imaging systems are usually used for this purpose. The clinical gold standard entails magnetic-resonance thermometry. This imaging technique ensures accurate spatial localization of the lesion and allows to simultaneously measure the local temperature and thermal dose [7]. However, magnetic resonance is available only in a few centers and

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it requires long imaging sessions, often encountering scarce acceptance among patients. Several new techniques for thermal therapy control are being studied. Among them, optical imaging techniques, and particularly hyperspectral imaging (HSI) are very promising. HSI combines a spectrometer to a photocamera, and this technology, through the light-tissue interaction (reflectance, absorbance, scattering, etc.), can track molecular structure modifications caused by the thermoablative procedure. These modifications alter tissue light scattering and absorption. In particular, light absorption is dependent on the distribution of chromophores within tissue [8]. Additionally, previous studies have shown that increasing temperature causes absorption spectra change for water, hemoglobin, and oxyhemoglobin [9], [10]. During photocoagulation induced by temperature increase, a dramatic variation in the absorption of blood could occur due to bathochromic shift and methemoglobin formation [5]. Therefore, the monitoring of changes in tissue absorption and scattering properties is an optics-based approach to objectively assess LA outcomes. HSI has recently been introduced in the medical field and its use is providing promising results in the detection and assessment of different diseases [11]. Only a few studies using HSI in the thermal treatment scenario presently exist. For instance, Gil et al. introduced the use of HSI to assess cardiac tissue damage during radiofrequency ablation [12]. On the contrary, this work presents a preliminary investigation of using HSI to monitor spectral signatures of laser-induced thermal damage in an *in vivo* liver.

2. MATERIALS AND METHODS

2.1 Animal study and experimental protocol

The study received full approval from the Institutional Ethical Committee of the Institute of Image-Guided Surgery of Strasbourg (Protocol No. 38.2015.01.069), and from the French Ministry of Superior Education and Research (Protocol No. APAFiS##19543-2019030112087889). It was conducted in compliance with French laws for animal use and care and according to European directives (2010/63/EU). One female pig (Large White, mean weight: 34.6kg) was involved. The animal was fasted 12 hours before the procedure with free access to water. Intramuscular ketamine (20mg/kg) and azaperone (2mg/kg) (Stresnil; Janssen-Cilag, Belgium) were used for premedication. Induction was achieved with intravenous propofol (3mg/kg) combined with rocuronium (0.8mg/kg). Anesthesia was maintained with 2% isoflurane. After the procedure, the pig was sacrificed with an intravenous injection of sodium pentobarbital (40mg/kg) (Exagon®, AXIENCE, France), under a 5% isoflurane anesthesia. A laparotomy was performed. Exposure of the liver was achieved and two distinguished areas on the liver surface underwent LA. One optical fiber of 400μm conveying an 808nm laser light was used to perform a contactless ablation of the tissue surface using a collimator placed at the applicator's tip ((a) in Fig. 1A). The procedure was recorded with a thermographic camera capturing images of the scene at 10fps ((b) in Fig. 1A). The real-time measurement of the temperatures in the area of interest, the ablated zone on the liver surface (red circle in Fig. 1B), allowed control for chosen temperature thresholds. These were selected as maximum temperatures measured within the selected ablated zone. Laser current (3000mA) was controlled in accordance with set temperature thresholds (i.e. 60, 70, 80, 90, 100, and 110° C). During the hyperspectral recording time, the laser system was switched off (once the temperature threshold was reached) to prevent any misleading tissue spectral reflection due do the laser light at 808nm, and consequent loss of spectral information. The HSI acquisition has been gated with the breathing protocol implemented for animal anesthesia, and HSI was recorded in the absence of breathing motion (~6s) in order to record a fixed scene. Hypercubes were collected before starting the ablation (around 36° C), for the six maximum temperature values and immediately after (1, 2, 3, 4, and 5 minutes). Target areas have been chosen in order to prevent light specular effects in the hyperspectral images (Fig. 1C). Markers surrounding the lesion were used as references to correct any potential motion error affecting images.

2.2 Experimental instrumentation

Two laser ablation procedures were performed on an *in vivo* liver surface by using a diode laser (LuOcean Mini 4, Lumics, Berlin, Germany). A thermographic camera, (FLIR T540, 464x368 pixels spatial resolution, 2° C accuracy) was used to record the superficial temperature of the liver undergoing ablation. The real-time temperature of the whole liver area during the procedure was visualized, hence allowing control to reach set thresholds. A region of interest (ROI) was placed in the ablated area for this purpose (red circle in Fig. 1B). Spectral and spatial information for the organ undergoing procedure were collected with a hyperspectral camera system in a prototype status with an internal push-broom imaging spectrograph (TI-CAM, Diaspective Vision GmbH, Germany).

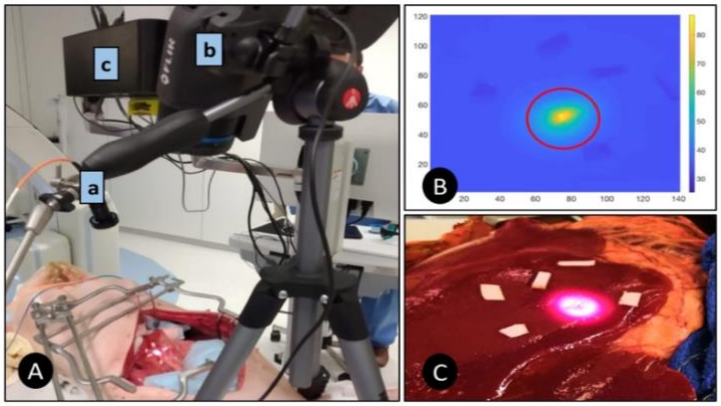

Figure 1: In (A) the experimental set-up consisting of: (a) a collimator placed at the laser applicator's tip; (b) a thermographic camera; (c) a hyperspectral camera. In (B), a thermal image displayed superficial temperature values acquired at a fixed time. Maximum temperatures used as control in the procedure were extracted within the red circle. In (B), a zoom for the *in vivo* liver subjected to the treatment also showed the laser pilot light.

By scanning over the tissue specimen or moving the camera across the tissue sample, an HSI camera collects 2D images for adjacent lines, creating a hypercube of spectral and spatial data [13]. The camera used in this investigation works in the range from 500 to 995nm with a spectral resolution of 5nm and a spatial resolution of 640 by 480 pixels. The camera sensor can collect image data of radiance in about 6s then converted to reflectance by recording a white reference object before measurements are initiated [14].

2.3 Analysis of hyperspectral images

The two LA procedures took an average time of approximately 15 minutes. Hypercubes were collected for set maximum temperature values corresponding to different times during the procedure. In order to minimize error due to pig breathing in this time interval, a motion correction algorithm was applied. Considering the starting point (before ablation) as the reference, images were rigidly rotated in accordance with markers designed to be visible with white color. Since pig breathing was stopped during HSI acquisition, motion correction was applied only among hypercubes. As for the single data-cube images recorded by varying wavelength, any motion is supposed to be negligible. For a better understanding of the organ optical behavior during LA therapy, ROIs of different dimensions were placed in the corrected images. ROIs of 16, 77, and 170 pixels of surface were chosen in order to cover the ablated tissue, and corresponding reflectance values were averaged to obtain one characteristic reflection spectrum for each area. Reflectance values were then converted to absorbance ones and optical response acquired during LA for the two tests were analyzed also relying on the analyzed area dimension.

3. RESULTS AND DISCUSSION

3.1 Spectra during *in vivo* liver laser ablation

Absorbance spectra in the ablated region for one of two tests are reported in Fig. 2 for the three different pixel groups in the defined ROIs. In order to investigate heat-induced spectral changes during the *in vivo* liver laser ablation, a basic description of the tissue optical behavior is necessary. In living tissue, the absorption spectrum in the visible wavelength range is dominated by oxyhemoglobin (HbO_2) and deoxyhemoglobin (Hb) chromophores. HbO_2 shows a double peak in the range between 500 and 600nm, whereas Hb has maximum absorptions at 556 and at 760nm. The temperature increase causes an additional absorption feature between 600 and 650nm, clearly visible in the shown spectra. In the literature, it has been shown that the substantial rise at ~630nm is due to methemoglobin (MetHb) formation. The reaction of MetHb formation requires a minimum activation energy. It is formed at temperatures above 65° C, with a maximum at approximately 72.5° C [15]. In the 700 to 1000nm range, water content (H_2O) is dominant with peculiar peaks at 750, 830, and 960nm [14].

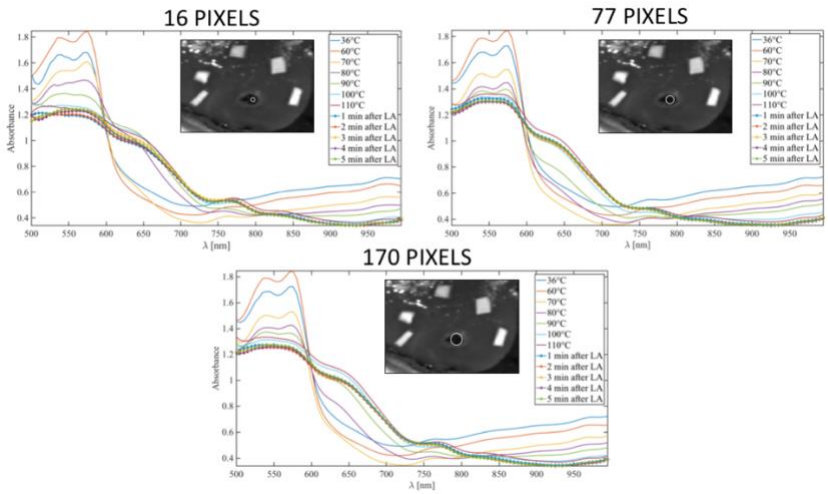

Figure 2: Absorbance spectra acquired during *in vivo* liver ablation in one test for three ROI dimensions (16, 77, and 170 pixels area).

According to the spectra in Fig. 2, when a specific maximum temperature is reached in the area of interest, the spectrum experiences a noticeable change. It can be noted that this temperature threshold varies depending on ROI dimension. In the case of 16 pixels, the ROI spectrum shape experiences a peculiar change for temperature higher than 70 °C. Conversely, this occurs when exceeding 90 °C, and around 80 °C for the regions of 77 and 170 pixels respectively. Above these temperatures, irreversible tissue damage occurs, leading to permanent alterations in spectral features. Indeed, spectral responses above those temperatures suggest a plateau phase possibly caused by tissue carbonization. The main shape variations, especially occurring after exceeding temperature thresholds involve the following: (i) smoothing of the HbO_2 double peak; (ii) MetHb peak formation and increasing (iii) Hb peak consistent variation, (iv) shape and sign changes for H_2O wavelength range [16]. Spectra evolution during and after the ablation procedure was also reported in Fig. 3 for the two tests in the range comprised between 600 and 1000nm. Within the two tests, the trends of tissue spectra are comparable. This confirms their dependence on tissue temperature. Indeed, by varying temperature and the consequent thermal damage, tissue optical properties change, although the magnitude and signs of these changes depend on the tissue area considered.

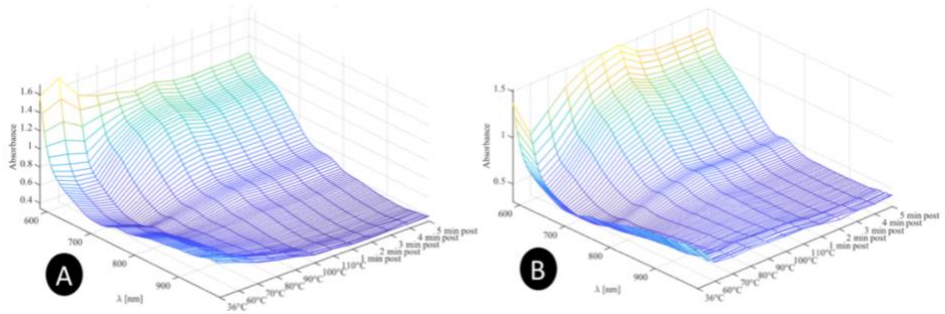

Figure 3: Spectra evolution in Test 1 (A) and Test 2 (B) during the procedure for the 77-pixel ROI in the range comprised between 600 and 1000nm.

3.2 Absorbance for tissue chromophores by increasing temperature

In order to quantify spectrum variation caused by thermal effect, emphasis was put on evaluating changes in metHb, Hb and H_20 absorption peaks for *in vivo* liver tissue during laser ablation. Relative variation for the absorbance values at 630, 760, and 960nm were measured considering values at the starting temperature (36 °C) as reference. This evaluation aims to define spectral features of specific tissue components in order to identify potential markers to monitor tissue thermal damage. Results obtained for absorbance values extracted from ROIs of different dimensions and for the two tests are shown in Fig. 4. Optical features at different temperatures during LA and after switching off the laser have been compared to the ones collected at body temperature. It is clearly visible that absorbance values for selected absorbance peaks experience comparable trends in the two tests and for the three areas. MetHb and Hb, after an initial decrease, start rising until they reach a maximum around 110° C. More specifically, if the MetHb content increases by approximately 50%, then

Hb percentage for 110 °C shows low and still negative values when the maximum temperature is reached. ROI size can affect temperature thresholds corresponding to the change of trend for MetHb and Hb chromophores.

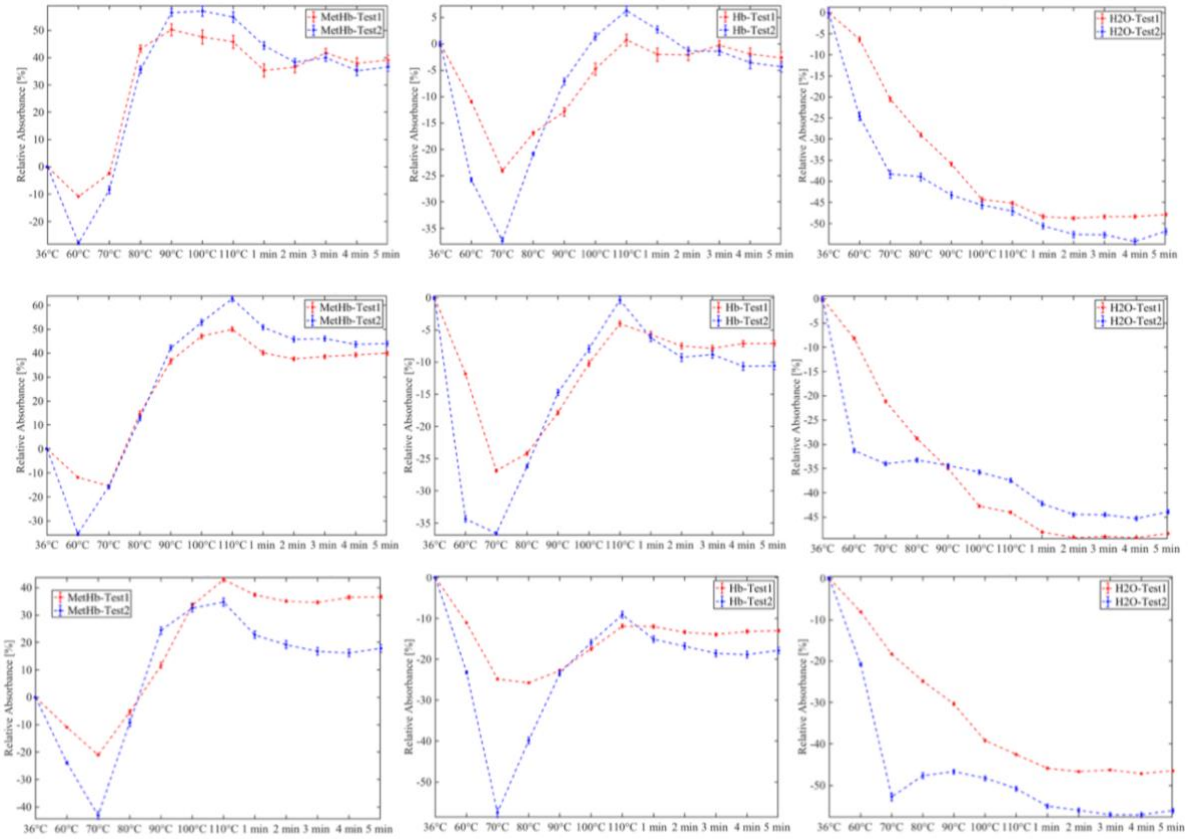

Figure 4: Relative absorbance variations and their standard deviations for MetHb (1° column), Hb (2° column), and H_20 (3° column) during the evolving procedure in the two tests. Absorption peaks were measured for the three ROI dimensions: 16 (1° row), 77 (2° row), and 170 (3° row) pixels.

This is more noticeable for MetHb (1° column in Fig. 4) where the absorbance value starts to increase for temperatures > 60 °C for the smallest area in Test 1, while for 77 and 170 pixels, this occurs when temperature exceeds 70 °C. However, the found temperature values leading to MetHb formation are in accordance with previous studies [17], [15]. The difference in optical response varying ROI size shows the strong dependence of the chosen region of interest with the definition of the therapy outcome. Since the damage is localized, the smallest area allows to lose less punctual information concerning caused thermal damage. On the other hand, choosing fewer pixels could lead to the easiest inclusion of artefacts in the analysis. Smoothing of error for single pixel handling a wrong absorbance value because of HSI working condition benefits from a higher number of pixels in the ROI. Relative absorbance peaks at 960nm (3° column in Fig. 4) exhibit negative values. Indeed, H_2O experiences a decrease during the whole procedure reaching a minimum value of around 50% at 110° C. After switching off the laser, a plateau is achieved for the three tissue elements corresponding to an advanced stage for thermal damage. Previous works showed the possibility to detect chemical and structural changes in tissue undergoing thermal ablation with spectroscopy [18] [19] [20] [21]. In the investigations, they used a probe inserted into the tissue in order to collect optical tissue response. Conversely, the approach analyzed here involves a non-invasive hyperspectral camera, which can collect tissue absorption and scatter information for every pixel in the images, hence allowing a contactless monitoring of laser therapy effects.

4. CONCLUSIONS

This study is a preliminary analysis of the possibility to monitor the LA therapeutic efficacy using HSI. In this investigation, the HSI camera was combined with a thermographic camera in order to relate the optical response of liver

tissue to maximum temperature values reached during a LA procedure. The influence of ROI size on the results has also been taken into account. Consistent variation in the spectra shape for two tests occurs once a specific temperature threshold has been reached (80-100 °C). After reaching such temperatures, carbonization occurs and modification of HbO_2, MetHb, Hb and H_2O wavelengths, already present with lower temperatures, are much more visible. The relative percentage variations for 630, 760, and 960nm were quantified in respect to starting condition for the overall procedure. MetHb and Hb show a starting decrease (falling until value > -50% for Hb in Test 2) and then they increase until reaching a maximum of 110 °C (e.g. ~60% for MetHb in Test 2 for the 77-pixel area), while H_2O content decreases during the whole procedure with a minimum value for Test 2 in the 170 pixels area > -50%. The findings of this work encourage a deeper analysis on the spectral behavior as a function of tissue temperature. In the near future, the proposed strategy holds the promise of supporting the development of a real-time monitoring tool for the optimization of thermal therapies for cancer treatment.

REFERENCES

[1] C. Brace, "Thermal tumor ablation in clinical use," *IEEE Pulse*, 2011.

[2] J. Park, Y. J. Chen, W. P. Lu, and Y. Fong, "The evolution of liver-directed treatments for hepatic colorectal metastases," *Oncol. (United States)*, 2014.

[3] C. M. Pacella, G. Francica, and G. G. Di Costanzo, "Laser Ablation for Small Hepatocellular Carcinoma," *Radiol. Res. Pract.*, 2011.

[4] C. Streffer, "Molecular and Cellular Mechanisms of Hyperthermia," 1995.

[5] A. J. Welch and M. J. C. Van Gemert, *Optical-thermal response of laser-irradiated tissue*. 2011.

[6] E. Schena, P. Saccomandi, and Y. Fong, "Laser Ablation for Cancer: Past, Present and Future," *J. Funct. Biomater.*, vol. 8, no. 2, p. 19, 2017.

[7] V. Rieke and K. B. Pauly, "MR thermometry," *J. Magn. Reson. Imaging*, vol. 27, no. 2, pp. 376–390, 2008.

[8] V. K. Nagarajan and B. Yu, "Monitoring of tissue optical properties during thermal coagulation of ex vivo tissues," *Lasers Surg. Med.*, 2016.

[9] L. Cordone, A. Cupane, M. Leone, and E. Vitrano, "Optical absorption spectra of deoxy- and oxyhemoglobin in the temperature range 300-20 K. Relation with protein dynamics," *Biophys. Chem.*, 1986.

[10] R. Sfareni, A. Boffi, V. Quaresima, and M. Ferrari, "Near infrared absorption spectra of human deoxy- and oxyhaemoglobin in the temperature range 20-40°C," *Biochim. Biophys. Acta - Protein Struct. Mol. Enzymol.*, 1997.

[11] M. A. Calin, S. V. Parasca, D. Savastru, and D. Manea, "Hyperspectral imaging in the medical field: Present and future," *Appl. Spectrosc. Rev.*, vol. 49, no. 6, pp. 435–447, 2014.

[12] D. A. Gil, L. M. Swift, H. Asfour, N. Muselimyan, M. A. Mercader, and N. A. Sarvazyan, "Autofluorescence hyperspectral imaging of radiofrequency ablation lesions in porcine cardiac tissue," *J. Biophotonics*, vol. 10, no. 8, pp. 1008–1017, 2017.

[13] G. Lu and B. Fei, "Medical hyperspectral imaging: a review," *J. Biomed. Opt.*, vol. 19, no. 1, p. 010901, 2014.

[14] A. Holmer, J. Marotz, P. Wahl, M. Dau, and P. W. Kämmerer, "Hyperspectral imaging in perfusion and wound diagnostics - Methods and algorithms for the determination of tissue parameters," *Biomed. Tech.*, 2018.

[15] L. L. Randeberg, J. H. Bonesrønning, M. Dalaker, J. S. Nelson, and L. O. Svaasand, "Methemoglobin formation during laser induced photothermolysis of vascular skin lesions," *Lasers Surg. Med.*, vol. 34, no. 5, pp. 414–419, 2004.

[16] M. De Landro, P. Saccomandi, M. Barberio, E. Schena, M. J. Marescaux, and M. DIana, "Hyperspectral imaging for thermal effect monitoring in in vivo liver during laser ablation," in *Proceedings of the Annual International Conference of the IEEE Engineering in Medicine and Biology Society, EMBS*, 2019.

[17] J. K. Barton, G. Frangineas, H. Pummer, and J. F. Black, "Cooperative Phenomena in Two-pulse, Two-color Laser Photocoagulation of Cutaneous Blood Vessels," *Photochem. Photobiol.*, 2001.

[18] C. R. Buttemere, R. S. Chari, C. D. Anderson, M. K. Washington, A. Mahadevan-Jansen, and W.-C. Lin, "In vivo assessment of thermal damage in the liver using optical spectroscopy," *J. Biomed. Opt.*, 2004.

[19] V. K. Nagarajan, J. M. Ward, and B. Yu, "Association of Liver Tissue Optical Properties and Thermal Damage," *Lasers Surg. Med.*, 2020.

[20] V. K. Nagarajan, V. R. Gogineni, S. B. White, and B. Yu, " Changes in optical properties during heating of ex vivo liver tissues ," in *Energy-based Treatment of Tissue and Assessment IX*, 2017.

[21] J. W. Spliethoff, E. Tanis, D. J. Evers, B. H. W. Hendriks, W. Prevoo, and T. J. M. Ruers, "Monitoring of tumor radio frequency ablation using derivative spectroscopy," *J. Biomed. Opt.*, 2014.