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Evgenii Zherebtsov, Andrian Mamoshin, Valery Shupletsov, Elena Potapova, Ksenia Kandurova, Viktor Dremin, Andrey Dunaev, "Optical needle biopsy for multimodal detection of the malignant liver tumours," Proc. SPIE 12147, Tissue Optics and Photonics II, 121470J (19 May 2022); doi: 10.1117/12.2622376

SPIE.

Event: SPIE Photonics Europe, 2022, Strasbourg, France

Optical needle biopsy for multimodal detection of malignant liver tumours

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ABSTRACT

Malignant tumours of the liver are one of the most common causes of cancer death worldwide. The presence and type of tumours can be verified only after histological and cytological analysis of a sample obtained during percutaneous needle biopsy. However, the relatively high probability of false-negative results caused by insufficient tissue samples remains a problem. The introduction of real-time methods to navigate the needle during the biopsy can serve as a solution that reduces the number of mistakes. The paper presents the results of measurements in the murine model of hepatocellular carcinoma by a system equipped with fluorescence lifetime and diffuse reflectance channels, as well as a novel needle optical probe compatible with 17.5G standard biopsy needles. The experimental setup developed allowed us to evaluate the parameters of short and slow fluorescence lifetimes, fluorescence intensities and blood oxygen saturation in tissues. We demonstrated that the set of recorded parameters allows us to distinguish the malignant tissue, control liver tissue and metabolically changed liver tissues near the tumour. We suppose that the proposed technique, supported by machine learning, can significantly decrease the rate of false-negative results obtained with percutaneous needle biopsy.

Keywords: fluorescence lifetime, diffuse reflectance spectroscopy, liver cancer, hepatocellular carcinoma, adenocarcinoma metastasis, optical biopsy, reactive oxygen species, glutathione.

1. INTRODUCTION

At the moment, liver cancer is among the most common causes of cancer mortality in the world. Malignant tumours can develop from liver cells, but mostly they are caused by metastasis of primary tumours located in lungs, pancreas, kidneys, breast, stomach or intestines[1,2]. Despite the high mortality rate in the later stages of cancer development, the early detection of tumour type can significantly increase the effectiveness of treatment[3].

According to modern medical views, objective verification of the presence and nature of tumour pathology is possible only with histological and cytological analysis. The procedure of percutaneous needle biopsy (PNB) remains the gold standard for the following diagnostics. However, the probability of obtaining a sample that leads to false-negative results can be up to 10%[4], due to factors such as poor visualization of a puncture needle tip or involuntary movements. Thus, the development of new methods to improve the diagnostic effectiveness of the standard PNB procedure remains an important matter for timely diagnosis and the choice of a treatment strategy for patients with verified cancer.

Improvement of the diagnostic technologies and the introduction of new approaches for optical biopsy can help surgeons to identify pathological changes at the initial stages of the cancer process and determine the treatment strategy faster and in a more individual way in each specific case. The functional state of the liver can be estimated by various optical technologies capable of providing new opportunities and diagnostic criteria. Spectroscopy methods are especially promising for researchers, as they allow one to assess the perfusion-metabolic state of tissue in real time and can be used through standard biopsy tools for biopsies of different organs[5–7].

Another key aspect is the possibility to combine several methods within a multimodal approach. This concept allows us to comprehensively assess the state of energy metabolism in liver cells and identify changes that characterize tumour genesis and growth at different stages.

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Tissue Optics and Photonics II, edited by Valery V. Tuchin, Walter C. P. M. Blondel,
Zeev Zalevsky, Proc. of SPIE Vol. 12147, 121470J
 · 0277-786X · doi: 10.1117/12.2622376

In particular, this paper continues to explore the possibility of applying combined fluorescence and diffuse reflectance measurements[7–9]. Fluorescence measurements are widely used in both research and clinical studies for monitoring cellular and tissue metabolism[10]. Several key substances involved in cell biochemical processes, such as NAD(P)H and FAD^{++} are also known to have specific endogenous fluorescence spectra[11,12]. Their content alters under malignant transformation, leading to changes in recorded fluorescence spectra[13]. However, standard fluorescence spectroscopy does not allow researchers to distinguish NADH and NADPH forms, which have similar spectra, but play different roles in cells. NADH is mostly needed for energy production, while NADPH is involved in antioxidant protection[14]. These endogenous fluorophores are bound to proteins in different ways that can be revealed using a time-resolved approach. The measurement of fluorescence lifetime (FSLT) has been proven to be an effective method for studying biological tissues and detecting the shift in the energy-type production from oxidative phosphorylation to glycolysis that occurs in cancerous cells[15,16].

Another method frequently used together with fluorescence techniques is diffuse reflectance spectroscopy (DRS), which allows one to study the morphological structure of the tissue as well as evaluate the blood content and oxygen saturation[17]. Architectural changes at the cellular and intracellular levels under malignant transformation affect the absorbing and scattering properties of biological tissues.

The aim of this work is to study the approach implementing the aforementioned optical biopsy techniques for differentiating healthy liver parenchyma and primary tumours of hepatocellular carcinoma (HCC), as well as to test the possibility of using these methods with a novel needle optical probe compatible with 17.5G standard biopsy needles.

2. MATERIALS AND METHODS

We used an experimental setup that included channels for FSLT measurements and DRS, which was used to calculate the tissue oxygen saturation (Fig. 1). The FSLT channels were based on the time-correlated single-photon counting system (Becker&Hickle, Germany) consisting of MonoScan2000 (Ocean Insight, USA) monochromator; BDL-SMN 375 nm laser to excite the fluorescence of NAD(P)H; the SPC-130-EMN photon counting board; HPM-100-40 detector; and MF445 (Thorlabs Inc., USA) filter (445±25 nm) to pick out the bandwidth of NAD(P)H fluorescence[18]. The radiation power of the laser measured at the end of the probe used did not exceed 0.2 mW for safety reasons[19] and to reduce photobleaching. The DRS channel included an HL-2000-FHSA halogen lamp (Ocean Insight, USA) as a broadband light source. Diffuse reflected radiation from tissue in the range of 400–900 nm was analysed after recording with a FLAME-T-VIS-NIR-ES spectrometer (Ocean Insight, USA). The optical fine needle probe connected with the sources and detectors for both methods had a diameter of 1 mm, ensuring compatibility with the standard 17.5G Chiba-type biopsy needle for the PNB procedure.

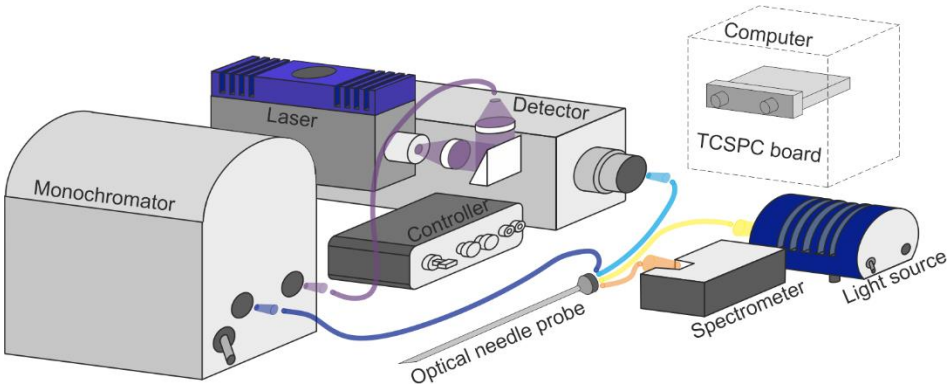

Figure 1. Experimental equipment including FSLT and DRS setups and optical needle probe.

The study was approved by the Ethics committee of Orel State University (record of the meeting No. 12 of 06.09.2018) in accordance with GLP principles. Hybrid BDF (C57Bl6xDBA) mice with inoculated hepatocellular tumours were provided by the N.N. Blokhin Russian Cancer Research Center (Moscow, Russia). Hepatocellular carcinoma line H33 cells[20] (100 µl/mouse, 50000 cells/µl) were inoculated into the right medial lobe of the liver of 6 laboratory mice. Measurements were taken no earlier than three months after inoculation at the end-stage of HCC tumour development, diagnosed according to dynamic observation of the animals' condition. The control group consisted of four healthy control animals. The mice were anesthetized with Zoletil 100 (Vibrac, France) at standard dosages. Each animal was fixed on a special platform in

position on its back. A laparotomy was performed to gain access to the liver, perform the optical *in vivo* measurements and obtain samples for histological study of grown tumours (Fig. 2).

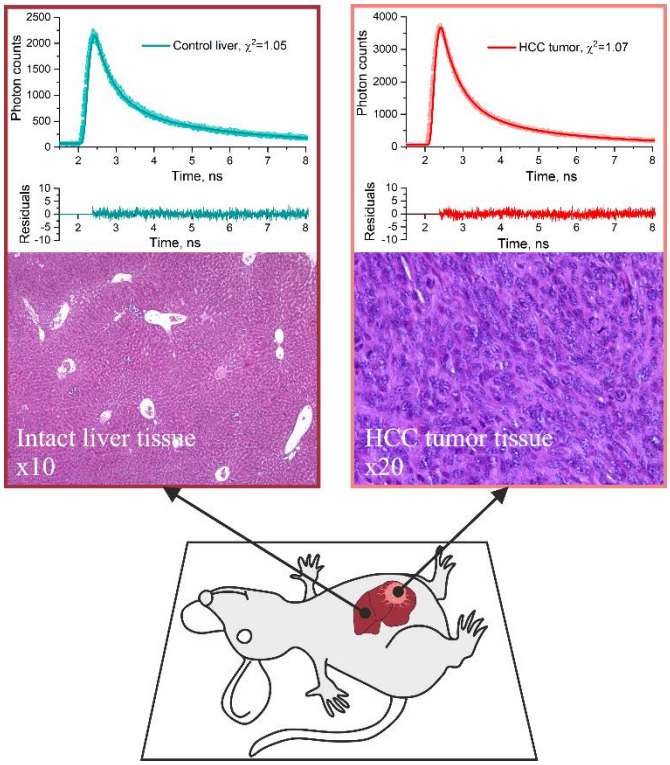

Figure 2. The measurement scheme and analysed results: fluorescence decay curves in both groups and tissue slices examined after standard haematoxylin and eosin staining.

The non-parametric Mann-Whitney U-test was used to validate statistical differences. A linear discriminant analysis (LDA) with leave-one-out cross-validation was used to determine the discriminant function.

3. RESULTS AND DISCUSSION

Calculation of the tissue oxygen saturation (StO_2) in HCC tumours, adjacent liver tissue and healthy liver tissue in control mice showed the following statistically significant differences ($p<0.001$). The highest values of StO_2 were observed in the HCC tumours (84.6±0.1%), while the lowest values were obtained in control liver tissue (80.9±0.1%). The adjacent liver tissues demonstrate the values of 83.0±0.1%. These changes affect mitochondrial energy production and indicate the increase of arterial blood supply caused by the tumour growth, whilst healthy liver tissue has lower tissue oxygen saturation[21,22].

The fluorescence intensity I_f measured in the tumours was more than two times higher than in the control liver tissues ($2.30\cdot10^6\pm0.1\cdot10^6$ and $0.9\cdot10^6\pm0.03\cdot10^6$ photons, $p<0.001$). The adjacent liver fluorescence intensity was not significantly different from that of the tumour ($2.07\cdot10^6\pm0.1\cdot10^6$ photons).

The amplitude of the short decay component α_1 was significantly higher in the tumour lumps in comparison with the adjacent liver tissue and the intact liver tissues (4600±260, 1840±100 and 1107±48 photons correspondingly, statistical significance of $p<0.001$, between the malignant and intact liver tissues). The amplitude of the long decay component α_2 was also highest in the HCC tissues (1430±75 photons) and the lowest in the healthy liver tissue (680±27 photons). The values of α_2 in the adjacent tissues also took an intermediate position, as in the previously mentioned parameters (1080±60 photons). The amplitude ratio α_1/α_2 demonstrated no significant difference between the control and adjacent liver tissues but a significant increase in the tumour tissues (61.9±0.5% in the adjacent tissues, 61.4±0.4% in the liver tissues of control animals and 75.6±0.6% in the HCC tissues, $p<0.001$). The parameter of short fluorescence lifetime τ_1 was significantly higher in the adjacent liver tissues compared to the control and malignant tissues (820±24, 552±15 and 540±5 ps

respectively, $p<0.001$). The long fluorescence lifetime τ_2 parameter has a similar trend (3150±30 ps in the adjacent liver tissues, 2720±10 ps in the control liver tissue and 2530±25 ps in the HCC tissue, $p<0.001$). The decrease of τ_2 is a known feature of cancer cells indicating the increase of the free NADH content due to glycolysis[23]. The obtained data emphasize the necessity of applying fluorescence decay parameters in addition to fluorescence intensities as a reliable marker to distinguish cancerous and non-cancerous liver tissue.

The combination of fluorescence intensities (I_f), the short and long fluorescence lifetimes (τ_1, τ_2) and the amplitude ratios (α_1/α_2) was used to assess the diagnostic value of the feature space by linear discriminant analysis (LDA). The classifiers were built on pairs of the independent parameters (τ_1, I_f), (τ_2, I_f), (τ_1, α_1/α_2) and (τ_2, α_1/α_2).

The estimated parameters of sensitivity, specificity and areas under the curve (AUC) for the obtained results of tissue discrimination are presented in Table 1. In general, the selected pairs of parameters display significant levels of classification quality.

Table 1. The parameters of sensitivity, specificity and AUC for the discrimination of HCC tissue from liver tissue in control animals.

Parameters	**Control liver - murine HCC**			**Adjacent liver - murine HCC**		
	Sensitivity	**Specificity**	**AUC**	**Sensitivity**	**Specificity**	**AUC**
τ_1, I_f	0.97	0.91	0.86	0.92	0.72	0.96
τ_2, I_f	0.92	0.99	0.94	0.99	0.99	0.99
τ_1, α_1/α_2	0.9	1.0	0.98	0.65	1.0	0.99
τ_2, α_1/α_2	0.87	0.97	0.97	0.79	0.96	0.99

Fig. 3 demonstrates the coordinate spaces and decision boundary lines for the (τ_1, I_f) and (τ_2, I_f) pairs.

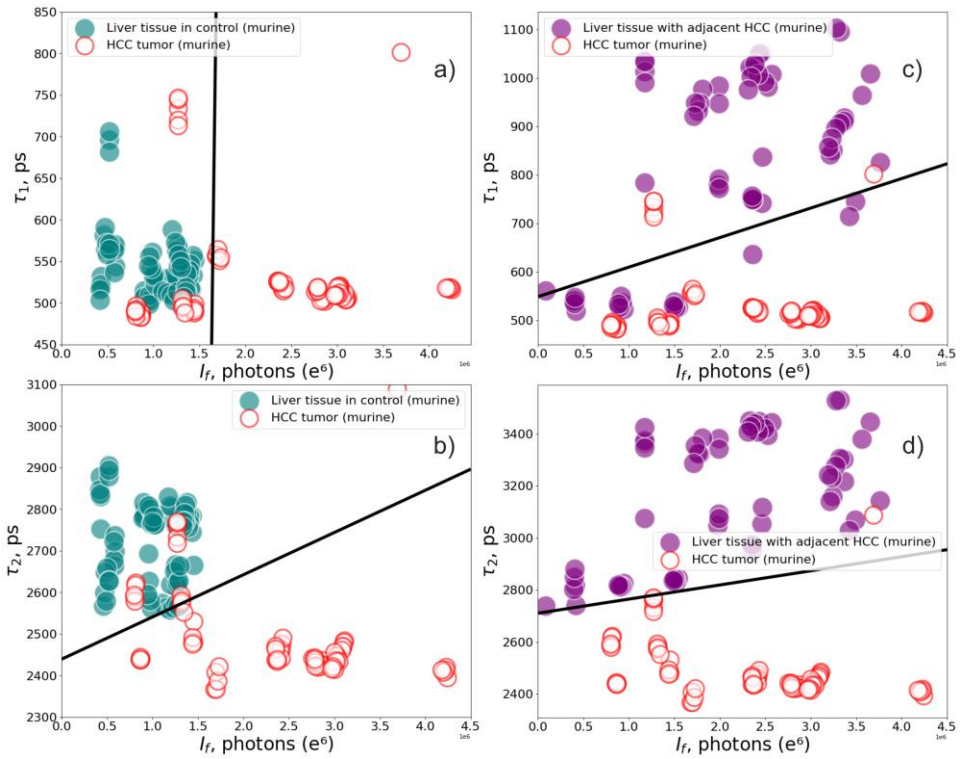

Figure 3. The LDA classification based on the parameters of lifetime (τ_1 and τ_2) and fluorescence intensity I_f. in the control liver and H33 tissues (a, b); in the adjacent liver and H33 tissues (c, d).

The fluorescence intensity I_f demonstrates the lowest diagnostic significance for compared tissue classes. The predictive value of the τ_1 is not equal between the cases of distinguishing HCC tumours from control and adjacent liver tissues. The (τ_1, I_f) classifier shows the worst quality indicators, with the AUC estimated as 0.86 for the "control liver - murine HCC" pair and 0.96 for the "adjacent liver - murine HCC" pair.

Fig. 4 shows the coordinate spaces and decision boundary lines for the (τ_1, α_1/α_2) and (τ_2, α_1/α_2) parameters.

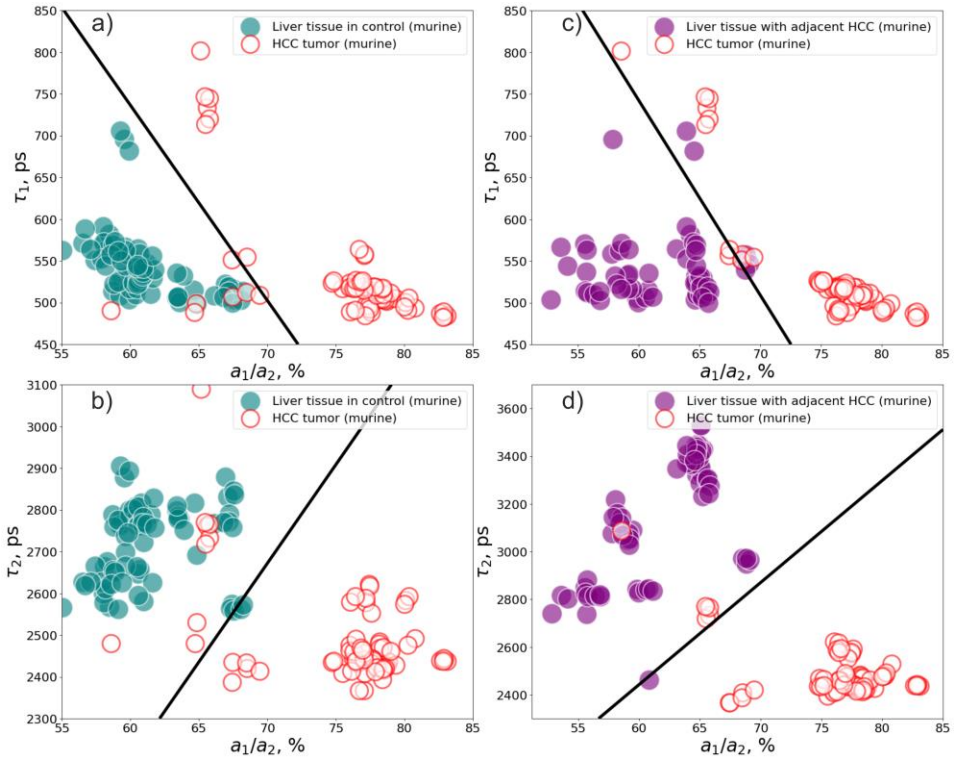

Figure 4. The LDA classification based on the parameters of lifetime (τ_1 and τ_2) and ratio α_1/α_2 in the control liver and H33 tissues (a, b); in the adjacent liver and H33 tissues (c, d).

By replacing the fluorescence intensity I_f parameter with the α_1/α_2 ratio, the performance of the obtained LDA classifiers was improved. The τ_2 and α_1/α_2 parameters demonstrated the highest diagnostic significance, with AUC values of 0.97 and 0.99 for "control liver - murine HCC" and "adjacent liver - murine HCC" pairs, respectively.

The results show that the comparison of the preliminary reference data obtained in healthy intact liver with the data recorded during the biopsy can lead to errors when applied to distinguishing the tissues of tumours and adjacent liver. The tumour growth may significantly alter the metabolic processes in the cells of liver tissue surrounding the tumour itself, which in turn changes measured fluorescence lifetime parameters. Another problem is uncertainty regarding the degree of pathology when gradual transition from healthy tissue to malignant tissue can take place. In that respect, the proposed vector of input parameters (τ_2, α_1/α_2) may be quite effective.

4. CONCLUSION

In this work, we presented a suggested multimodal approach and a developed optical biopsy system which implements FSLT and DRS measurements. The application of a basic classification algorithm of LDA for a set of fluorescence lifetime parameters allowed us to reliably distinguish primary liver cancer tissue, healthy liver tissue and metabolically changed liver tissues which surrounded developed tumours. The results obtained show the high sensitivity and specificity of the proposed optical biopsy technique. The results seem promising for further upgrades of the fluorescence lifetime needle optical biopsy setup with real-time classification, based on more advanced machine learning algorithms. The research protocol of the preliminary study conducted was not designed to assess more different stages of tumour growth, so this is considered for future studies, as well as the identification of benign neoplasms for further transition to full-scale clinical

research. The approach proposed to study the characteristic states of tissues in healthy and malignant conditions using FSLT and DRS will allow us to improve the accuracy of the PNB procedure, as well as assess the functional reserves of the rest of liver parenchyma and predict the course of the postoperative period after surgical cancer treatment.

ACKNOWLEDGMENTS

The study was supported by the Russian Science Foundation (project 21-15-00325).

REFERENCES

[1] Bruix, J., Reig, M. and Sherman, M., "Evidence-based diagnosis, staging, and treatment of patients with hepatocellular carcinoma," Gastroenterology 150(4), 835–853 (2016).
[2] Stavraka, C., Rush, H. and Ross, P., "Combined hepatocellular cholangiocarcinoma (cHCC-CC): an update of genetics, molecular biology, and therapeutic interventions," J. Hepatocell. carcinoma 6, 11 (2019).
[3] Roayaie, S., Obeidat, K., Sposito, C., Mariani, L., Bhoori, S., Pellegrinelli, A., Labow, D., Llovet, J. M., Schwartz, M. and Mazzaferro, V., "Resection of hepatocellular cancer≤ 2 cm: results from two Western centers," Hepatology 57(4), 1426–1435 (2013).
[4] Park, S., Lee, D., Jin, S., Kim, D., Jeen, Y. and Choi, I., "Fine-needle aspiration cytology as the first pathological diagnostic modality in breast lesions: A comparison with core needle biopsy," Basic Appl. Pathol. 3(1), 1–6 (2010).
[5] Mathieu, M.-C., Toullec, A., Benoit, C., Berry, R., Validire, P., Beaumel, P., Vincent, Y., Maroun, P., Vielh, P. and Alchab, L., "Preclinical ex vivo evaluation of the diagnostic performance of a new device for in situ label-free fluorescence spectral analysis of breast masses," Eur. Radiol. 28(6), 2507–2515 (2018).
[6] Tanis, E., Evers, D. J., Spliethoff, J. W., Pully, V. V, Kuhlmann, K., van Coevorden, F., Hendriks, B. H. W., Sanders, J., Prevoo, W. and Ruers, T. J. M., "In vivo tumor identification of colorectal liver metastases with diffuse reflectance and fluorescence spectroscopy," Lasers Surg. Med. 48(9), 820–827 (2016).
[7] Dremin, V., Potapova, E., Zherebtsov, E., Kandurova, K., Shupletsov, V., Alekseyev, A., Mamoshin, A. and Dunaev, A., "Optical percutaneous needle biopsy of the liver: a pilot animal and clinical study," Sci. Rep. 10, 14200 (2020).
[8] Kandurova, K., Shupletsov, V., Potapova, E., Dremin, V., Zherebtsov, E. and Dunaev, A., "Fluorescence lifetime optical biopsy of the hepatocellular carcinoma in murine model," Proc. SPIE 11919, 1191922 (2021).
[9] Zherebtsov, E. A., Potapova, E. V, Mamoshin, A. V, Shupletsov, V. V, Kandurova, K. Y., Dremin, V. V, Abramov, A. Y. and Dunaev, A. V., "Fluorescence lifetime needle optical biopsy discriminates hepatocellular carcinoma," Biomed. Opt. Express 13(2), 633–646 (2022).
[10] Ramanujam, N., "Fluorescence spectroscopy of neoplastic and non-neoplastic tissues," Neoplasia 2(1–2), 89–117 (2000).
[11] Koenig, K. and Schneckenburger, H., "Laser-induced autofluorescence for medical diagnosis," J. Fluoresc. 4(1), 17–40 (1994).
[12] Croce, A. C. and Bottiroli, G., "Autofluorescence spectroscopy and imaging: A tool for biomedical research and diagnosis," Eur. J. Histochem. 58(4), 320–337 (2014).
[13] Awasthi, K., Moriya, D., Nakabayashi, T., Li, L. and Ohta, N., "Sensitive detection of intracellular environment of normal and cancer cells by autofluorescence lifetime imaging," J. Photochem. Photobiol. B Biol. 165, 256–265 (2016).
[14] Ju, H.-Q., Lin, J.-F., Tian, T., Xie, D. and Xu, R.-H., "NADPH homeostasis in cancer: Functions, mechanisms and therapeutic implications," Signal Transduct. Target. Ther. 5(1), 1–12 (2020).
[15] Becker, W., Bergmann, A., Hink, M. A., König, K., Benndorf, K. and Biskup, C., "Fluorescence Lifetime Imaging by Time-Correlated Single-Photon Counting," Microsc. Res. Tech. 63(1), 58–66 (2004).
[16] Marcu, L., French, P. M. W. and Elson, D. S., [Fluorescence lifetime spectroscopy and imaging: principles and applications in biomedical diagnostics], CRC Press (2014).
[17] Baltussen, E. J. M., Snæbjörnsson, P., De Koning, S. G. B., Sterenborg, H. J. C. M., Aalbers, A. G. J., Kok, N., Beets, G. L., Hendriks, B. H. W., Kuhlmann, K. F. D. and Ruers, T. J. M., "Diffuse reflectance spectroscopy as a tool for real-time tissue assessment during colorectal cancer surgery," J. Biomed. Opt. 22(10), 106014 (2017).

[18]Heikal, A. A., "Intracellular coenzymes as natural biomarkers for metabolic activities and mitochondrial anomalies," Biomark. Med. 4(2), 241–263 (2010).
[19]The International Commission on Non-Ionizing Radiation Protection., "Guidelines on Limits of Exposure to Ultraviolet Radiation of Wavelengths Between 180 nm and 400 nm (Incoherent Optical Radiation)," Health Phys. 87(2), 171–186 (2004).
[20]Lazarevich, N. L., Cheremnova, O. A., Varga, E. V, Ovchinnikov, D. A., Kudrjavtseva, E. I., Morozova, O. V, Fleishman, D. I., Engelhardt, N. V and Duncan, S. A., "Progression of HCC in mice is associated with a downregulation in the expression of hepatocyte nuclear factors," Hepatology 39(4), 1038–1047 (2004).
[21]Chen, J.-A., Shi, M., Li, J.-Q. and Qian, C.-N., "Angiogenesis: multiple masks in hepatocellular carcinoma and liver regeneration," Hepatol. Int. 4(3), 537–547 (2010).
[22]Kim, S. K., Kim, H., Koh, G. Y., Lim, D.-S., Yu, D.-Y., Kim, M. D., Park, M.-S. and Lim, J. S., "Mouse hepatic tumor vascular imaging by experimental selective angiography," PLoS One 10(7), e0131687 (2015).
[23]Rodimova, S., Kuznetsova, D., Bobrov, N., Elagin, V., Shcheslavskiy, V., Zagainov, V. and Zagaynova, E., "Mapping metabolism of liver tissue using two-photon FLIM," Biomed. Opt. Express 11(8), 4458–4470 (2020).