

FIBER PROBE FOR SIMULTANEOUS MID-INFRARED AND FLUORESCENCE SPECTROSCOPIC ANALYSIS

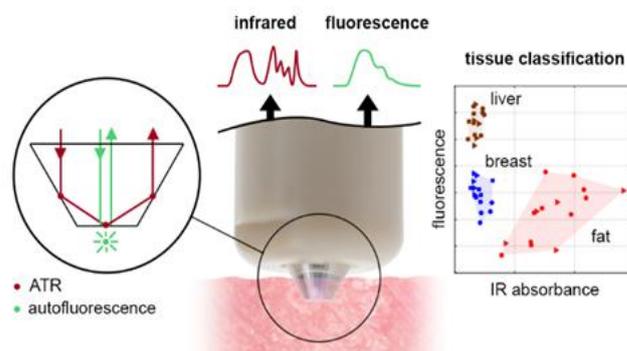
A multimode fiber optic probe has been developed that enables simultaneous analysis of various liquid and solid samples using attenuated total reflection mid-infrared spectroscopy and fluorimetry. The capability of the probe to deliver complementary chemical information from the same measurement point has been illustrated using qualitative analysis of biological tissue samples.

A. Bogomolov, T. Sakharova, I. Usenov, C. Mizaikoff, V. Belikova, S. Perevshikov, V. Artyushenko, O. Bibikova. Fiber probe for simultaneous mid-infrared and fluorescence spectroscopic analysis. *Anal. Chem.* **93** (2021) 6013–6018.

Optical spectroscopy offers a number of flexible analytical techniques for studying the sample properties at the molecular level. Depending on the chosen spectral range and measurement geometry, optical spectra can deliver various chemical and morphological information about an object under study. Fiber optics and probes on its basis enable sampling-free measurements *in situ*, thereby essentially expanding the scope of optical analysis compared to the traditional lab spectroscopy.

Considerable progress in this area is associated with the advent of new optical fiber materials on the basis of chalcogenide glasses and polycrystalline silver halides having sufficiently high transmittance in different intervals of the mid-infrared (IR) spectral region (4000–400 cm^{-1}). The modern optical fiber probes together cover the entire practical range of the optical spectroscopic analysis, thereby extending the applicability of ultraviolet, visible, near- and mid-IR spectroscopy. They are designed to measure various effects of the light-to-sample interaction: absorbance, diffuse reflectance, attenuated total reflection (ATR), Raman scattering, and fluorescence.

The application of fiber optic probes can be moreover indispensable for the spectroscopic analysis of solid inhomogeneous samples, such as biological tissues, where focusing on the local analysis of the chemical composition is required. For complex samples and challenging analytical tasks, any single spectroscopic technique can be insufficient for reliable analysis. Thus, in the case of tumor diagnostics, multiple chemical biomarkers and morphological changes of the tissue should be determined at a time to distinguish between the normal and malignant cells, especially near the border of the tumor. Preliminary investigations on the clinical samples have shown that optical diagnostics



using a combination of fluorimetry with ATR IR spectroscopy results in much better accuracy of the tumor margin detection than each of the methods individually. This synergy was explained by the capability of methods to deliver complementary chemical information, i.e., indicate the presence of different tumor biomarkers. The main challenge of such local multispectral analysis is related to the necessity to perform different spectroscopic measurements at the same point simultaneously. Alternating multispectral measurements cannot guarantee complete coincidence of the spots analyzed by both methods.

The developed probe provides the optimal measurement geometry and the choice of necessary materials in order to provide sufficient quality of the signals detected by each technique.

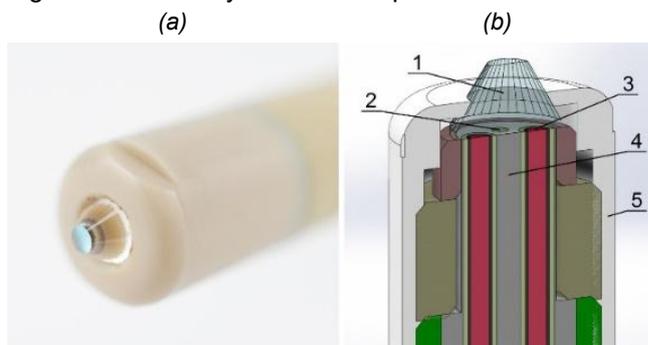


Fig.1. Multispectral probe head design: (a) the probe photo and (b) axial cross-section, where 1 – ATR crystal, 2 – Silica fibers, 3 – CIR fibers, 4 – ferrule, and 5 – probe shaft.

Mid-IR spectroscopy and fluorimetry are very different in physical phenomena and wavelength ranges used for the chemical analysis, and therefore, their coupling within one probe is a challenging task. The proposed solution (Fig. 1) is based on a precise determination of the optical configuration of the ATR crystal head, which, in this case, plays the role of a common measurement interface for both methods. ZrO_2 crystal was chosen due to its high (close to the diamond) refractive index, high transparency in the working spectral regions of both chalcogenide infrared (CIR)

and UV silica fibers, as well as small intrinsic fluorescence.

The frustum geometry provides three total internal reflections of the IR light with 60° angle of incidence. A round platform at the top of the truncated cone simultaneously serves to record fluorescence spectra. Silica fiber end faces are located exactly below it, providing the orthogonal sample illumination and reception of the fluorescence signal emitted back by the sample. At the same time, the CIR fiber cross-sections are positioned right below the slopes of the cone beside its truncated apex (Fig. 1). The frustum tip diameter of 1.1 mm was found to be optimal for the fluorescence measurement.

The developed multispectral probe was tested in the practical analysis of biological tissue samples using different organs and parts of the chicken: breast, fat, and liver. The general chemical similarity of biological objects makes spectroscopic recognition of different tissue types a nontrivial task, which require careful optimization of the measurement technique.

Three samples of each tissue type were prepared. Each sample was represented by five distributed points resulting two data matrices: a matrix of Fourier-transform (FT) IR and a matrix of fluorescence spectra. Preliminary visual exploration of the obtained spectra (Fig. 2) revealed the different abilities of the two techniques to recognize certain tissue types. Fat samples have band of the ester carbonyl C=O at about 1740 cm^{-1} in mid-IR, which is characteristic of saturated triglycerides (Fig. 2a). At the same time, all three types of samples have amide I and II bands of the protein peptide group at about 1548 and 1640 cm^{-1} , respectively. Low measurement reproducibility of the fat samples is explained by inhomogeneity of this tissue type. The fluorescence spectra after standard normal variate (SNV) correction look very similar below 600 nm (Fig. 2b). The most selective peak at 613 nm , corresponding to porphyrins, allows the liver to be distinguished from other measured samples.

Class separation is easily achieved by using multivariate analysis methods, e.g. principal component analysis (PCA), on combined data from the two methods. Each method alone is not capable of providing unambiguous tissue classification. However, the same result can be achieved without the use of chemometrics. Class separation is achieved in the plane of two selected wavelengths (Fig. 3), which carry the most important chemical information. This example illustrates the gain that can be obtained using the proposed multispectral probe in the qualitative analysis of biological tissue.

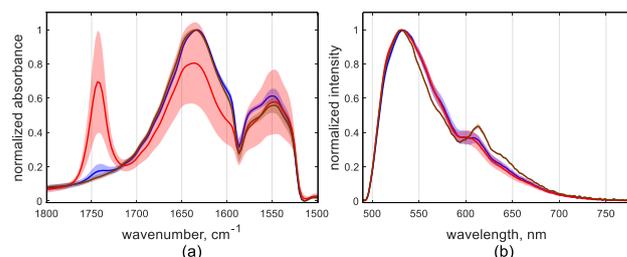


Fig. 2. Normalized experimental spectra of chicken breast (blue), fat (red), and liver (brown) acquired with the multispectral probe: (a) IR spectra and (b) fluorescence spectra preprocessed by SNV.

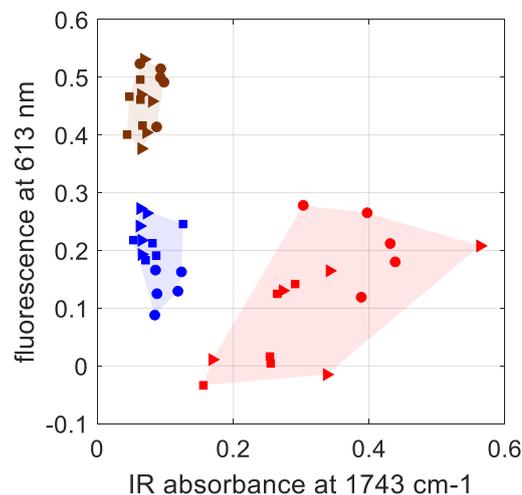


Fig. 3. Multispectral probe measurements of chicken fat (red), breast (blue) and liver (brown) presented in the coordinate plane of two single-variable intensities of normalized spectra presented in Fig. 2.

The main focus of the proposed technology is on analyzing non-homogeneous solid samples, because it enables simultaneous measurements at the same point on the sample surface. The proven ability of combined fluorimetry and ATR IR spectroscopy is expected to have an impact on the oncological diagnostics. The developed concept of combining infrared and fluorescence spectroscopy provides a viable solution for the measurement interface in a multisensor system of this kind. The potential of limiting the analysis to only two relevant information channels is illustrated in Fig. 3 (full separation of different types of the chicken tissue was achieved on a plane of two spectral variables).

Multispectral probes of this family are highly practicable and can be indispensable for the local analysis of complex heterogeneous samples, such as biological tissue, when combining spectroscopic techniques results in a synergic effect. The developed probe provides a well suited measurement interface for low-cost multisensor systems optimized for various particular applications as an alternative to the universal lab spectroscopy.