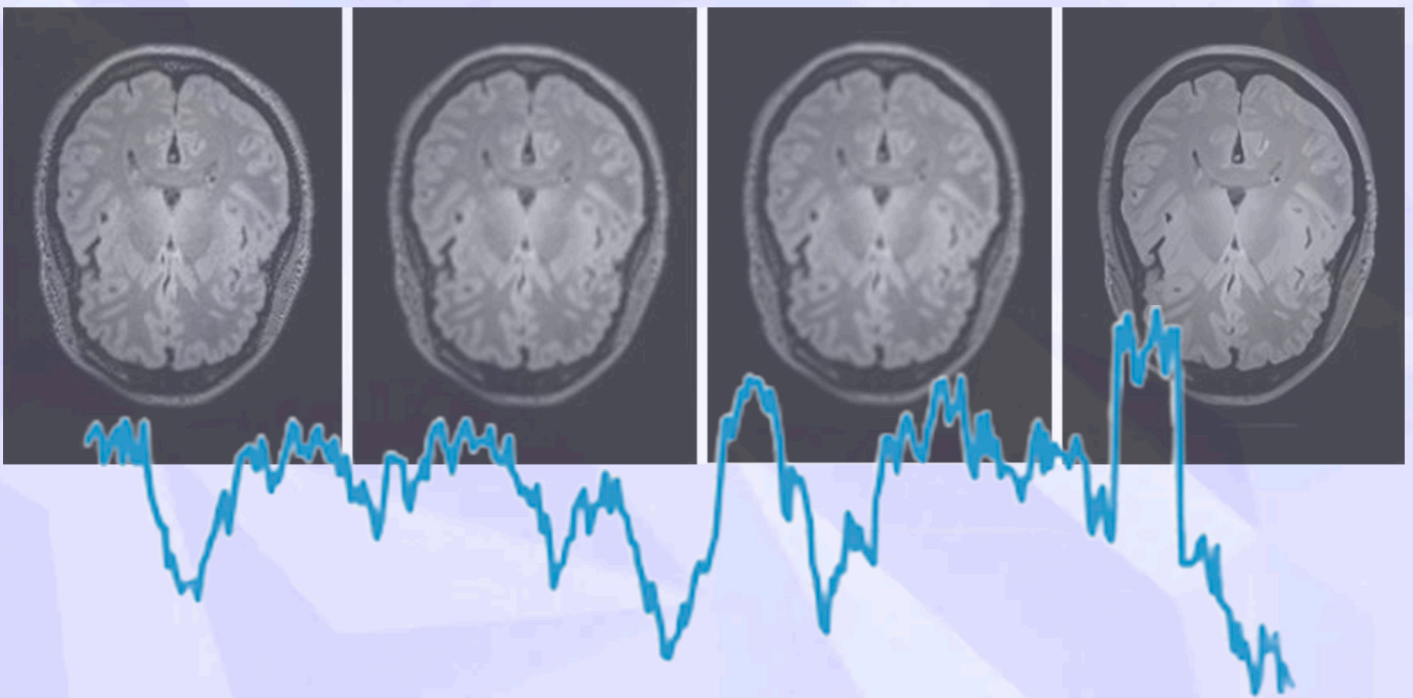


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Hyperspectral Image Segmentation and Clustering for Intraoperative Diagnosis of Intestinal Ischemia

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Abstract. We present an intraoperative hyperspectral imaging (HSI) approach for the assessment of intestinal ischemia, integrating deep learning-based segmentation with unsupervised spectral clustering. Sixteen patients were enrolled, of whom hyperspectral datasets from three representative cases were analyzed in detail. The developed convolutional neural network (1D-CNN) demonstrated high classification performance between intestinal tissue and background, enabling consistent delineation of clinically relevant regions. For ischemia assessment, oxygen saturation was estimated using a two-wavelength method, and principal component analysis combined with Gaussian mixture modeling (PCA-GMM) was applied to spectral features. The clustering revealed progressive separation of intact, moderately ischemic, and severely ischemic tissue, consistent with intraoperative surgical observations. This pipeline enables pixel-wise visualization of ischemic alterations with quantitative metrics, providing surgeons with objective and spatially resolved information to support resection decisions. The proposed framework demonstrates feasibility in clinical practice and establishes a foundation for large-scale validation and integration into intraoperative decision-support systems.

Keywords: hyperspectral imaging; intestinal ischemia; spectral clustering; convolutional neural network; tissue oxygenation; intraoperative diagnostics.

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1 Introduction

Intestinal ischemia is a life-threatening surgical condition characterized by impaired blood supply to the intestinal wall, leading to necrosis and severe clinical complications if timely treatment is not provided [1]. In modern abdominal surgery, the most common method for intraoperative assessment of intestinal viability remains visual inspection and palpation, including such indicators as the color of the serosal surface, the presence and intensity of peristalsis, pulsation of marginal mesenteric vessels, and the degree of their blood filling [2, 3]. However, these criteria are largely subjective and depend on the surgeon's experience, which may result in variability of interpretation and, consequently, an

increased risk of suboptimal decisions regarding the extent of resection or the preservation of potentially ischemic tissue [4].

In this regard, there is a need in clinical practice for objective and quantitative methods that allow intraoperative evaluation of the intestinal wall condition and detection of ischemic changes. Several optical approaches are already in use or are being actively developed for this purpose. The most widely applied technique is indocyanine green (ICG) fluorescence angiography, which provides real-time visualization of blood flow but requires administration of a contrast agent and is limited in penetration depth [5–7]. Diffuse reflectance spectroscopy (DRS) makes it possible to record spectral characteristics of reflected light and

extract information on hemoglobin concentration and tissue oxygenation levels [8–10]. Laser speckle contrast imaging (LSCI) is used to assess microcirculation by analyzing the dynamics of speckle patterns [11, 12]. In addition, optical coherence tomography (OCT) and photoacoustic imaging are actively investigated as promising techniques for quantitative characterization of tissue status [13–16]. All these methods rely on the interaction of light with tissue to provide objective information about blood supply and oxygenation; however, they have limitations related to spatial resolution, probing depth, or the need for contrast agents.

Against this background, hyperspectral imaging (HSI) appears to be a particularly promising technology, as it combines spectral and spatial resolution, allowing analysis of reflected light across a wide wavelength range at each pixel of the image [17, 18]. This capability makes it possible to construct tissue oxygenation maps and detect ischemic changes at early stages. A number of studies have already demonstrated the feasibility of HSI for assessing intestinal viability, including in combination with machine learning methods for automatic classification and refinement of resection margins [19, 20]. However, classification models should be complemented with quantitative physiological parameters such as tissue oxygen saturation, which can be calculated from hyperspectral data and can improve the reliability of ischemia assessment [21].

2 Materials and Methods

2.1 Hyperspectral Imaging Method

For this study, a custom-developed hyperspectral imaging system was used, consisting of a broadband light source (OSL2, Thorlabs, USA) coupled to a fiber-optic ring illuminator (FRI61F50, Thorlabs, USA) and a hyperspectral camera (Specim, Spectral Imaging Ltd., Finland). The camera operated in the spectral range of 400–1000 nm with a spectral resolution of approximately 7 nm, providing 204 contiguous spectral channels and a spatial resolution of 512×512 pixels. Illumination was provided by a 50 W halogen lamp coupled into the ring light guide to ensure uniform illumination of the surgical field. The average irradiance at the tissue surface was 4 ± 0.5 mW/cm². The system was mounted on a medical stand with a reinforced articulated arm, which allowed flexible positioning of the camera above the surgical area.

During clinical procedures, hyperspectral images were recorded intraoperatively with an exposure time of 150 ms per frame. According to the protocol, hyperspectral data were obtained from 16 patients with acute abdominal pathology complicated by intestinal ischemia. In total, 16 hyperspectral cubes were collected, of which 13 were used for algorithm training, while 3 representative cases were selected for detailed demonstration of the developed approach. For calibration, a diffuse reflectance standard (fluoroplast F4 material) was applied, and all hyperspectral data were normalized to this reference using the standard formula. Prior to each measurement, the reference standard was sterilized and the optical components of the

system were cleaned with alcohol wipes to comply with surgical asepsis requirements.

All clinical procedures were carried out at Orel Regional Clinical Hospital and were approved by the Ethics Committee of Orel State University (Protocol No. 2, dated September 18, 2023).

2.2 Case Report

In total, hyperspectral datasets were collected from 16 patients. For detailed illustration of the proposed pipeline, we present three representative clinical cases with different types of intestinal wall lesions. Patient 1 was a 73-year-old man diagnosed with perforated ulcer complicated by peritonitis. Patient 2 was a 66-year-old woman diagnosed with adhesive intestinal obstruction. Patient 3 was a 57-year-old man with a diagnosis of strangulated hernia. Hyperspectral arrays were recorded using the developed system for each of these patients.

3 Developed Algorithm

3.1 Data Preprocessing

Preliminary hyperspectral data processing involved several sequential steps. Initially, raw data cubes were corrected for the dark current signal by subtracting the corresponding dark reference data for both the measured and calibration cubes. Subsequently, normalization was performed relative to the diffuse reflectance standard by dividing the dark-corrected measured data by the dark-corrected calibration data. To reduce spectral noise and smooth the obtained spectra, a Savitzky-Golay filter was applied along the spectral dimension for each pixel.

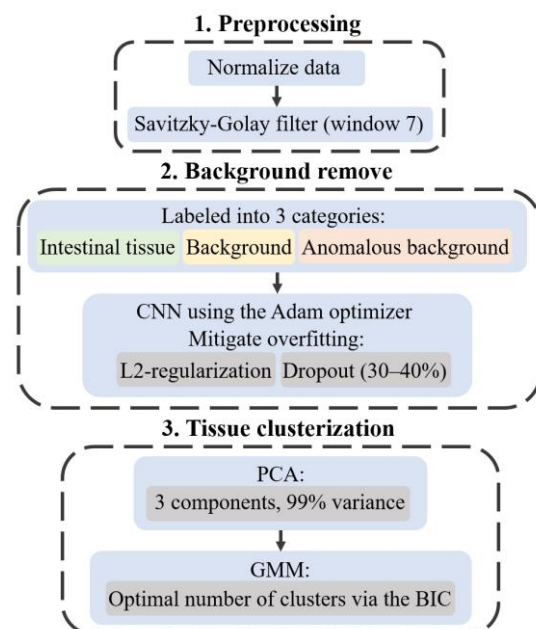


Fig. 1 Schematic representation of the data processing algorithm. The workflow includes spectral preprocessing, removal of background using CNN-based classification, and tissue clustering with PCA and GMM.

The filtered data cubes were then reshaped into a two-dimensional array suitable for subsequent machine learning-based analysis. The overall pipeline is illustrated schematically in Fig. 1.

3.2 Tissue Segmentation Using Convolutional Neural Network

Automated analysis of intraoperative hyperspectral images is complicated by multiple background elements such as surgical instruments, gauze, illumination probes, and adjacent skin, which must be differentiated from the intestinal tissues of interest. Due to the spectral complexity of these background components, conventional spectral peak analysis is insufficient. To address this, a one-dimensional convolutional neural network was designed and trained to classify individual pixel spectra independently, disregarding spatial context.

Hyperspectral cubes were manually annotated into three categories: intestinal tissue, background, and anomalous background (areas lacking proper reflectance normalization). Each pixel was represented as a one-dimensional spectral vector, yielding approximately 1.3 million spectra for training. The CNN architecture consisted of sequential convolutional layers for feature extraction, followed by fully connected layers. A sigmoid activation function was used in the output layer for binary classification. Regularization strategies included L2-penalty, dropout (30–40%), and batch normalization. Training was performed with the Adam optimizer and binary cross-entropy loss. Class imbalance was mitigated using class weighting. Early stopping and learning-rate reduction were applied to prevent overfitting.

3.3 Tissue Oxygen Saturation Estimation

Tissue oxygen saturation was estimated pixel-wise using a two-wavelength approach in the near-infrared range (750 and 795 nm), exploiting the distinct absorption of oxygenated and deoxygenated hemoglobin [22]. The algorithm was applied to intestinal regions identified by CNN-based segmentation, ensuring that background and non-relevant tissue were excluded from analysis. This approach provided spatial maps of relative oxygen saturation across the intestinal wall.

3.4 Unsupervised Spectral Analysis Using PCA-GMM

To investigate ischemic alterations without relying on histological validation or predefined labeling, an unsupervised approach was employed. Spectral data were first transformed using PCA, reducing dimensionality while retaining 99% of the variance. GMM was then applied within the PCA feature space, with the optimal number of clusters determined using the Bayesian Information Criterion (BIC).

Each hyperspectral dataset was clustered individually within the shared PCA coordinate system, ensuring comparability across cases. This framework enabled grouping of intestinal tissue spectra according to their optical characteristics.

4 Results and Discussion

The proposed approach was validated on representative intraoperative hyperspectral datasets obtained from patients with different forms and severities of intestinal pathology. The processing pipeline (Fig. 1) included CNN-based segmentation of intestinal tissue, oxygen saturation mapping, and unsupervised clustering of spectral features using PCA and GMM. Below, we present quantitative and qualitative results for each component of the pipeline and discuss their clinical interpretation.

4.1 Performance of CNN Segmentation

The one-dimensional convolutional neural network, trained on a large set of pixel spectra, demonstrated high performance in distinguishing intestinal tissue from background. On independent hyperspectral cubes not used in training, the model achieved an overall accuracy of 0.996, with precision and recall values above 0.98 and an F1-score of 0.99. These results ensured consistent identification of clinically relevant intestinal regions while reliably excluding surgical instruments, gauze, and adjacent skin. Such robust segmentation provided a stable foundation for subsequent oxygen saturation analysis and spectral clustering.

4.2 Tissue Oxygen Saturation Maps

Following segmentation, tissue oxygen saturation was estimated using the two-wavelength method (750 and 795 nm). Representative intraoperative images and corresponding saturation maps are shown in Fig. 2.

Areas of reduced oxygen saturation (blue-green regions) consistently coincided with tissue zones later assessed as ischemically compromised, whereas intact regions exhibited higher values (orange-red). This pixel-wise mapping enabled detection of localized hypoxic areas not always evident upon visual inspection and provided complementary physiological information to the surgeon's intraoperative assessment.

4.3 Spectral Clustering with PCA-GMM

Spectral vectors of the segmented intestinal tissue were projected into a PCA space retaining 99% of the original variance. GMM was then applied to identify distinct clusters, with the optimal number of components determined using the Bayesian Information Criterion. Representative results are presented in Fig. 3, where cluster maps illustrate the differentiation of intestinal tissue states. The green clusters corresponded to spectrally stable, intact tissue; the yellow clusters reflected intermediate alterations; and the red clusters indicated severe ischemic changes. Importantly, regions classified into the yellow cluster also showed reduced oxygen saturation compared to surrounding green areas, demonstrating consistency between clustering results and physiological oxygenation estimates. In the PCA feature space, clusters were clearly separated, with no overlap, confirming the robustness of spectral differentiation.

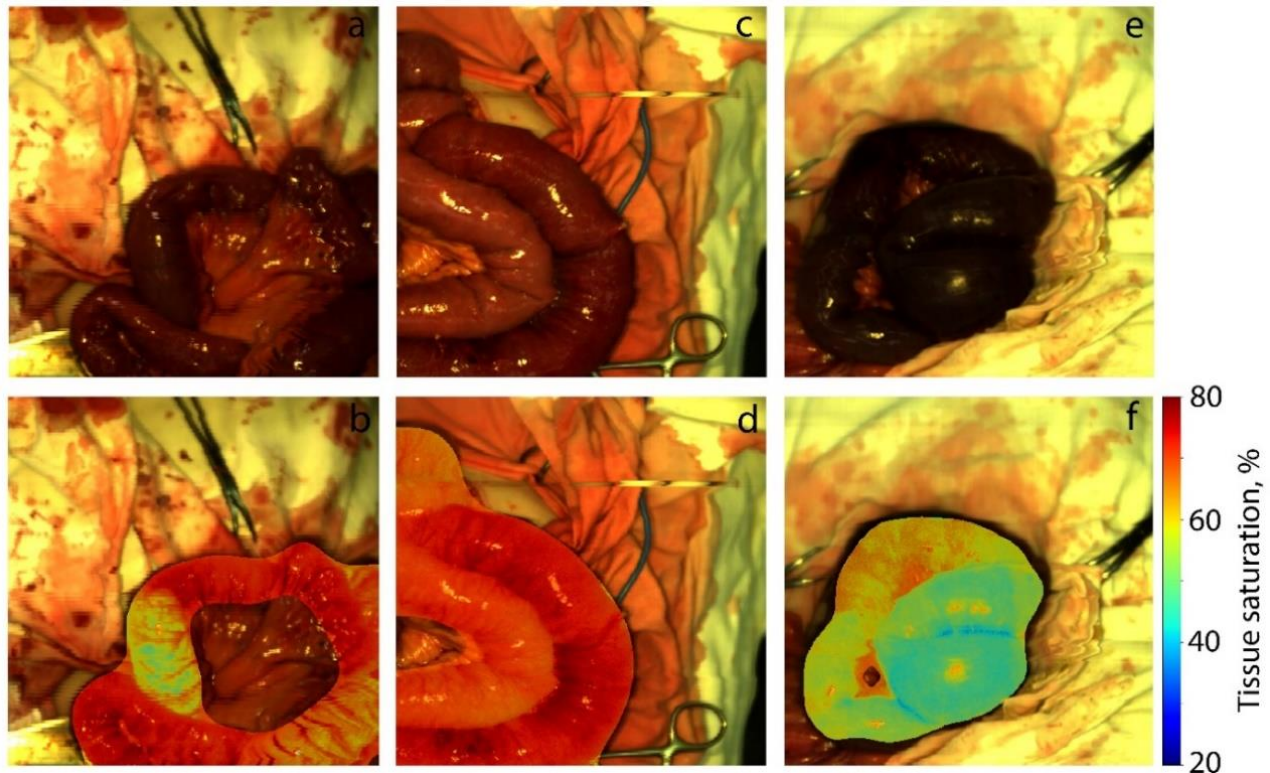


Fig. 2 Intraoperative hyperspectral imaging of the small intestine. (a, c, e) Original images recorded during surgery; (b, d, f) corresponding tissue oxygen saturation maps calculated from hyperspectral data.

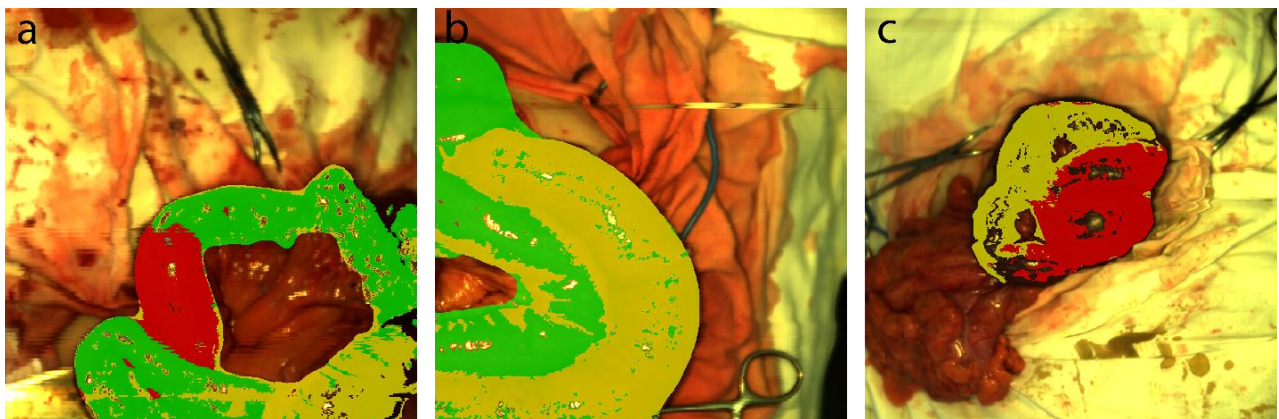


Fig. 3 Classification of intestinal tissue based on hyperspectral data. Pixel-wise cluster maps obtained using the GMM are shown for three representative cases: (a) perforated ulcer, (b) adhesive obstruction, (c) strangulated hernia. Clusters occupied distinct regions in PCA space without overlap, confirming spectral separation of tissue states.

Quantitative analysis supported these findings:

- Case (a), perforated ulcer: average saturation values were $74.0 \pm 2.1\%$ in green (intact) tissue, $68.6 \pm 3.6\%$ in yellow regions, and $61.9 \pm 5.4\%$ in red ischemic areas. These findings matched the surgeon's intraoperative assessment, where progressive ischemia surrounded the ulcerative defect.
- Case (b), adhesive obstruction: green clusters showed $70.0 \pm 2.3\%$, while yellow regions reached $74.1 \pm 2.3\%$. The paradoxically higher oxygenation in altered tissue reflected post-reperfusion measurement,

when blood flow was partially restored, leading to transient hyperoxygenation.

- Case (c), strangulated hernia: yellow clusters averaged $59.2 \pm 3.2\%$, while red clusters decreased to $49.2 \pm 3.4\%$, indicating severe oxygen depletion. The clustering successfully separated the area with pronounced ischemic alterations at the hernia site from adjacent areas of progressive ischemia.

Taken together, these results demonstrate that the combined spectral-physiological analysis not only distinguished intact from ischemically altered tissue but also reflected clinically observed differences in ischemic progression across patients.

5 Conclusions

This study demonstrated the feasibility of intraoperative hyperspectral imaging for the evaluation of intestinal ischemia by integrating three analytical components: CNN-based segmentation, pixel-wise oxygen saturation mapping, and unsupervised clustering of spectral features with PCA-GMM. The combined pipeline allowed both anatomical delineation of the intestine and functional assessment of its viability.

The convolutional neural network consistently separated intestinal tissue from complex surgical backgrounds, forming a reliable basis for further analysis. Oxygen saturation maps derived from hyperspectral data highlighted localized hypoxic regions not always visible to the naked eye, providing surgeons with complementary physiological insight. Spectral clustering revealed distinct groups of tissue states – ranging from intact to progressively ischemic and severely altered – that correlated with intraoperative

observations and demonstrated meaningful differences in tissue oxygenation levels. By combining anatomical, physiological, and spectral information, the proposed approach revealed a continuous transition from viable to severely altered tissue, reflecting the progressive nature of ischemic injury. This integrative framework can be applied to new clinical data, offering a pathway toward scalable validation, standardization of spectral features, and eventual incorporation into intraoperative decision-support systems for improved surgical outcomes.

Disclosures

The authors declare no conflicts of interest in this paper.

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Phase Retardation for Dehydrated Human Tears Fluid Samples in Diabetes Mellitus

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Abstract. New analytical capabilities are reported for the V. N. Shabalin – S. N. Shatokhina wedge-shaped dehydration method in the study of biochemical mechanisms of eye diseases development under diabetes mellitus, based on the value of phase retardation of dehydrated samples of human tear fluid. The phase retardation was measured for three sample groups of patients: examined and recognized as healthy patients, patients with undetermined diagnosis of diabetes mellitus, patients with confirmed diagnosis of type 2 diabetes mellitus. The proposed new methodological approach enriches the known morphological methods for the analysis of dehydrated samples of various biological fluids with quantitative parameters over the entire sample area. The approach might prove useful in the research practice of medical and biological fields.

Keywords: wedge-shaped dehydration; tear fluid; phase retardation; phase distribution visualization.

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1 Introduction

An increasing use of biological fluids such as mixed saliva, blood, or tear fluid is observed in recent research to identify significant biomarkers of various diseases. Blood and mixed saliva are required both for the diagnosis of non-communicable cardiovascular or oncological diseases, and for the diagnosis of infectious diseases, such as COVID-19 [1–3]. In the 2000s, researchers discovered that tear fluid also carries information about eye diseases [4]. Tear fluid, being the secret of the lacrimal gland and located directly on the eyeball surface, plays an important role in maintaining its healthy condition and functioning [5]. As for the classification of tears, they are divided into basal, reflex and mental tears. The basal tears role is to constantly keep the cornea moist [6]. Reflex tears occur when foreign particles and substances (onions, perfumes, pepper spray, tear gas) irritate the eyes [7]. Mental tears are caused by emotional stress, joy, anger, physical pain, etc. [8]. Under normal conditions, the volume of tear fluid for a healthy person averages about 5–10 μl , while the level of secretion is about 1.2 $\mu\text{l}/\text{min}$ [9].

A great number of methods for the biological fluids and tissues analyses are known [10–13]. These include high performance liquid chromatography (HPLC), ultra high-performance liquid chromatography (UPLC), electrophoresis (SDS-PAGE, CE), enzyme immunoassay (ELISA), mass spectrometry, light scattering and morphology of biological fluids [14]. For the first time in an extensive medical research practice, a comprehensive analysis of the crystalline structures of biological fluids in polarized light was proposed in the 1990s by S. N. Shatokhina in her works on structuring during dehydration and further morphological analysis of the obtained samples of biological fluids [15].

In paper [16] by S.V. Zenin, the tear fluid is viewed and analyzed. The tear fluid represents a complex multicomponent colloidal system that forms specific patterns while being crystallized upon drying. These patterns depend on the tear biochemical composition, surface tension and external environmental parameters. The tear fluid consists of 98 percent water, and the remaining 2 percent includes proteins, lipids, electrolytes, and mucins [17]. In this work, to describe

and analyze the tear fluid, we studied its dehydrated structures. We used wedge-shaped dehydration. Unlike non-wedge-shaped dehydration, this process is controlled and forms specific zones.

The dehydration process proper can be divided into several stages, conventionally distinguished are three of them: a) evaporation of water, b) crystal nucleation, c) crystal structures growth. Figure 1 illustrates the process of the tear fluid dehydration during the formation of different types of its structure [18]. The first stage is water evaporation. The evaporation intensity depends on humidity, temperature, and film thickness. In the course of the crystal nucleation, NaCl ions form primary centers of crystallization, and proteins and mucins act as an anisotropic seed, thereby affecting the shape of crystals. With the crystal structures growth, certain patterns are emerging. Each such pattern is a characteristic type of the structure organization. There are 3 of these types: crystalline, amorphous-crystalline and clumpy-crystalline clumpy-crystalline (Fig. 1) [18]. The 4th column in the Fig. 1 shows images of crystallized tear fluid for each type of organization in polarized light.

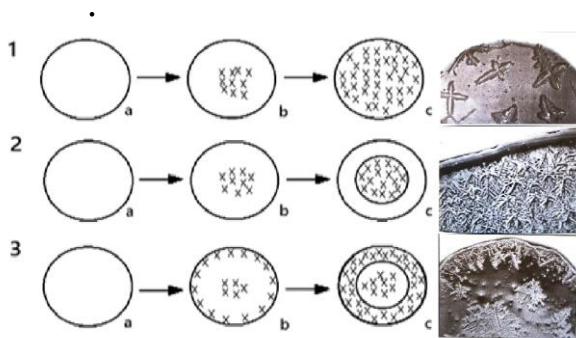


Fig. 1 Types of the tear fluid structure organization: 1 – crystalline, 2 – amorphous-crystalline, 3 – clumpy-crystalline. Explanations of panels (a–c) are given below in the text. The 4th column shows images of crystallized tear fluid for each type of organization in polarized light.

A qualitative analysis of the dehydrated structures of biological fluids proved a high informative value of the detected markers of such common pathological conditions as hypoxia, hyperglycemia, stagnation in tissues, intoxication, ischemia, sclerosis, etc., which form a “mosaic” of typical pathophysiological processes [15]. The dehydrated structures of most biological fluids are polarization-inhomogeneous media. In such structures, the phase retardation value of the light wave and the state of polarization ellipticity change when light passes through them. Analyzing the sample pattern in a polarizing interferometer (in a scheme with crossed polarizers), it is possible to draw conclusions about certain diseases, such as blepharitis, dry eye syndrome, inflammatory and dystrophic eye diseases, including diabetic retinopathy, etc., based on the type of such structures [15, 18]. The phase retardation value is determined by the thickness of the medium and the refractive index for the area under study. The magnitude

of the phase retardation can also be different in various parts of the sample and for different diseases.

Morphological studies of biological fluids are mainly a qualitative analysis. By supplementing it with the quantitative measurements results we can expand the analytical resource of the wedge-shaped dehydration method. The purpose of our work was to visualize and measure the phase retardation of dehydrated tear fluid samples for patients without a diagnosis of diabetes mellitus and for patients with the confirmed diagnosis of type 2 diabetes mellitus and to carry out its statistical analysis.

2 Object, Material, and Methods

Dehydrated tear fluid samples achieved by the V. N. Shabalin-S. N. Shatokhina method of wedge-shaped dehydration [15] are glass substrates with tear fluid applied onto them. Reflex tear fluid caused by the mechanical stimulation was noninvasively obtained at the T. I. Eroshevsky Ophthalmoeocrinological Consultative and Diagnostic Division of the Samara Regional Clinical Ophthalmological Hospital. These samples were then dehydrated at a constant room temperature until their complete drying. During the dehydration process, the salt components are drawn together towards the center by surface tension forces, while the protein formations remain at the sample edges. Fig. 2 shows a typical structure of the dehydrated tear fluid sample. In the micrograph, the numbers mark the sample zones. Below is the central (salt) zone, filled with numerous fern-like crystalline structures, and the marginal (protein) zone, which is amorphous. Our measurements were carried out in the shown areas of the obtained samples.

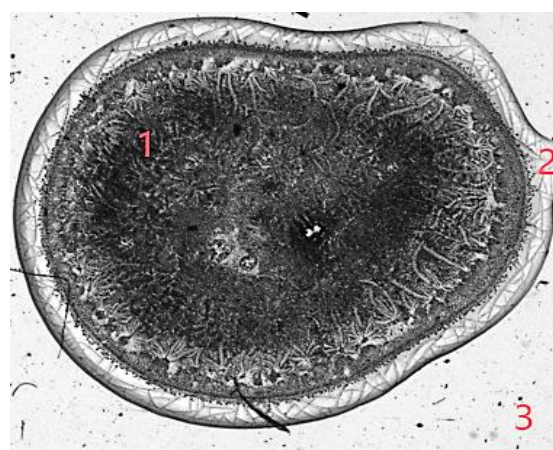


Fig. 2 The typical structure of the dehydrated tear fluid sample in polarized light. The following areas marked with numbers are shown: 1 – central zone (salt structure), 2 – marginal zone (protein substrate), 3 – slide.

Phase retardation visualizer (THORLABS LCC7201B) was used for the measurements. It provides measurement of the phase retardation in the 0 to π range for a wavelength of 633 nm, while the accuracy level of 9.93×10^{-6} rad is ensured. The accuracy of the device is

determined by the interferometric method with the aid of the phase retardation. It consists in the following: a liquid crystal light modulator capable of setting various phase retardations between the arms of the interferometer, is fixed on one of the arms. Thus, the increase in the measurement accuracy during the interference patterns processing is achieved. During the measurement process, about 103 thin dried structures were examined, and phase retardation values ranged from 0.01 nm to 30 nm. Therefore, it can be said that the phase shift visualizer can detect even thin and complex structures.

To measure the phase retardation of the dehydrated tear fluid samples [12], the five-point method was used, often applied in evidence-based medicine. This method consists in the selected parameters measurement at 5 equidistant points (4 points are in the sample marginal (protein) zone and 1 point is located in the central (salt) zone). Figure 3 illustrates the application of the five-point method. The described method is recognized for such tasks, as the marginal (protein) area is homogeneous. However, we also tested this method, calculated errors and deviations (at the stage of calculating non-parametric criteria). We also compared the average value of the obtained values using the 5-point method with the value obtained by averaging the phase delay over the entire boundary area (more than 20 points). We obtained relatively similar results for one sample.

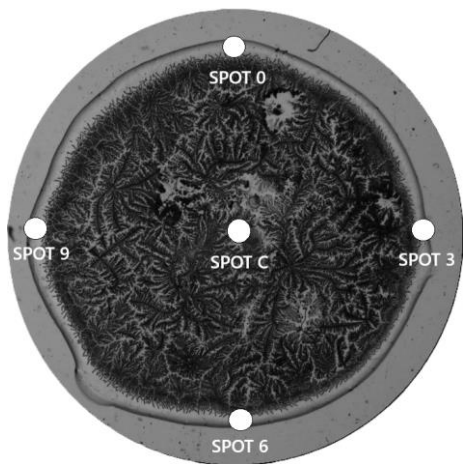


Fig. 3 Estimation of the phase retardation at 5 equidistant points on the surface of a dehydrated tear fluid sample. Polarization microscopy.

In total, 103 samples were measured (10 samples from the healthy patients group (Reference Group), 68 samples from a group of patients without a diagnosis of diabetes mellitus but with combined dystrophic pathology of the visual organ, i.e. the initial stage of glaucoma, mild myopia and incomplete complicated cataract (Group 1), and 25 samples – from a group of patients diagnosed with type 2 diabetes mellitus with the nonproliferative stage of diabetic retinopathy in combination with dystrophic pathology of the visual organ i.e. glaucoma in its initial stage, mild myopia and incomplete complicated cataract (Group 2), all the measurements were entered in the electronic database.

Statistical data processing in this work was carried out with the subsequent calculation of nonparametric criteria so that to evaluate the reliability of differences between the groups. For each measurement point the median (Me, second quartile), the 25th and 75th percentiles (first and third quartiles), maximum (Max) and minimum (Min) values were found.

There is a lot of nonparametric criteria for comparing two independent samples, whereas a special place belongs to the criteria most frequently applied in biomedical data statistics [4, 19–21], they are the Mann-Whitney *U*-test, the Kolmogorov-Smirnov test and the Wald-Wolfowitz test.

3 Results and Discussion

Basing on the data obtained, a bar graph shown in Fig. 4 was built. It shows the median values for the phase retardation in nanometers (for a wavelength of 633 nm, the phase retardation of π corresponds to 316 nm) for 5 points of three different groups (Reference group, Group 1, Group 2). The groups are marked with different colors, points 0, 3, 6, 9 are located in the sample marginal (protein) area, and the point c is in the central (salt) zone. It can be seen from the presented data, that the phase retardation for Group 2 exceeds this value for the Reference Group more than by 1.5 times. Statistically reliable differences between Group 1 and the Reference Group for this parameter are observed at point C.

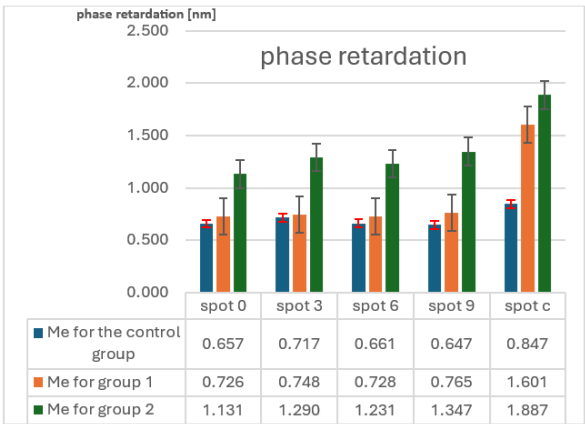


Fig. 4 The value of the phase retardation distribution depending on the measurement point for the three groups of the examined patients.

As a result of the preparatory stage of statistical processing, we did not find a normal distribution for any of the parameters studied. This circumstance determined our subsequent use of only nonparametric tests.

To verify the validity of differences in phase retardation between the groups of patient’s nonparametric criteria were used, such as the Mann-Whitney *U*-test, the Kolmogorov-Smirnov test, and the Wald-Wolfowitz test. These criteria were calculated for different pairs of samples: Reference Group – Group 2 and Reference Group joint with Group 1 – Group 2.

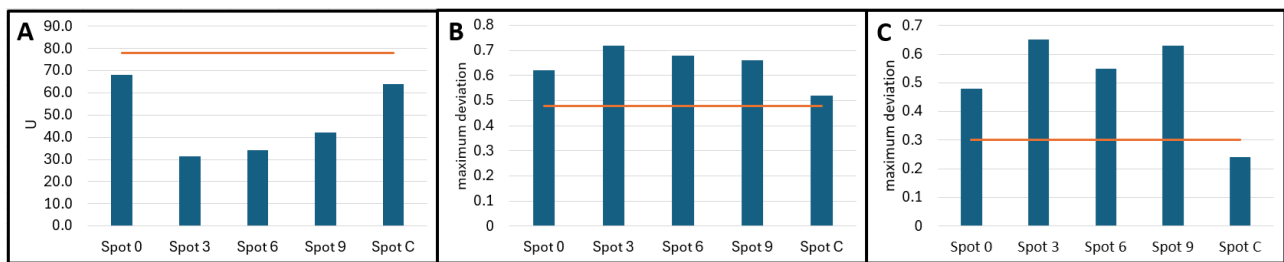


Fig. 5 Graphical representation of the statistical criteria values: (A) the Mann-Whitney U -test, (B) the Kolmogorov-Smirnov test for the samples of Reference group and Group 2; (C) the Kolmogorov-Smirnov test for the samples of Reference Group joint with Group 1 and Group 2. The red line on the charts represents the critical value.

For illustration purposes, some nonparametric criteria are presented in the form of bar graphs (Fig. 5). From diagram A, corresponding to the Mann-Whitney U -test, it can be seen that the differences are statistically significant, since the value of the phase retardation for each point does not exceed the critical value. In the following two bar graphs, according to the Kolmogorov-Smirnov test, the situation is reversed, the differences are statistically significant if the value obtained is higher than the critical one. In accordance with the Wald-Wolfowitz test, the statistical significance of the differences is also confirmed.

The results of these criteria calculation prove that the differences in phase retardation between the samples of groups of the examined patients are statistically reliable.

4 Conclusion

In the course of the work fulfilled, the distributions of the phase retardation of the tear fluid dehydrated structures were studied for three types of samples: from a group of healthy patients (Reference Group), a group of patients without a diagnosis of diabetes mellitus but with combined dystrophic pathology of the visual organ (Group 1) and a group of patients with a confirmed diagnosis of type 2 diabetes mellitus with diabetic retinopathy in the nonproliferative stage in combination with dystrophic pathology of the visual organ (Group 2).

The phase retardation was measured for 103 studied samples, and an electronic database was formed based on

these measurements. It can be seen from the presented data, that the phase retardation for Group 2 exceeds this value for the Reference Group more than by 1.5 times. All the results obtained were statistically processed by 3 different non-parametric criteria (the Mann-Whitney U -test, the Kolmogorov-Smirnov test, the Wald-Wolfowitz test), confirming that the phase retardation is an informative value.

Thus, the applied methodological approach based on the phase retardation measurements, can take its rightful place in research practice for analyzing the optical characteristics of biological objects of this type, which is proved by statistically significant differences between groups of examined healthy patients and those with various eye diseases.

It is worth noting that this study does not violate ethical policy. All tear fluid samples were obtained at the Eroshevsky Ophthalmological Clinic, where each patient signs an informed voluntary consent to medical intervention (order of the Ministry of Health of the Russian Federation dated November 12, 2021, number 1051n).

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Disclosures

The authors declare no conflicts of interest in this paper.

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Modeling of PpIX Fluorescence of a Two Layered Medium: A Monte-Carlo Study

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Abstract. An advancement of an exponential model of protoporphyrin IX (PpIX) fluorescence of two-layered media has been accomplished to mimic healthy and tumor brain tissues. The optical properties of these layers were selected to mimic meningioma as a tumor tissue and white matter as a healthy tissue at 405 nm excitation and 635 nm emission wavelengths. The media were considered to be homogeneous and the mismatched boundary was not taken into account. Then, the refractive index of these two layers was the same and equal to 1.4. The thickness of the tumor layer turned out to be varied between 0.1 μm and 0.6 cm, and 10 cm thick of the lower normal layer (infinity). Fluorescence was estimated theoretically based on the Monte-Carlo algorithm and fitted to the developed model. Finally, the relative error of the presented model was less than 5%. The simplicity and robustness of the presented model make it a good choice to estimate fluorescence and reproduce the thickness of the upper layer in real-time.

Keywords: Fluorescence; meningioma; Monte Carlo method; PpIX.

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1 Introduction

Meningioma is a central nervous system tumor that means it begins in the brain and spinal cord, which is classified into three grades (grade I, II & III). The most common type of meningioma is low-grade meningioma, which is characterized by slow growth and can be totally resected without recurrence. Predominantly, meningioma is a benign tumor that accounts for approximately 20% overall of the intracranial tumors of humans [1–3]. The widely spread approach used to cure meningioma, regardless the grade, is surgery during which a biopsy can be taken to determine which grade of the tumor is [1–3]. It is worth noting that the recovery rate depends mainly on the complete resection of the tumor, which requires a deep knowledge of the tumor structure. That could be achieved by using an assistant tool to determine the tumor's structural characteristics in real time during surgery [2–6].

On the other hand, laser-induced fluorescence is one of the promising modalities used to discriminate tumor margins in real-time, which depends on accumulation of endogenous and exogenous fluorophores in tumor tissue [2–4]. Protoporphyrin IX (PpIX) fluorescence spectroscopy, as an example, is currently utilized as a pointer for surgeons during surgery to distinguish brain

tumor margins. However, fluorescence spectra carry potential information of tumor characteristics if it is well interpreted due to the relationship between the spectrum and the optical and morphological tissue properties [2–4]. In that context, Richards-Kortum and co-authors developed an exponential model of fluorescence of semi-infinite medium and evaluated the robustness of the presented model for determining atherosclerosis, which exceeded 88% in distinguishing normal aorta [7]. Furthermore, a spatially resolved fluorescence model was developed for multi-layered media based on diffusion approximation [8]. Although the time of calculation could not be neglected, the validity of that model was examined, and the differences were found in a small radial distance, which approximated 2% [8]. An analytical model of fluorescence spectra of a two layered medium mimicked epithelial tissue was developed based on diffusion theory and Beer-Lambert law. The model used to detect the fluorescence spectra of the tumor with mean square error equaling 15% [9]. On the other hand, Yudovsky et al. developed a semi-empirical model based on the two-flux approximation of layered media with different fluorescent slab thicknesses. The optical properties of slabs were identical and matched human tissue optical properties (dermis, muscle) [10]. However, the

fluorescence calculated for transport albedo varied between 0.7 and 0.9 and the relative error enhanced 25% for small transport albedo [10]. An analytical model of fluorescence from a layered medium was developed that was based on diffusion theory to estimate fluorescent layer thickness. In spite of its simplicity and reproducibility to estimate optical properties, the relative error enhanced 30% in some cases of thickness varying between 0.1 and 2 mm [11]. To the best of our knowledge, there is no fluorescence model for two layered medium with distinct optical properties, the peripheral layer mimics meningioma.

The aim of the current work is to develop a simple exponential model of fluorescence for two-layered media mimicking the optical properties of tumor and normal brain tissue, respectively. For these purposes, a Monte Carlo simulation has been implemented to predict the fluorescence of this medium, and the dataset has been fitted to the developed exponential model.

2 Materials and Methods

2.1 Exponential Model

As known, the radiative transport equation (RTE) describes precisely light-tissue interaction in spite of its complexity. Thus, an analytical solution of RTE has been developed to simplify its solution, such as diffusion and two-flux approximations. Exponential approximation was widely used to simulate light propagation through biological tissues, and the validity was evaluated. In this work, there are some presumptions regards the studied case: 1) the optical properties of these two layers are distinct for excitation and emission wavelengths, 2) both layers are optically homogenous with matched refractive index, and 3) the light source is a pencil homogenous beam that incident perpendicularly to the surface. Then, the photon density of fluorescence can be regarded as the sum of two terms as follows [10]:

$$d\phi = d\phi_1 + d\phi_2. \quad (1)$$

Then, the first term determines the attenuation of the excitation and emission light through the first layer, which is attenuated exponentially upon passing a dz thickness. Furthermore, the emitted photon density is linearly proportional to absorbed intensity and quantum yield; then, it can be expressed as follows [7, 11]:

$$d\phi_1 = I_{absorbed} \cdot \varphi \cdot e^{-2(\mu'_{t(ex_1)} + \mu'_{t(em_1)}) \cdot z}, \quad (2)$$

where $I_{absorbed}$ is the absorbed excitation intensity, φ is the fluorescence quantum yield, $\mu'_{t(ex_1)}$ is the reduced extinction coefficient of the first layer at excitation wavelength and $\mu'_{t(em_1)}$ is the reduced extinction coefficient of the first layer at emission wavelength. In the second layer, the excitation and emission light is attenuated exponentially by absorption and scattering

events; then, the second term can be expressed as follows:

$$d\phi_2 = k \cdot e^{-2(\mu'_{t(ex_2)} + \mu'_{t(em_2)}) \cdot z}, \quad (3)$$

where $\mu'_{t(ex_2)}$ is the reduced extinction coefficient of the second layer at excitation wavelength, $\mu'_{t(em_2)}$ is the reduced extinction coefficient of the second layer at emission wavelength and k is a constant that represents the amount of light passing through the boundary between the two layers and propagating through the second layer. In order to calculate total photon density, an integration must be applied as follows [5, 6]:

$$\phi = \int_0^L I_{absorbed} \cdot \varphi \cdot e^{-2(\mu'_{t(ex_1)} + \mu'_{t(em_1)}) \cdot z} dz + \int_L^\infty k \cdot e^{-2(\mu'_{t(ex_2)} + \mu'_{t(em_2)}) \cdot z} dz. \quad (4)$$

Then:

$$\phi = \frac{I_{absorbed} \cdot \varphi}{2(\mu'_{t(ex_1)} + \mu'_{t(em_1)})} \cdot (1 - e^{-2(\mu'_{t(ex_1)} + \mu'_{t(em_1)}) \cdot L}) + \frac{k}{2(\mu'_{t(ex_2)} + \mu'_{t(em_2)})} e^{-2(\mu'_{t(ex_2)} + \mu'_{t(em_2)}) \cdot L}. \quad (5)$$

Although Eq. (5) is simple to use and able to reproduce to predict any variables involved, the difference between its results and the real measurements cannot be neglected; then, semi-empirical parameters can be proposed to reduce the relative error of this equation. Thus, the equation can be rewritten as Ref. [5, 6]:

$$\phi = \frac{k_1 \cdot I_{absorbed} \cdot \varphi}{2(\mu'_{t(ex_1)} + \mu'_{t(em_1)})} \cdot \left(1 - e^{-k_2(\mu'_{t(ex_1)} + \mu'_{t(em_1)}) \cdot L}\right) + \frac{k_3}{2(\mu'_{t(ex_2)} + \mu'_{t(em_2)})} e^{-k_4(\mu'_{t(ex_2)} + \mu'_{t(em_2)}) \cdot L}, \quad (6)$$

where k_i ($i = 1, 2, 3$, and 4) are the fitting parameters of the presented model which can be determined by fitting the equation to the standard results.

2.2 Monte Carlo Simulation

In the present work, the Monte-Carlo algorithm was used to solve the radiative transport equation (RTE), which can be considered as a gold standard [12]. A custom Monte-Carlo code (FLMC) was used to predict the emission photon density (fluorescence) of a two-layered medium [13]. The simulated fluorophore is PpIX that has an absorption peak at 405 nm and two emission peaks, the highest corresponds 635 nm. This type of fluorophore was widely used in vivo to point out tumor margins with high sensitivity and specificity [2–4]. Fluorescence quantum yield was 0.011 that corresponding to the

concentration 3.26 mg/L [14]. The peripheral layer mimics the optical properties of meningioma at 405 nm (excitation wavelength) and 635 nm (emission wavelength) of PpIX [2–4]. The lower layer was chosen to mimic the optical properties of normal brain tissue (white matter) at these wavelengths with a thickness enhanced to 10 cm (infinity), Table 1 [15]. The thickness of the upper layer turned out to be varied between 0.1 μ m and 6 mm. In the literature, the refractive index of both layers is not determined at excitation and emission wavelengths; then, the refractive indexes of both layers were supposed to be 1.4 [5, 6].

An infinitely thin pencil beam was implemented with 0.5 million photons that incident perpendicularly on the surface of the presented medium [12, 13]. FLMC code simulates the excitation light firstly to map the absorption of the excitation wavelength that is called the absorbed intensity matrix in which the absorbed intensity is depicted as a function of radial distance and depth (r, z) [13]. Then, the Monte-Carlo simulation is performed in second time to map light propagation of the fluorescence wavelength that depicted as a function of radial distance and depth, which is called the emission matrix. Finally, a custom FLMC code is supplied by a MATLAB interface to begin simulation and show the matrices of fluorescence and absorbed intensity [13, 14].

Table 1 Depicts the optical properties of both two layers at excitation and emission wavelengths [15].

Optical Properties	Meningioma		Normal Tissue	
	405 nm	635 nm	405 nm	635 nm
Absorption Coefficient (cm^{-1})	4.5	0.3	3	0.8
Scattering Coefficient (cm^{-1})	200	170	400	400
Anisotropy Factor (...)	0.86	0.95	0.75	0.85

3 Results

Depending on the FLMC code, the fluence of the absorbed and emitted light was predicted as a function of the peripheral layer thickness that mimics meningioma at both wavelengths 405 nm and 635 nm. Figure 1 (a) depicts the correlation of absorbed light as a function of the thickness of the upper layer. The total absorbed light increased dramatically as the thickness increased until the thickness approximated 0.05 and; then, the absorbed light increased slightly as the thickness increased until reaching the end of the studied thickness region. That is resulted from increasing of the number of fluorophores that absorb light until thickness exceeds the optical penetration depth at excitation wavelength that approximated 0.05 cm for meningioma. After reaching this distance, a saturation occurred and absorbed light increased slightly. On the other hand, the fluorescence of the surface decreased exponentially as the thickness of

the peripheral layer increased. That could be explained by a distribution of fluorescence light inside tissue and decreases at the surface, Fig. 1(b). Fluorescence light emitted from an embedded fluorophore could be absorbed or scattered inside tissue after reaching the surface. Furthermore, it is worth noting that the detected fluorescence at the surface is higher due to a contribution of the lower layer that has high scattering property. That can increase the detected fluorescence at the surface compared to a large thickness.

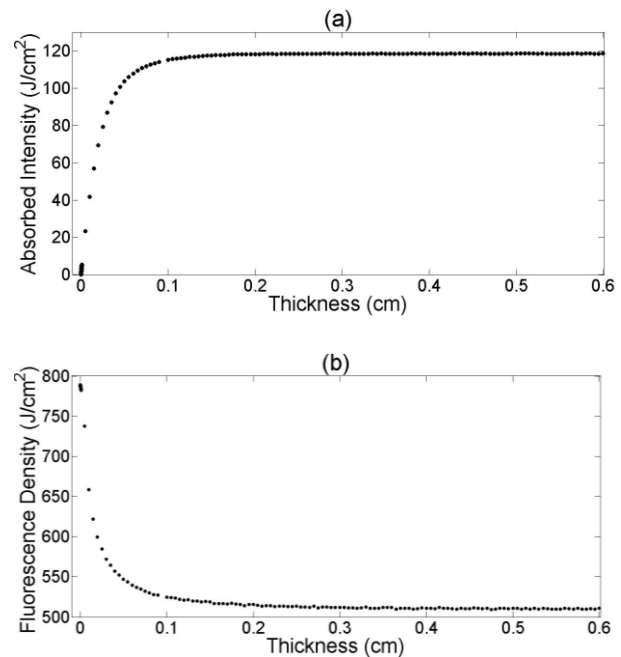


Fig. 1 (a) Depicts the correlation of absorbed light estimated by Monte-Carlo simulation as a function of thickness at 405 nm, (b) depicts the relationship of fluence of fluorescence light at surface to thickness of peripheral layer at 635 nm.

Using the fitting function procedure in MATLAB (Matlab 2015, Mathworks Inc, USA), the Monte-Carlo results were fitted to the exponential equation developed for two-layered homogenous media (Eq. (6)). The fitting parameters were predicted and depicted in the following Table 2.

Table 2 Depicts the fitting parameters of Eq. (6).

	$k_1(\dots)$	$k_2(\dots)$	$k_3(\text{J}/\text{cm}^2)$	$k_4(\dots)$
Fitting Coefficient	-17214.5	12.87802	21.42996	$3.91 \cdot 10^{-5}$

Then, the fluence of fluorescence light was estimated by the developed model (Eq. (6)) and the relative error predicted by the following Eq. (7):

$$\text{relative error} = \left(\frac{(F_{MC} - F_{predicted})}{F_{MC}} \right) \cdot 100\%. \quad (7)$$

Figure 2 (a) represents fluorescence fluence predicted by Eq. (6) as a function of upper layer thickness in comparison with the Monte-Carlo results. The convergence was obvious, and the relative error for our developed model was less than 5% for all situations. Large differences were observed for five thicknesses, and the relative error was less than 1% in general.

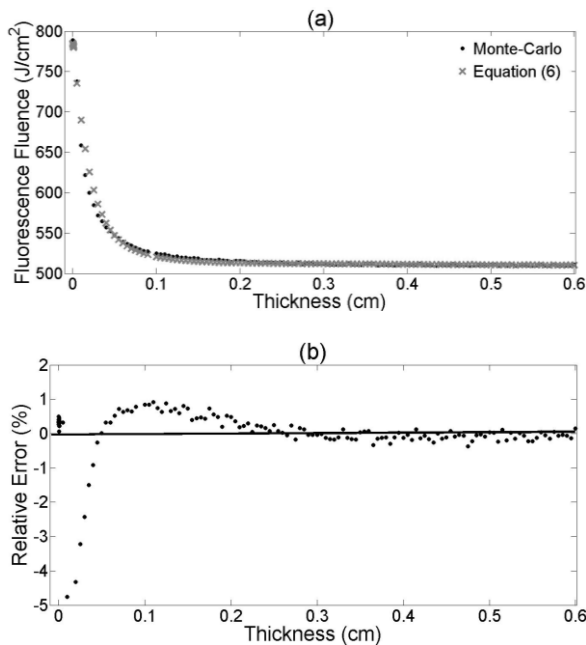


Fig. 2 (a) Comparing of fluence estimated by Monte-Carlo simulation (black) and Eq. (6) (grey) as a function of meningioma thickness, (b) relative error relationship to thickness.

4 Discussion

Fluorescence of two-layered media, which mimicked the optical properties of meningioma and healthy brain tissue, respectively, were estimated using Fluorescence-Layered Monte-Carlo simulation (FLMC) of PpIX as an endogenous fluorophore. That has an absorption peak corresponding to 405 nm and an emission peak at 635 nm [2–4]. Absorbed light was estimated and depicted as a function of thickness. It is noted that the absorbed light increased as the fluorescent layer thickness increased because of the increase in the number of fluorophores. After a specific thickness that is comparable to 0.05 cm, the absorbed light increased slightly due to exceeding the optical penetration depth of the first layer at the excitation wavelength, Fig. 1(a). The fluorescence of the presented medium decreased exponentially as the thickness increased, Fig. 1(b). It is supposed that the increase of thickness may increase the number of fluorophore, and, then, the fluence of fluorescence intensified. However, fluorescence distribution increases inside tissue as thickness increases, but the peripheral fluence that can be measured, decreases exponentially. Furthermore, we could not neglect the contribution of the lower layer that has a highly scattering property, which intensifies the

fluorescence at the surface of thin peripheral layer thickness. In that context, all these points explain why the total photon density of fluorescence at the surface decreased as the thickness increases. After a specific thickness, that is comparable to 0.3 cm, the fluence remains the same that could be explained by the optical penetration depth of meningioma at 635 nm that is equal to 0.3 cm.

Then, the developed model based on exponential decay was fitted to the Monte-Carlo results to estimate fitting coefficients that were proposed for scale invariance to reduce the divergence between both methods, Table 2. This model was sensitive to thickness variation, and the relative error, in comparison to Monte-Carlo, did not exceed 5%. However, the developed model's error was less than or equal to the error of other models developed by other research groups in spite of its simplicity [9, 10]. In that context, the relative error of the fluorescence model for a two-layered medium developed using diffusion theory was 15% [9]. Another semi-empirical model developed by the two-flux approximation has a relative error comparable to 30% [10]. Furthermore, the robustness of the presented model was comparable to an analytical model developed by solving the radiative transport equation using the Fourier transform and modified spherical harmonics for semi-infinite and multi-layered media with distinct optical properties [16]. Then, the small relative error of the presented model makes it more likely to predict the photon density that emitted from meningioma, which is supplied by PpIX and excited by a violet laser with an operating wavelength being 405nm. A reproduction of the thickness of meningioma can be made after measuring the emitted fluence of meningioma in real-time using the abovementioned equation (Eq. (6)).

5 Conclusion

In the present work, an exponential model of fluence emitted by a two-layered medium with a peripheral fluorescent layer was developed to mimic PpIX fluorescence of a two-layered medium with optical properties corresponding to meningioma, and normal tissue, respectively. Monte-Carlo simulation was used to generate a data set of our work, and the developed model was fitted to these results. Then, fitting parameters were predicted and used to generate fluorescence of the same medium with thickness varied between 0.1 μm and 0.6 cm. Since the relative error of the presented model did not exceed 5%, the model could be utilized to estimate PpIX fluorescence for known thickness and vice versa. Finally, the thickness of the peripheral layer (meningioma) can be reproduced upon measuring the fluence of the fluorescence signal. Furthermore, the simplicity of our model makes it more likely to help surgeons to determine the structural properties of the tumor in real-time.

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Disclosures

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A Hybrid Super-Resolution Method for Medical Images Using Transfer Learning

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Abstract. High-quality medical imaging is essential for accurate diagnosis and effective treatment planning. However, limitations such as noise, artifacts, high acquisition costs, and patient safety concerns often restrict the ability to obtain high-resolution (HR) scans. Super-resolution (SR) techniques offer a promising alternative by reconstructing HR images from low-resolution (LR) inputs, thereby enhancing image quality without additional scanning. This capability is particularly important in clinical contexts, where improving image resolution directly impacts diagnostic confidence and therapeutic decision-making. In this work, we propose a hybrid SR framework that integrates transfer learning with a generative adversarial network (RESRGAN) and low-rank total variation (LRTV) regularization. This combination leverages both local neighborhood features and global contextual information to recover fine structural details while preserving overall image fidelity. The approach was evaluated on three brain imaging modalities – magnetic resonance imaging (MRI), computed tomography (CT), and ultrasound – using volumetric datasets acquired at different time intervals. Quantitative experiments show that the proposed method consistently outperforms bicubic interpolation, achieving an average peak signal-to-noise ratio (PSNR) of 40.05 dB and a structural similarity index (SSIM) of 0.9808. Notably, ultrasound images exhibited the most substantial improvement. These findings highlight the potential of the proposed framework as a robust tool for enhancing medical image quality and supporting more reliable clinical decision-making.

Keywords: medical image processing; super-resolution; deep neural network; MRI; CT Scan; ultrasound.

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1 Introduction

In recent years, researchers in both image system manufacturing and software development have strived to create digital images with high quality and resolution. Medical images are particularly challenging due to their importance in image reconstruction and generation [1]. As doctors rely on images for patient diagnosis and treatment, the significance of this challenge will only continue to grow [2]. A thorough comprehension of medical image observation and review is essential for

informed decision-making and improved treatment of patients. Recent technological advancements have significantly increased the accuracy and quality of medical imaging systems. However, obtaining high-quality images in medical centers remains a persistent challenge due to factors such as noise, artifacts, and the high cost of modern equipment. Improving image quality using super-resolution methods has become a popular research topic among experts in the medical, industrial, and laboratory fields due to the necessity of each level of image processing, such as noise removal, segmentation,

registration, object recognition, etc. [3–7]. Most of these techniques have been applied to general digital images. As image quality increases, it is critical to preserve important medical information after translations. It is important to compare and review the current proposed methods, particularly for medical images. The super-resolution will be mainly divided into two categories:

- 1 – multi image super-resolution (MISR);
- 2 – single image super-resolution (SISR).

In the initial approach, we acquire multiple images of an object with average qualities using techniques such as image averaging, conversion of images to the frequency domain and amplification of high frequencies, or merging information from several images. As a result, we obtain high resolution, also referred to as super-resolution MISR. For our study, we will employ the SISR method. Due to existing limitations, such as time-consuming medical imaging processes, the risk of high-dose radiation exposure and tissue ionization, as well as high costs, recording multiple patient images is not feasible. This presents a significant challenge for super-resolution of medical images compared to other digital images. Thus, we will utilize the SISR method. Starting with the original image, known as high resolution (HR), we will create a low-resolution (LR) image. The quality and resolution will be improved via various methods, including interpolation and up-sampling, using only the information from the HR image. Notably, previous studies on medical images implementing super-resolution methods utilized single-modality images [8]. In this study, we evaluated three distinct image modalities. Both non-neural network methods [9, 10] and deep neural networks [11] can generate super-resolution images. Artificial neural networks have made this algorithm highly popular in machine learning due to its efficiency. Deep neural networks have shown their capability to solve problems such as classification and regression across multiple domains.

In the past few years, progress has been made in the use of deep networks for super-resolution applications, with promising results. Generative adversarial networks, known as GAN, will be very powerful in this field [12–15]. The GAN architecture was first described in the 2014 paper by Goodfellow et al. [16]. They must compete with an adversary by generating samples directly. In addition, the discriminator network tries to distinguish between samples from the training data and samples generated by the generator [17, 18].

The primary aim of this research is to develop and evaluate a novel approach for enhancing the quality of medical images through super-resolution techniques, leveraging transfer learning and the RESRGAN network [19] combined with the total variation method [20]. This study focuses on three distinct medical imaging modalities – computed tomography (CT) scan, magnetic resonance imaging (MRI), and ultrasound – to improve the resolution of low-quality images while preserving critical medical information. It addresses key challenges in medical imaging, including time

constraints, high costs, and radiation exposure risks, thereby contributing to more effective diagnostic and therapeutic outcomes.

1.1 Previous Works

Akbari et al. [21] used the random forest method for super-resolution of abdominal CT scan images. In this work, they first divided CT slice images into non-overlapping patches and used deep learning networks to cluster them during the training phase. To prepare LR images, a Gaussian filter was first applied to the image, and the dimensions of the image were reduced, finally, non-overlapping patches with a size of 5×5 pixels were considered as input to the random forest algorithm to reconstruct HR images. Finally, they validated their work using peak signal-to-noise ratio (PSNR) and structural similarity index measure (SSIM) metrics, and the obtained results show an improvement in the values compared to the bicubic method. The proposed algorithm for PSNR and SSIM showed an improvement of 5.6 dB and 0.11, respectively.

Deeba et al. [22] also introduced a wavelet-based super-resolution method using a convolutional neural network. Instead of a discrete wavelet transform, they utilized a stationary wavelet transform. Then for training, a 3-layer neural network is considered, after the hidden layer, using the sub-pixel convolution technique, this technique directly creates HR images by using an upscaling filter for each feature map of the LR image [23]. Their method was trained and tested using 662 radiology images, and the reported scores for the two PSNR and SSIM metrics are 31.65 and 0.92, respectively.

Gu et al. [24] increased the resolution of CT scans and MR images using an adversarial generative network based on deep learning. In this work, in addition to using the residual whole map attention network (RWMAN) network as a generator of all aspects of the image, they focused more on the significant parts of the image and also benefited from the weighted sum of losses during training. Training and validation of the network have been done using 242 CT images and 110 MR brain images, and the final result of the algorithm, in addition to the common metrics of PSNR and SSIM, has been reviewed and evaluated by the empirical metrics of the average opinions of 5 radiologists as experts. The PSNR metric increased by 3.3 and the SSIM metric increased by 0.005 compared to the bicubic method for CT scan images.

In another work, Chen et al. [25] also implemented a super-resolution algorithm for medical images based on deep learning. Their convolutional neural network is constructed using adaptive weighted recurrent connections, which was named feedback adaptively weighted dense network (FAWDN). The output information from the image is passed to the LR image property page through recursive connections in this method. Also, to obtain more information from the image, the adaptive weight feature has been used to avoid receiving overlapping data by adaptively selecting advanced features that contain important information from the image. The network was trained by using

multiple medical datasets that contained MR images of various body organs in this work. The results obtained on average from one dataset for PSNR metric was 31.57 and for SSIM was 0.90.

In another method based on non-deep neural networks, Salvador et al. [26] working on public images were able to present a new method in super-resolution. A set of public digital images was used to implement their algorithm. In this work, by applying Bayes theorem and bimodal trees for clustering, and in the next step, mapping each image patch using local Bayesian, they increased the work speed and improved the PSNR metric of the output images. According to the results, there will be a slight increase in PSNR amount with the increase in clusters. The only work evaluation index used in this work was PSNR, and the changes in the results caused by the increase of clusters were not taken into account.

2 Materials and Methods

2.1 Data

In this work, to make the results comprehensive and positive performance of the model on medical images, we used three different modalities of medical images. Different reconstructed images are obtained using different technologies. There is no overlap between the three databases used in this project and they are completely separate from one another. The first database is the 3D brain MR images of 23 men and women, from (St. Olav's University Hospital, Trondheim, Norway), which was obtained after the informed consent of the patients during the years 2011 to 2016. The images were created using a 3T Magnetom Skyra MRI scanner (Siemens, Erlangen, Germany) with a 20-channel head coil for 12 min. T2 images were utilized in this project. Also, this database contains brain ultrasound images of the same people after skull removal. The obtained 3D ultrasound images were prepared using the Sonowand Invite neuronavigation system (Sonowand AS, Trondheim, Norway). Also, a linear probe in the frequency range of 6 to 12 MHz was used. The Sonowand Invite neuronavigation system utilized a Polaris infra-red camera (manufactured by NDI, Waterloo, Canada) to accurately record the position and orientation of the ultrasound probe. This was achieved through the optical trackers that were securely attached to the probe [27]. Another database deals with 3D brain CT scan images from the Patient Contributed Image Repository (PCIR) [28] open-access database, which includes medical images of different organs and different techniques. The brain CT scan data recorded in 2006 in digital imaging and communications in medicine (DICOM) format was utilized in this work. A sample of the data used can be seen in the Fig. 1.

It should be mentioned that Mango software was used to display images [29] and MATLAB software [30] was used for 3D volume display. Representative 3D samples of the imaging modalities are shown in Fig. 2.

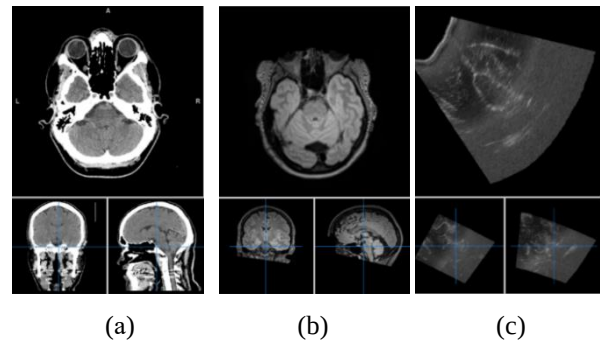


Fig. 1 Two-dimensional representations of sample data images from three imaging modalities. (a) CT scan sourced from the PCIR database; (b) MRI and (c) ultrasound images obtained from the REtroSpective evaluation of cerebral tumors (RESECT) database [27].

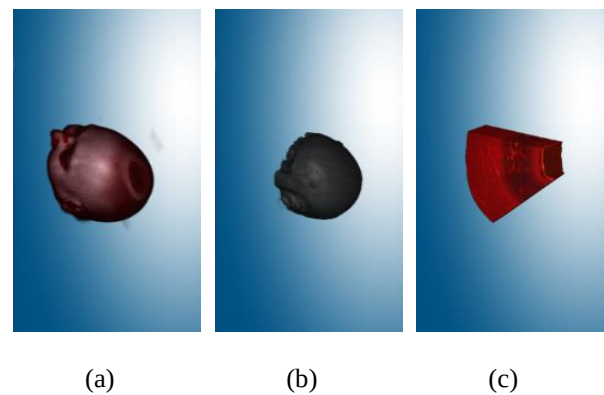


Fig. 2 Representative samples of volumetric 3D data images, showcasing diverse medical imaging modalities. (a) CT scan with dimensions $64 \times 256 \times 256$ voxels, sourced from the PCIR database; (b) MRI and (c) ultrasound images obtained from the RESECT database [27], with MRI dimensions of $192 \times 256 \times 256$ voxels and ultrasound dimensions of $348 \times 313 \times 275$ voxels.

2.2 Pre-Processing

All high-resolution images will be available to us because the data sets were not recorded specifically for super-resolution processing. To obtain LR images as training data, it is necessary to create LR images that correspond to each of the data using existing processes and use them as input to the proposed algorithm.

The Gaussian function filter shown in Eq. (1) was used to smooth all the images first. We picked 0.5 for the variance of the considered filter.

$$G_{(x,y)} = \frac{1}{2\pi\sigma^2} e^{-\frac{x^2+y^2}{2\sigma^2}}. \quad (1)$$

In the next step, we reduced the dimensions of the images by applying Eq. (2) with a factor of 1/2 for all images in all directions. This resulted in an output image with half the dimensions of the input image. Bicubic interpolation was used as the interpolator function for each slice of the image during this translation.

$$\begin{bmatrix} x_0 & y_0 & 1 \end{bmatrix} = \begin{bmatrix} x_i & y_i & 1 \end{bmatrix} \begin{bmatrix} 1 & 0 & 0 \\ 0 & 1 & 0 \\ t_x & t_y & 1 \end{bmatrix}. \quad (2)$$

In this equation, y_i and x_i represent the dimensions of the input image, y_0 and x_0 represent the dimensions of the output image, also t represents the displacement of each pixel in different directions.

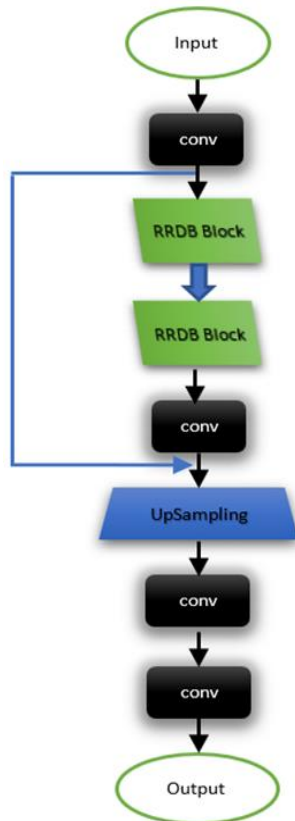


Fig. 3 Schematic representation of the RESRGAN network architecture employed for super-resolution reconstruction in medical imaging. The diagram delineates the sequential layers: black blocks correspond to convolutional operators responsible for feature extraction and transformation; the blue block indicates the upsampling module, which facilitates spatial resolution augmentation through techniques such as pixel shuffling; and green blocks represent residual in residual dense blocks (RRDB), designed to enhance deep feature propagation and mitigate vanishing gradient issues via nested residual connections.

2.3 Proposed Method

The LR image is inputted into the low-rank and total variation (LRTV) method to super-resolution. This method utilizes the neighborhood information of the pixels, as well as the general information and voxel placement of the image to improve the quality of the reconstruction. Neighborhood information is the sole factor that can retain the sharpness of edges in a given

image. To optimize this method, alternating direction method of multipliers (ADMM) is employed as the optimizer function. Finally, the pre-trained RESRGAN network receives the image for further processing. Just like other adversarial generator networks, this network has two parts including generator and discriminator. The generator network receives the LR image as an input and the discriminator network tries to minimize the difference between the output image made by the generator network and the original image. The generative network does not just upscale the image, but also returns the details of the image according to the initial information obtained from the previous method. Also, as shown in Fig. 3, this network RRDB to enhance the quality of the final reconstructed image. The *U*-net model is followed by the adversarial network in this architecture, which utilizes spectral normalization to enhance stability while training. Figure 4 presents an overview of the steps taken in this project to create high-resolution medical images.

3 Results

To evaluate this work, image similarity measurement methods are necessary. Because of the absence of a specific standard for these issues, it is essential to utilize the current metrics. In previous articles and other research, SSIM and PSNR Metrics were used to evaluate their methods [31, 32]. It should be noted that other Metrics are also available, such as sum of squared differences (SSD), correlation coefficient (CC), etc. To compare with previous works, we concentrate on two metrics, PSNR and SSIM, in this project. The two LR images super-resolved by the proposed method and the original HR image are examined from the point of view of similarity, and the higher the similarity, the better the result.

The SSIM index is a measure of image structural similarity that measures the similarity of two images by examining the dependence of pixels on their neighbors. Since the neighborhood of pixels in the image also contains important information, it can be concluded that by examining this information, the structure of the objects in the images can be compared [33]. Eq. (3) shows how to calculate this index:

$$SSIM_{(x,y)} = \frac{(2u_x u_y + C_1)(2\sigma_{xy} + C_2)}{(u_x^2 + u_y^2 + C_1)(\sigma_x^2 + \sigma_y^2 + C_2)}, \quad (3)$$

where u_x, u_y represent the mean and σ_x, σ_y represent the standard deviation of x and y values. Also, σ_{xy} is the covariance of x, y and the values indicated by the letter C are constant values. The final range of SSIM is between the numbers 0 and 1, where the number 0 indicates no similarity and the number 1 indicates complete similarity between the two images.

The PSNR metric is also a similarity index based on the amount of signal (here pixels of an image) to the amount of noise [34]. Equations (4–5) is the method of calculating this metric:

$$MSE = \frac{1}{mn} \sum_{j=0}^{m-1} \sum_{k=0}^{n-1} [I_{HR}(j, k) - I_{SR}(j, k)]^2, \quad (4)$$

$$PSNR = 20 \log \left(\frac{255}{\sqrt{MSE}} \right). \quad (5)$$

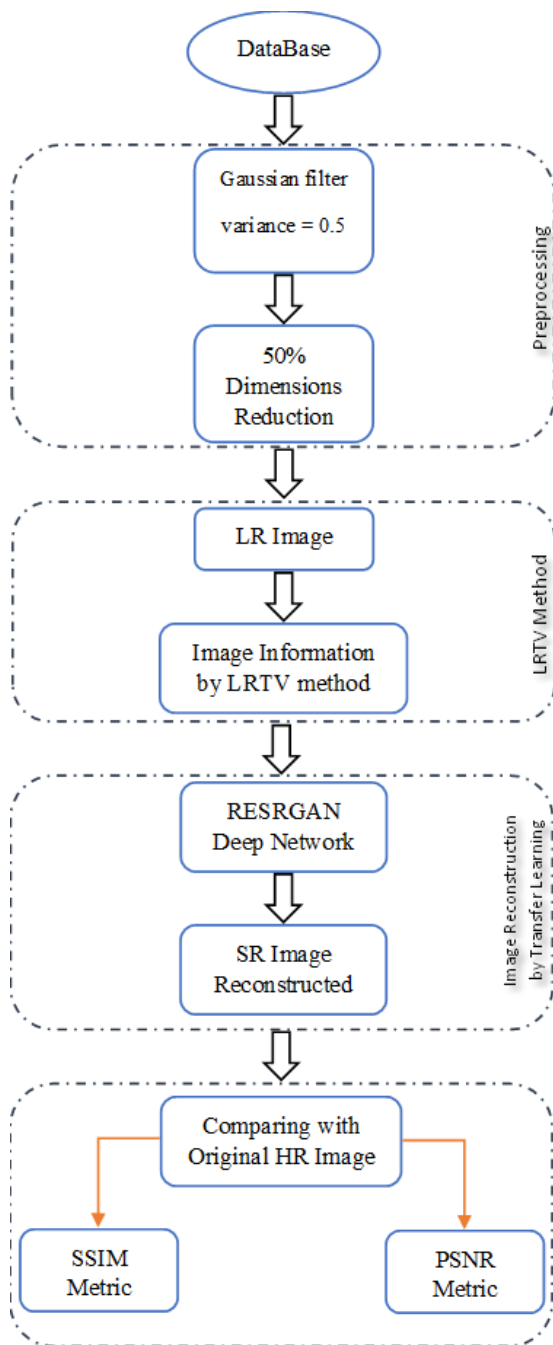


Fig. 4 A detailed workflow of the proposed image super-resolution approach, starting with a Database, applying a Gaussian filter (variance = 0.5) and 50% dimension reduction to create LR images. LRTV method enhances image details, followed by RESRGAN Deep Network with transfer learning for SR reconstruction. Quality is assessed by comparing with original HR image using PSNR and SSIM metrics.

The mean squared error (MSE) represented in Eq. (5), where I_{HR} and I_{SR} represent the original HR image (input)

and the super-resolution image (output of the method), respectively. Also, mn shows the dimensions of the images in pixels. PSNR is introduced in terms of decibels (dB) and the higher the value of this number, the greater the similarity between two images. It is necessary to mention that the number obtained from the PSNR metric only compares the intensity values of the pixels of two images and its main focus is on the sensitivity of the noise level [21]. Quantitative results for PSNR and SSIM across the three modalities are summarized in Table 1.

Figures 5–6 present a concise review and comparison of the proposed method and the bicubic method, showcasing the obtained results. The reconstructed images using the proposed method demonstrated a notable improvement compared to the bicubic method. Graphs demonstrate this improvement clearly.

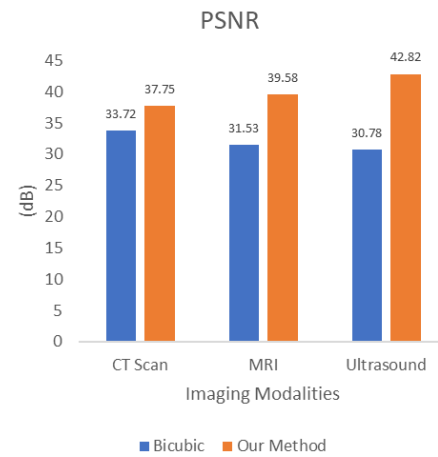


Fig. 5 This chart compares PSNR values (in dB) between bicubic and our method across three imaging types: CT Scan, MRI, and ultrasound. The X-axis represents the imaging types, while the Y-axis shows the PSNR values, indicating image quality with higher values reflecting better quality.

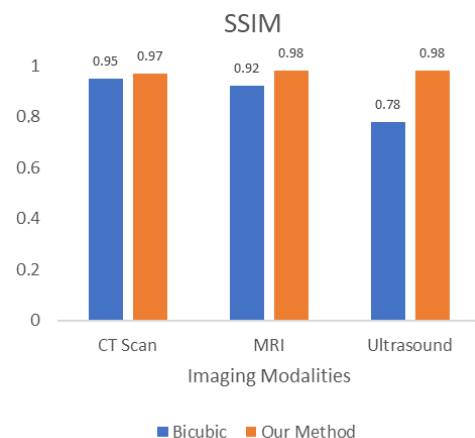


Fig. 6 This chart compares SSIM values between bicubic and our method across three imaging types: CT Scan, MRI, and ultrasound. The X-axis represents the imaging types, while the Y-axis shows the SSIM values, indicating image quality with higher values reflecting better quality.

Table 1 Mean and standard deviation of PSNR and SSIM for quantitative evaluation of the super-resolution approach across different brain image modalities.

Modalities	Metrics	Bicubic method	The proposed method
CT	PSNR	33.77 ± 1.40	37.75 ± 0.90
	SSIM	0.95 ± 0.005	0.97 ± 0.002
MRI	PSNR	31.53 ± 1.62	39.58 ± 2.08
	SSIM	0.92 ± 0.01	0.98 ± 0.005
Ultrasound	PSNR	30.78 ± 1.02	42.82 ± 0.79
	SSIM	0.78 ± 0.03	0.98 ± 0.007

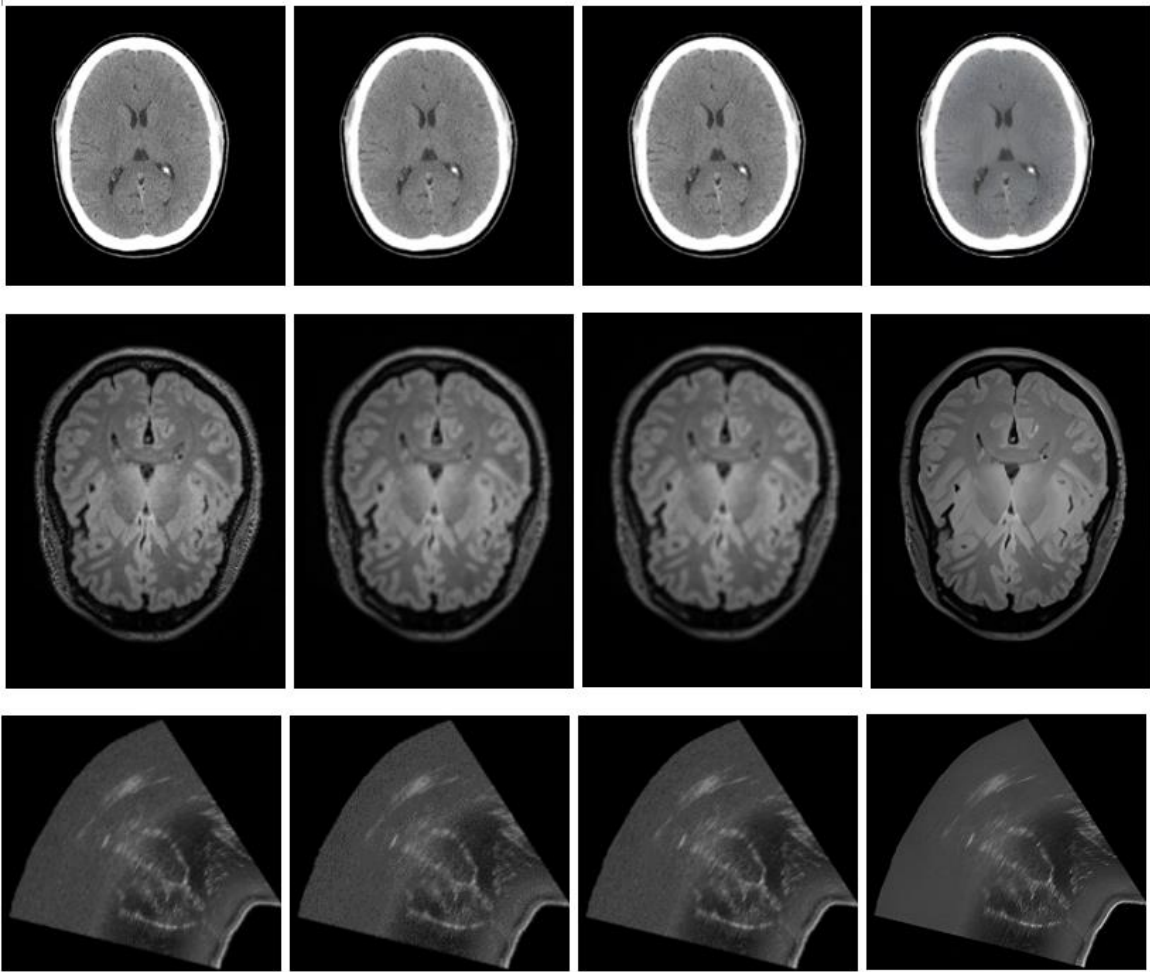


Fig. 7 Comparative analysis of original and enhanced images using various resolution improvement methods. Column (a) original images sourced from databases; Column (b) constructed LR images; Column (c) images reconstructed via the bicubic interpolation method; Column (d) super-resolved images generated by the proposed method.

Figure 7 displays the conversion steps for the original image, including the LR image and the super-resolution image. All three image modalities are depicted in this figure with the first row showing CT scan images, the

second row showing MR images, and the third row depicting ultrasound images. The reconstructed images can be visually evaluated to compare the quality of the bicubic method to the proposed method.

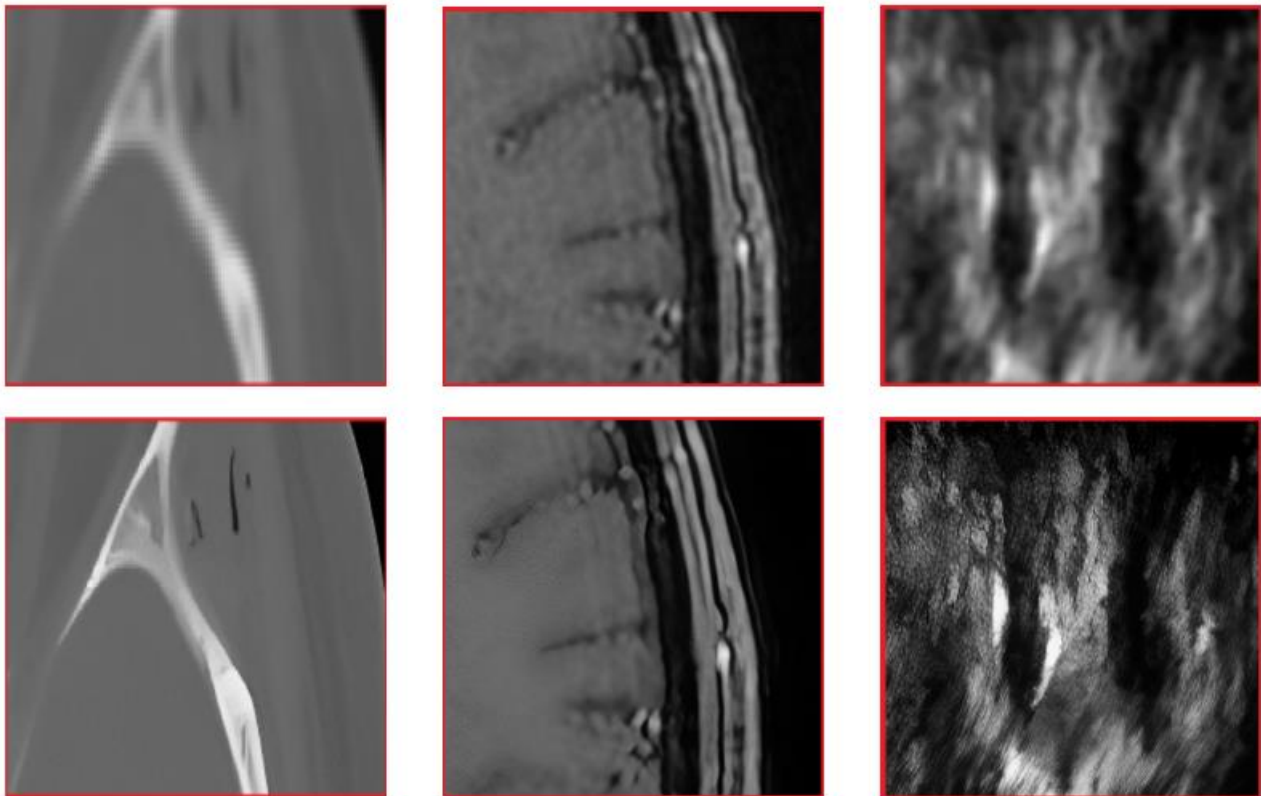


Fig. 8 Visual comparative assessment of original images (top row) versus their corresponding super-resolved images (bottom row), with magnified identical patches provided for detailed inspection, utilized for validating the proposed method. (a) CT scan sourced from the RIRE database [37]; b) MRI and c) ultrasound images obtained from the BITE database [35]. These images, derived from two entirely distinct databases, were employed to ensure robust validation and generalization of the proposed methodology across diverse medical imaging modalities.

One important aim of this study is to establish a strong and complete method for medical imaging systems. For further validation of the proposed method's performance on other systems, we have utilized additional legacy databases. Brain images of tumors for evaluation (BITE) database [35], which includes MRI and ultrasound images of 14 men and women with glioma tumors. A 1.5 Tesla GE Signa EXCITE (General Electric, Milwaukee, WI) was used to acquire the images. the slice thickness is 1 mm and the Images are T1-weighted. Ultrasound images were also acquired with the image acquisition system known by IBIS NeuroNav [36]. The ultrasound machine that used to acquire the images was HDI 5000 (ATL/Philips, Bothell, WA), also the ultrasound probe was a P7-4 MHz phased array transducer. images were displayed with B-mode.

Retrospective image registration evaluation (RIRE) database [37], is used for CT scan images, which is from Siemens DR-H scanner with 28 to 34 slices. The thickness of the voxels in the x and y directions was equal to 0.65 mm and 4 mm in the z direction. These data were solely utilized to evaluate the proposed method's generalization. As access to their corresponding HR images was unavailable, their investigation will be conducted qualitatively and visually.

As shown in Fig. 8, implementing the proposed method on LR images obtained from other databases produces satisfactory results. The images possess superior quality due to the significant details present, such as borders and edges. In addition, the primary information of the images remains intact, and no detrimental alterations are detected.

4 Discussion

An important challenge is to increase the resolution of digital images for higher level processes and stages [38]. After conducting extensive research on super-resolution using deep networks, we decided to specifically investigate the results on medical images. To determine the accuracy and generality of the methods used, three medical images of different modalities were used in this study. In this work, single image super-resolution methods were used to obtain brain images using MR, CT scan, and ultrasound modalities. Due to the absence of two predetermined image qualities, the initial images underwent Gaussian smoothing at a rate of 0.5. Subsequently, the image dimensions were reduced by a factor of 0.5 to create a low-resolution image, which was subsequently used as an input for the neural network to reconstruct the image at high resolution. Combining RESRGAN networks with a method that involves both

neighborhood pixels and general image information has led to a novel approach for reconstructing images with higher resolution. Our results demonstrate improvement over previous works, with the PSNR and SSIM validation metrics averaging 40.05 and 98.05, respectively, across all three groups of medical images. These values indicate an improvement of 8.03 dB and 9.49 when compared to the bicubic method for the same metrics. The ultrasound image modality exhibited the highest rate of increase, particularly in the PSNR metric. Based on initial observations, it can be concluded that the method used for this modality outperformed the other two as evidenced by the significantly superior SSIM criterion compared to the other modalities. The PSNR metric measures results in terms of signal-to-noise ratio. In future studies, additional medical image modalities and data can be utilized to evaluate networks with different data sets. Other methods for measuring similarity are necessary as PSNR and similar methods cannot be significantly relied upon. Thus, there is a need for more valid standards to evaluate super-resolution techniques.

5 Conclusion

Enhancing the resolution of medical images is of critical importance, as it directly impacts diagnostic accuracy and clinical decision-making, while also addressing the limitations and high costs of modern imaging systems. In this study, we proposed a hybrid super-resolution framework that combines transfer learning, a RESRGAN, and LRTV regularization. By integrating deep learning with classical approaches, the proposed method achieved superior performance in both quantitative metrics and visual quality. Experimental results confirmed that our framework significantly

outperformed conventional bicubic interpolation. Specifically, the proposed method achieved an average PSNR of 40.05 ± 1.25 and SSIM of 0.97 ± 0.004 , compared to bicubic interpolation, which yielded 32.02 ± 1.34 and 0.88 ± 0.015 , respectively. These improvements demonstrate the method's ability to preserve fine anatomical details and reduce artifacts, leading to clearer and more reliable reconstructions for medical interpretation.

In conclusion, the proposed hybrid framework represents a promising tool for medical image super-resolution. Future research will focus on validating the model on larger and more diverse datasets, exploring additional imaging modalities such as histopathology, and developing more reliable evaluation protocols, such as the feature-based similarity index (FSIM) and the universal image quality index (UIQ), to establish more comprehensive benchmarks. Such efforts will contribute to advancing the field by providing standardized and clinically relevant assessments of super-resolution techniques.

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Conflicts of Interest

The authors declare that they have no conflicts of interest or competing financial interests related to this work.

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Photodynamic Inactivation of Pigmented and Non-Pigmented *Pseudomonas Aeruginosa* in Biofilms

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Abstract. This study presents a method to improve the efficacy of photodynamic inactivation against both pigmented and non-pigmented *Pseudomonas aeruginosa* strains in biofilms through pre-treatment with acoustic shock waves. Biofilms were exposed to photosensitizers in solution, and shock waves were generated using laser pulses (10 ns duration, 5–10 MW/cm² power density). Pre-treatment with shock waves significantly enhanced photodynamic bacterial damage, increasing inactivation efficiency by twofold for the non-pigmented strain and sixfold for the pigmented strain. The observed improvement is likely due to shock wave-induced permeabilization of the biofilm matrix and bacterial cell membranes, facilitating greater uptake of photosensitizers and oxygen.

Keywords: biofilms; pyoverdine; shock waves; photodynamic inactivation.

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1 Introduction

The development of resistance by pathogenic bacteria to the action of traditional antibiotics stimulates the search for alternative methods of microbial inactivation. One promising approach is antibacterial photodynamic therapy (aPDT), which combines photosensitizers (PS), light, and oxygen to kill microorganisms [1–4]. aPDT offers several advantages: it is safe for in vivo use (no chemically toxic reagents, high temperatures), selectivity of action (bacterial damage only in areas where PS and light are delivered), and a low probability of bacteria developing photodynamic immunity. The main disadvantage of aPDT is its low efficiency in suppressing bacteria in the inner layers of tissues and biofilms, particularly against Gram-negative bacteria.

Biofilms are a special form of existence of a group of microorganisms, when bacteria produce a special matrix of polymers (DNA, proteins, polysaccharides) and then multiply within this environment. In biofilms, bacteria become significantly more resistant to antibiotics, disinfectants, and the host's immune response. The matrix limits the penetration of antimicrobial agents and creates a gradient of nutrients and oxygen, which slows down the metabolism of some cells but increases their survival [5–7].

In this work, the efficiency of aPDT of *Pseudomonas aeruginosa* bacteria in biofilms is assessed. *P. aeruginosa* bacteria were chosen because they are one

of the main pathogens of hospital-acquired infections, including pneumonia, sepsis, urinary tract infections, skin lesions and soft tissues [8–10]. In addition to the ability to form biofilms, these bacteria produce a number of virulence factors, including pigments (pyocyanin, fluorescein) and exotoxins, which help them colonize tissues and suppress the host immune response. However, some bacterial strains do not produce pigments, which can be caused by random mutations (fixed as an adaptation to stress or immune response), as well as a lack of oxygen in the environment and excess iron [11–12]. Pyoverdine exhibits antioxidant and antibiotic properties and is a siderophore used for iron uptake by the cell [13]. Pyocyanin has its own antibiotic [14] and photodynamic activity [15], these properties can be used to inactivate pigmented strains.

A promising method for increasing the efficiency of aPDT is pre-treatment of biofilms with shock waves propagating in an elastic medium. Shock waves can cause mechanical damage in biofilms, increase the permeability of the biofilm matrix as a whole and the walls of individual bacterial cells for photodynamic agents (PSs and oxygen), increasing their sensitivity to photodynamic treatment. Various authors reported on the successful use of shock waves in the inactivation of biofilms in stents [16], in urinary catheters [17] and other medical instruments [18–19].

In Ref. [20], it was proposed to form shock waves directly in a solution with PS. For this, it is necessary to

use molecules with a high quantum yield in the triplet state as PS in order to simultaneously ensure efficient production of singlet oxygen and the generation of shock waves as a result of local heating of the medium after excitation by pulses of focused laser radiation. The aim of this work is to evaluate the efficiency of photodynamic inactivation of *P. aeruginosa* bacteria in biofilms after exposure to shock waves and to determine the role of the bacterial pigment in this process.

2 Materials and Methods

2.1 Biofilm Preparation

The work involved *P. aeruginosa* strains that produce the blue-green pigment characteristic of these bacteria (which primarily includes pyocyanin and pyoverdine), as well as those that do not produce such a pigment. In what follows, we will refer to such strains as pigmented and non-pigmented. To obtain multiple identical biofilm samples, the technique described in Ref. [21] was used. A combination of cetrimide both and cetrimide agar (Microgen, Russia) in a 3:2 ratio was used as a culture medium. It has a liquid consistency at an incubation temperature of 37 °C, but solidifies at room temperature. To obtain standard samples, a sterile metal punch with a diameter of 8 mm was used, which allows cutting out biofilm fragments of identical volume (cylindrical blocks) with minimal damage to the structure. The variation between fragments in bacterial cell density did not exceed 5%, which was confirmed in a series of control experiments with seeding of destroyed blocks and subsequent counting of colony-forming units (CFU).

2.2 Generation of Shock Waves

Erythrosine (tetraiodofluorescein, Sigma-Aldrich, Switzerland) was used as a sensitizer. The dye is quite photostable, an effective generator of singlet oxygen $^1\text{O}_2$, which allows it to be used both as a photodynamic agent and as a source of shock waves. Erythrosine is known to be used as a photodynamic agent in antimicrobial therapy [22–23]. To generate shock waves and subsequent photodynamic irradiation of biofilms, an experimental setup was used, schematically shown in Fig. 1.

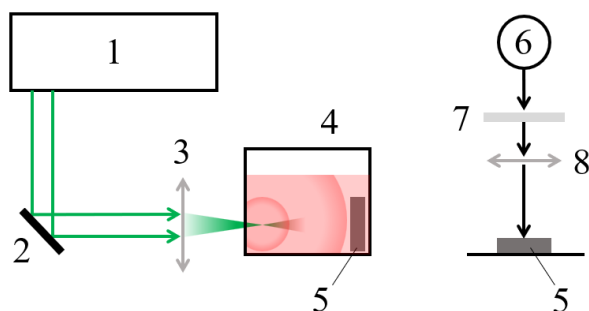


Fig. 1 Experimental setup for generating shock waves and photodynamic irradiation of biofilms. 1 – YAG:Nd³⁺ laser, 2 – mirror, 3 – cylindrical lens, 4 – cuvette with samples, 5 – biofilm on an agar substrate, 6 – KGM-150 lamp, 7 – light filter, 8 – collecting lens.

Excitation of erythrosine molecules in the solution was produced by the second harmonic radiation $\lambda = 532$ nm of a pulsed solid-state YAG:Nd³⁺ laser (Solar LQ-215, Belarus). The laser radiation was focused by a cylindrical lens 3 in a volume of $3 \times 0.3 \times 0.3$ mm³ inside a cuvette 4 near its end wall. Block 5 with the biofilm was placed on the opposite side of the cuvette at a distance of 15 mm from the focus of the lens. At a power density of 10 MW/cm² and a dye concentration of 0.5 mM, the excitation radiation penetrated into the solution to a distance of less than 10 mm, thus eliminating photodynamic or thermal effects in the biofilm. The energy of the excitation pulses was measured by a Field Master GS power meter with an LM-10HTD measuring head (Coherent, Inc., USA).

2.3 Photodynamic Treatment

For photodynamic treatment of biofilms, the radiation of a KGM-150 incandescent lamp (Institute of Light Sources named after A. N. Lodygin, Russia) was used (indicated by number 6 in Fig. 1). The biofilm treated with shock waves was removed from the cuvette and placed in a well of the plate with the FS solution so that the thickness of the liquid layer above the biofilm surface did not exceed 1 mm. With this arrangement, the oxygen concentration above the biofilm surface is the highest. Using lens 8, the radiation was focused on the biofilm (light spot diameter 8 mm). Radiation coinciding with the spectral absorption region of erythrosine and bacterial pigments was isolated from the continuous spectrum of the lamp by light filter 7 of the SZS-22 type (PhotonTechSystem, Russia). The power density of the light radiation was 0.8 W/cm².

3 Results and Discussion

3.1 Intrinsic Photodynamic Activity of Bacterial Pigment

After centrifugation and filtration through a bacterial filter of the enrichment culture of the pigmented strain *P. aeruginosa* in the cetrimide broth, a solution with the pigment pyoverdine was isolated [24]. The absorption spectrum of the solution shows a characteristic maximum in the region of $370 \div 380$ nm, coinciding with the absorption maximum of pyoverdine. The maximum fluorescence of the solution was recorded at a wavelength of 470 nm (Fig. 2).

The photodynamic activity of the pigment was estimated experimentally. *P. aeruginosa* biofilms were placed in wells of a plate filled with water or a solution with the isolated pigment so that the thickness of the liquid layer above the biofilm surface did not exceed 1 mm (shown schematically in Fig. 3 under the histograms). There were three groups of samples in the experiment:

- non-pigmented biofilms;
- pigmented biofilms;
- non-pigmented biofilms immersed in a solution with a pigment.

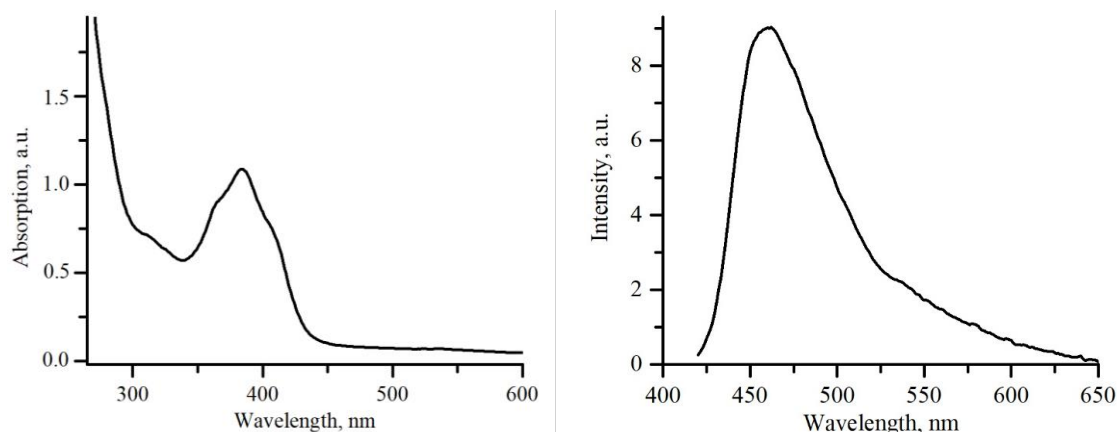


Fig. 2 (a) Absorption and (b) fluorescence spectra of pigment isolated from bacterial suspension.

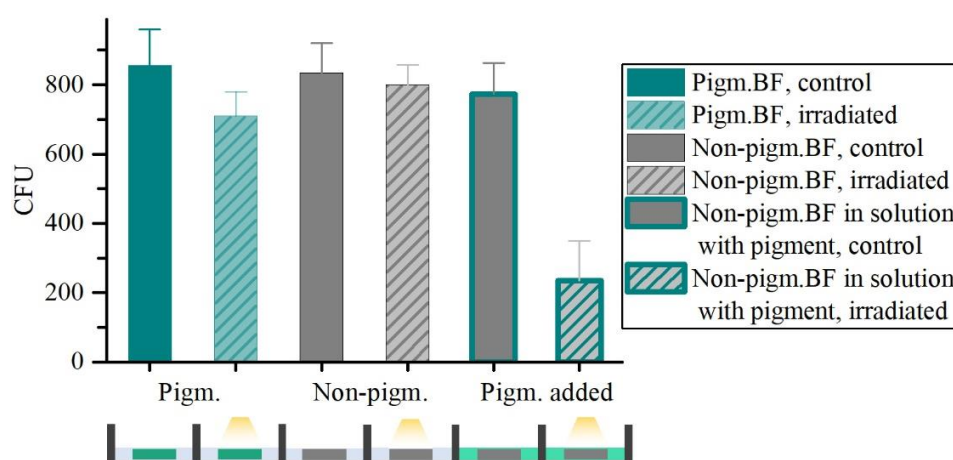


Fig. 3 Histograms of *P. aeruginosa* CFU after photodynamic treatment of pigmented and non-pigmented biofilms, as well as non-pigmented biofilms with added pigment. Non-shaded columns are the control, shaded columns are the experimental samples.

Photodynamic exposure was carried out using a KGM-150 lamp with a SZS-22 light filter for 15 min to obtain a dose of 50 J/cm^2 . Each biofilm was then crushed, the resulting suspension was diluted and plated on a nutrient medium, as described above. The CFU histograms for each of the three groups are shown in Fig. 3 in comparison with their dark controls.

Irradiation of native biofilms (non-pigmented and pigmented) does not affect cell viability. The number of CFU from non-pigmented biofilms immersed in a pigment solution and in water is practically the same without a light dose. This indicates the absence of a chemical (dark) effect of the pigment on the cells. And only in the case of irradiation of non-pigmented biofilms “colored” with pigment, the number of CFU decreases almost fourfold, which confirms the photodynamic activity of the pigment.

It is noteworthy that in the case of a pigmented strain, 90% of cells survive after photodynamic irradiation, and in the case of a “colored” strain-less than 25% of cells. Apparently, the concentration of PS in samples with added pigment was significantly higher than in pigmented samples. An alternative assumption is that

pigmented cells have greater tolerance to photodynamic action than cells grown without pigment.

3.2 Combined Action of Shock Waves and Photodynamic Treatment on Biofilms

In the experiment, CFU were counted for pigmented and non-pigmented strains for four groups (Fig. 4):

- samples without exposure (control);
- biofilm samples exposed only to shock waves (SW);
- biofilm samples after photodynamic (PD) treatment (dose 50 mJ/cm^2);
- biofilm samples exposed first to shock waves (1000 pulses), then to photodynamic treatment (dose 50 mJ/cm^2) (SW + PD).

All samples, including controls, were kept in erythrosine solution (0.5 mM). After shock wave treatment, biofilms were removed from the cuvette, placed in wells of the plate with PS solution and exposed to photodynamic treatment according to the method described above.

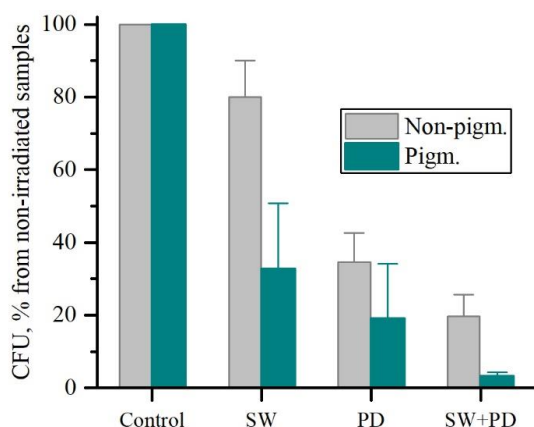


Fig. 4 Histograms of CFU of bacteria from *P. aeruginosa* biofilms exposed to 0 and 1000 pulses without subsequent photodynamic treatment, as well as 5 min of irradiation with a KGM-150 lamp with an energy density of 50 J/cm² for 15 min. Light columns are for the non-pigmented strain, dark columns are for the pigmented strain.

The action of shock waves generated by laser pulses in an erythrosine solution leads to a decrease in viable bacteria in the biofilm (approximately three times). Shock acoustic waves with a peak pressure close to the tensile strength threshold of the cell wall material are capable of destroying biofilms by the ablation mechanism, mechanically separating the resulting fragments into viable bacteria [21]. At the same time, the relatively low efficiency of damaging bacteria in biofilms by such shock waves has been experimentally proven. Probably, the elastic protective matrix of biofilms protects bacteria from mechanical damage. The photodynamic effect of erythrosine on gram-negative bacteria is low-effective. Figure 4 shows that with an accumulated energy of 50 J/cm², the number of viable cells in the biofilm remains virtually unchanged. However, after preliminary treatment with shock waves, the picture changes dramatically: the number of CFU decreases almost 20 times! Apparently, shock waves

disrupt the structural integrity of the biofilm as a whole and increase the permeability of cell membranes for PS and oxygen.

For the pigmented strain, physical effects (both shock waves and photodynamic treatment) were more destructive than for the non-pigmented strain. The pigment has weak photodynamic activity in relation to the cells in which it is synthesized and does not harm *P. aeruginosa* bacteria. However, the pigment can suppress the growth of other competing microorganisms under conditions of food resource deficiency, including during biofilm formation. It is possible that under stressful effects on *P. aeruginosa* (shock waves, photodynamic action), the pigment under light irradiation makes an additional contribution to the inactivation of bacteria. This assumption is confirmed by the fact that pigmented *P. aeruginosa*, all other things being equal, survived somewhat worse than non-pigmented ones.

Thus, preliminary treatment of the medium with acoustic shock waves significantly increases the efficiency of photodynamic inactivation, which can find wide application in clinical practice. This synergistic effect is due to the disruption of the structural integrity of the biofilm matrix and permeabilization of cell walls, which improves the penetration of PS and molecular oxygen into the deep layers of the biofilm. The data obtained indicate the prospects for developing combined physical methods for combating biofilm infections, where mechanical destruction of the extracellular matrix enhances the efficiency of subsequent antimicrobial action.

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Conflicts of Interest

The authors declare that they have no conflicts of interest or competing financial interests related to this work.

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Universal Analytical Modeling of the Optical Properties for Coated Plasmonic Nanoparticles

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Abstract. We describe a new analytical method for coated plasmonic nanoparticles, combining the modal expansion method (MEM) with the dipole equivalence method (DEM). The universality and accuracy of our approach are demonstrated through comparisons of extinction and scattering spectra for variously shaped two-layer gold and silver nanoparticles (rods, disks, triangle prisms, bicones, and bipyramids) with dielectric coatings from 0 to 100 nm, benchmarked against numerical calculations using COMSOL. Due to its universality, the MEM + DEM approach accurately reproduces the dependence of extinction and scattering spectra on particle size, shape, shell thickness, and orientation relative to the incident light polarization. We also investigate the effects of size-corrected dielectric functions for gold and silver, along with related damping mechanisms, on the spectra. As expected, size-dependent damping broadens and reduces plasmonic peaks, especially for small silver nanoparticles. Although MEM inherently does not depend on particle shape, the MEM + DEM method fails for coated bicones with sharp tips but performs satisfactorily for rounded bicones. This limitation is due to strong localization of plasmonic fields near the tips, which violates the assumption of homogeneous internal fields and the applicability of DEM. To avoid this difficulty, we introduce a factorized version of MEM that ensures accurate analytical calculations for coated sharp bicones. In summary, the MEM + DEM approach provides a simple yet accurate analytical tool for simulating various plasmonic properties of coated metal nanoparticles and for developing machine learning models.

Keywords: gold and silver nanoparticles; coated nanoparticles, localized plasmon resonance; modal expansion method; dipole equivalence method; COMSOL.

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1 Introduction

Biomedical applications of plasmonic nanoparticle conjugates require functionalization of their surface with various functional molecules, which ensure their stability in the bloodstream, invisibility for immune system cells, targeting specific biotarget sites, etc. [1–23]. Often, such properties are achieved in hybrid multilayer structures with silica-type dielectric shells. Thus, from a structural point of view, multifunctional bioconjugates are at least bilayer particles with a metal core and a dielectric coating. The optical properties of such conjugates differ

from those of the plasmonic core and, in principle, can be simulated by modern numerical methods, such as the finite element method [4], the boundary element method [5, 6], the finite difference time domain method [7], and the discrete dipole approximation [8]. For applications of these methods to specific plasmonic problems and comparison of their efficiency the readers are referred to recent reviews [9, 10]. However, analytical models based on simple physical ideas are more in demand to control the various stages of synthesis in situ to predict given optical properties and their

correlation with the structure of particles. Besides, analytical methods can be useful in application of AI tools based on deep machine learning models.

In this paper, we describe an analytical model of this type [11], which is based on a combination of two methods: the modal expansion method (MEM) [12] and the dipole equivalence method (DEM) [13]. The main advantage of the DEM method is that the analytical MEM models for particles of various shapes are also applicable for particles with a single-layer or even multilayer dielectric coating. In this communication, the capabilities of our method are illustrated by comparing analytical and numerical (COMSOL) extinction and scattering spectra of gold and silver nanorods (NRs), nanodisks (NDs), triangular nanoprisms (NTs), bicones (BCs), and bipyramids (BPs).

In our previous papers [11, 14] we used the bulk dielectric functions of silver and gold. Here, we extend our consideration to include the effects of size-corrected dielectric function caused by limitation of the mean free path of conductive electrons. The history of this point dates back to pioneering works by Doyle [15], Hampe [16], and Fragstein and Römer [17] published in 1958. Doyle discovered that the plasmon resonance widths (0.05–0.5 eV) of NaCl crystals did not match the calculated bulk value of 0.02 eV. He suggested that this anomaly could be explained by the limitation of the electron mean free path, which cannot be larger than the nanoparticle size. Therefore, the classical electron mean free path (~20 nm) should be replaced with the actual size of the NaCl particles. Similarly, Hampe observed significant broadening of the absorption spectra of gold nanoparticles in a silicate matrix and proposed that this could also be explained by the limitation of the electron mean free path. Römer and Fragstein [18] measured the refractive index of gold nanoparticles ranging from 3 to 20 nm and found significant differences between the optical constants of bulk gold and those measured for the smallest 3.5 nm particles. Consistent with Doyle [15] and Hampe [16], they speculated that these anomalies might be related to size-correction effects. In 1969, Kreibig and Fragstein [19] developed a comprehensive classical model of the size-corrected dielectric function in metal nanoparticles, incorporating the size-dependent damping constant $\gamma_p(a)$

$$\gamma_p(a) = \gamma_b + \gamma_s = \gamma_b + A_s \frac{v_F}{a}, \quad (1)$$

where γ_b is the dumping constant of bulk metal, v_F is the Fermi velocity, a is the radius of a spherical nanoparticle, and the scattering constant A_s depends on the scattering model of conductive electrons at the particle surface. The modified dumping constant $\gamma(a)$ should be substituted into the classical Drude-Lorentz [20] theory for the dielectric function of conductive electrons. Since the publication of these early works, the size-corrected dielectric function, based on the modified dumping constant (Eq. (1)) within the Drude-Lorentz theory, has been widely used in many studies [21–26].

In line with many previous studies [26, 27], we demonstrate that size-dependent dumping results in

broadening and reduction of the plasmonic peaks of coated nanoparticles, especially for small silver nanoparticles. For the main localized plasmon resonance (LPR) peaks, the analytical method accurately describes the changes in extinction and scattering spectra with increasing dielectric shell thickness for all particle types. However, we observed noticeable differences between the analytical spectra and their numerical counterparts for bicones and bipyramids. These differences are due to strong near-field localization near sharp vertices, which violates the conditions for applying the dipole approximation. To improve the original MEM + DEM scheme, we propose a factorized MEM model where the effective path length is adjusted via an additional MEM parameter that depends on the dielectric coating thickness. This correction is shape- and size-independent, making it broadly applicable.

2 Methods and Materials

Our MEM models are similar to those previously described [11], with differences in parametrization and size-dependent corrections for the dielectric constants of gold and silver. Figure 1 shows the five particle shapes considered in this work. We also define the geometrical parameters in terms of the characteristic particle size L and a generalized aspect ratio AR . Panel B illustrates the parametrization of coated nanorods. The particle core is characterized by a complex permittivity ε_1 , while a homogeneous shell is described by its thickness s , and permittivity $\varepsilon_2 = 2.25$, which corresponds to a typical refractive index of 1.5 for most stabilizing ligands and functional coatings [28]. The surrounding medium is characterized by its dielectric function ε_m , (water in this work).

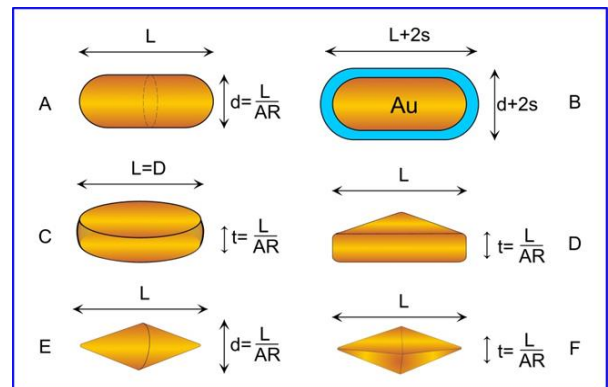


Fig. 1 The characteristic length L and a generalized aspect ratio AR characterize geometrical models for five particle morphologies. Panel B illustrates NP coating with a dielectric shell of constant thickness s ; (A) NRs have hemispherical caps at the tips; (C) nanodisks have semicircular edge profile; (D) nanotriangle prisms have smooth edges with rounding radius $(L + t)/40$; (E) bicones have rounded tips and circular base edge with rounding radii $d/40$ and $d/100$, respectively; (F) bipyramids have the pentagonal base and smooth tips edges.

To eliminate sharp edges for prisms and bipyramids, we used small smoothing parameters proposed by Yu et al. [12]. However, the experimental as-prepared and chemically etched bipyramids have variable tip and base radii, which are significantly larger than those in rounding model by Yu et al. [12]. Specifically, the radii of the tips and base increase as the overall length of the bipyramid decreases [29]. Since the bipyramid diameter remains nearly constant during chemical etching, the experimental data can be approximated by simple linear relationships.

$$\begin{aligned} R_{tip}(\text{nm}) &= 14 - 2AR_1, \\ R_{base}(\text{nm}) &= 16 - 2AR_1, \\ AR_1 &\leq 6. \end{aligned} \quad (2)$$

For benchmark calculations, we use the finite element method with the commercial package COMSOL Multiphysics 5.1 (Wave Optics module) and T-matrix codes [30]. For details concerning COMSOL and T-matrix simulations as well as the tabulated optical constants data and its approximations, the readers are referred to our previous works [11, 14].

To calculate the size-corrected dielectric functions of gold and silver we used the classical approach [19, 26, 27] in which the tabulated bulk data are corrected by difference between the Drude-Lorentz conductive electron terms with bulk and size-corrected dumping constants

$$\Delta\varepsilon(\omega, a) = \frac{\omega_p^2}{\omega(\omega + i\gamma_b)} - \frac{\omega_p^2}{\omega(\omega + i\gamma_p)}. \quad (3)$$

In Eq. (3), the particle dumping parameter γ_p is given by Eq. (1) with the scattering constant A_s which includes two contributions from the surface electron scattering [19] and the chemical interface dumping chemical interface damping (CID) caused by changing the nanoparticle's chemical interface [31, 32]

$$\gamma_s = \left(A_s^{surf} + A_s^{CID} \right) \frac{V_F}{l_s} = A_s \frac{V_F}{l_s}, \quad (4)$$

where the mean free path of electrons l_s is proportional to the ratio of the particle volume V and surface S according to the Coronado and Schatz [33] Eq.:

$$l_s = \frac{4V}{S}. \quad (5)$$

Note that we did not include radiation dumping term [34, 35] in Eq. (4) because the full wave finite element method (FEM) solution accounts for the radiation dumping automatically.

One important note concerning possible quantum mechanical (QM) effects in small metal particles is in order. Kawabata and Kubo [36] were the first to note that the classical interpretation of size effects, based on surface electron scattering by the nanoparticle surface, is not correct. According to QM treatment, the size-

correction effect stems from the appearance of surface discrete levels and an additional imaginary part of the dielectric function due to dipole transitions between these levels. However, it was an unexpected result that more sophisticated QM calculations led to the same inverse proportionality between the damping constant and the characteristic particle size [37–39]. Therefore, here we adopt the classical size-correction scheme (Eqs. (3–5)).

In our MEM + DEM method [11], the generalized polarizability of a coated plasmonic particle is given by the following analytical expression:

$$\alpha_{av} = R_{2ev}^3 \sum_j q_j(h_2) \frac{\bar{\varepsilon}_j - \varepsilon_m}{3\varepsilon_m + 3L_{2mj}(\bar{\varepsilon}_j - \varepsilon_m)}, \quad (6)$$

$$L_{2mj} = \frac{1}{1 - \eta_j(h_2)} - A_j(h_2, \varepsilon_m), \quad (7)$$

$$A_j(h_2, \varepsilon_m) = a_{j2}(h_2)x_{2m}^2 + i\frac{2}{9}q_j(h_2)X_{2m}^3 + a_{j4}(h_2)x_{2m}^4, \quad (8)$$

$$x_{2m} = \frac{L_2 k_m}{2\pi}, \quad X_{2m} = R_{2ev} k_m, \quad k_m = k\sqrt{\varepsilon_m}, \quad (9)$$

where the summation is over all modes “ j ” ($j = 1$ corresponds to the dipole eigenmode); R_{2ev} and L_2 are the equivolume sphere radius of the coated particle and its characteristic size, respectively, $q_j(h_2) = V_j^m(h_2)/V_2$ are the modal volume fractions for mode “ j ”, $V_j^m(h_2)$ and V_2 are the modal and total volumes of the coated particle. For metal particles without coating, the parameter $\bar{\varepsilon}_j$ is simply replaced by the metal dielectric function ε_1 reducing the above equations to the original MEM form [12]. Eigenvalues $\eta_j(h_1)$ and $\eta_j(h_2)$ are the key parameters of MEM. They depend on the particle shape only or, in other words, on the generalized aspect ratio $h_i = AR_i$, $i = 1, 2$. It should be emphasized here that $\eta_j(h_1)$ and $\eta_j(h_2)$ do not depend on the particle size, composition, or environment thus making MEM + DEM approach quite universal. Analytical expressions for all MEM parameters are available in our previous work [11].

The MEM polarizability (Eq. (6)) depends on the incident light's polarization but not on its direction relative to the particle frame. In practice, the extinction and scattering cross sections averaged over random particle orientations are given by following Eqs. [30]:

$$\langle C_{ext} \rangle = \pi R_2^2 \langle Q_{ext} \rangle = \frac{4\pi}{k'_m} \text{Im} \left(k_m^2 \frac{\alpha_{av}^x + \alpha_{av}^y + \alpha_{av}^z}{3} \right), \quad (10)$$

$$\langle C_{sca} \rangle = \pi R_2^2 \langle Q_{sca} \rangle = \frac{8\pi}{3} |k'_m|^4 \frac{|\alpha_{av}^x|^2 + |\alpha_{av}^y|^2 + |\alpha_{av}^z|^2}{3}, \quad (11)$$

where $k'_m = \text{Re}(k_m) = (2\pi/\lambda)\text{Re}(n_m)$, is the real part of the wave vector modulus, the angular brackets stand for averaging over random orientations, and superscripts x, y, z correspond to polarizations of the incident light along the frame axes related to the particle.

3 Results and Discussion

First, consider the effects of dielectric coating on plasmonic cores with bulk optical constants. Figure 2 shows the excellent accuracy of the analytical model in predicting the main LPR peaks for randomly oriented silver and gold nanorods as the shell thickness increases from 0 to 30 nm. In this and subsequent figures, the shell refractive index is 1.5, and the external medium is water. A similar agreement between analytical and numerical spectra was observed for scattering cross sections (data not shown).

Extensive COMSOL and MEM + DEM simulations show that the analytical MEM + DEM approach accurately predicts the extinction and scattering spectra of gold and silver nanorods with a maximum length of 200 nm, aspect ratios from 1 to 6, and dielectric shell thicknesses up to 100 nm.

The greatest difference between COM and MEM solutions for gold nanorods occurs at a moderate aspect ratio ($AR = 2$) and a maximum shell thickness of 30 nm. The COM model gives extinction and scattering peaks of $C_e = 1379 \text{ nm}^2$ and $C_s = 73.9 \text{ nm}^2$ at 646 nm, compared to MEM values of 1359 nm^2 and 64 nm^2 at 642 nm. This results in maximal errors of approximately 0.6% in peak position and 1.5% in extinction cross section. The error for the scattering resonance peak is significantly larger, about 14%. These trends are similar for silver nanorods. It is

important to note that all comparisons between MEM and COMSOL focus only on the main dipolar plasmonic peaks, as MEM does not capture minor multipolar extinction and scattering peaks.

Figure 3 compares numerical (blue) and analytical (red) extinction (A) and scattering (B) spectra calculated for in-plane excitation of silver disks with the coating thicknesses from 0 to 30 nm. Minor short wavelength peaks are omitted because MEM theory does not reproduce them. There is excellent agreement between analytical and numerical spectra in peak positions, amplitudes, and their dependence on the shell coating thickness. Based on extensive simulations, the analytical approach is valid for gold and silver disks with a core diameter of 120 nm, core thickness of 20 nm, and shell thickness of 60 nm. The above numbers should be considered upper boundaries. The maximal MEM errors for gold NDs occur for $20 \times 120 \text{ nm}$ disks with a 30-nm coating. In particular, the PR peak positions and the extinction and scattering cross sections are: MEM – 787 nm, $1.281 \times 10^5 \text{ nm}^2$, and $1.092 \times 10^5 \text{ nm}^2$, respectively; COM – 784 nm, $1.281 \times 10^5 \text{ nm}^2$, and $1.092 \times 10^5 \text{ nm}^2$, respectively. For silver NDs similar numbers are: 787 nm, $1.348 \times 10^5 \text{ nm}^2$, and $1.046 \times 10^5 \text{ nm}^2$, respectively. Thus, the corresponding relative MEM errors are 0.4%, 0%, and 4.4%. For AgNDs, similar estimations give a bit higher errors: 1% (peak position), 3.2% (extinction peak), and 1.2% (scattering peak).

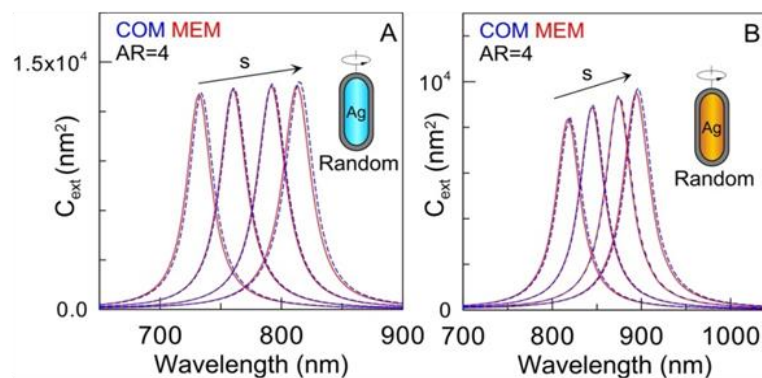


Fig. 2 (A) Extinction spectra of randomly oriented silver and (B) gold coated nanorods with a core diameter of 15 nm, aspect ratio 4, and coating thicknesses $s = 0, 3, 10$, and 30 nm. Calculations by COMSOL (blue) and MEM + DEM (red) methods.

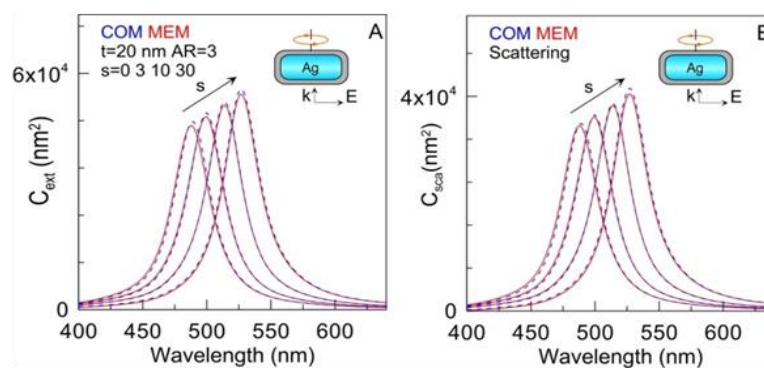


Fig. 3 (A) Extinction and (B) scattering spectra of silver nanodisks at in-plane excitation. The coating thicknesses $s = 0, 3, 10$, and 30 nm, and the disk thickness and aspect ratio are 20 nm and 3, respectively. Calculations by COMSOL (blue) and MEM + DEM (red) methods.

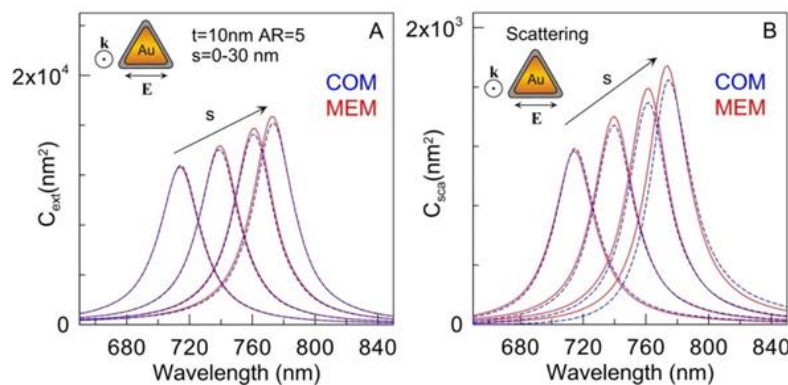


Fig. 4 (A) Extinction and (B) scattering spectra of gold nanotriangles with a thickness of 10 nm and aspect ratio of 5. Note that the scattering cross-section values are one order less than those for the extinction cross sections.

Figure 4 demonstrates the excellent accuracy of the analytical MEM + DEM method in predicting the extinction and scattering spectra of gold triangular nanoprisms with a 10 nm thickness and increasing dielectric coating from 0 to 30 nm. Similar results were obtained for silver and gold nanotriangles of various thicknesses. Minor short-wavelength multipolar peaks from COMSOL are not shown here. For gold NTs, most significant difference between MEM and COM spectra peaks occur for a thick 30×150 -nm particles without coating: MEM – 810 nm, $1.345 \times 10^5 \text{ nm}^2$, and $1.030 \times 10^5 \text{ nm}^2$, respectively; COM – 814 nm, $1.425 \times 10^5 \text{ nm}^2$, and $1.102 \times 10^5 \text{ nm}^2$, respectively. In other words, the percentage differences are 0.5% (peak position), 5.6% (extinction), and 6.5% (scattering). Finally, for bare 30×250 nm silver NTs, the percentage difference between MEM and COM peak position, extinction and scattering are 0.8%, 5.3%, and 5.45%, respectively.

Compared to rods, disks, and triangles, the analytical spectra of coated bicones and bipyramids did not match the numerical results obtained with COMSOL. We investigated this issue and hypothesize that the discrepancy between MEM + DEM and COMSOL may be due to strong localization of the local field near the sharp bicone tips [11]. We also compared the COMSOL and MEM dependencies of plasmonic peak positions on shell thickness, starting with very thin coatings of 0.2 nm. These dependencies differ significantly when plotted against both shell thickness and shell volume fraction. Specifically, COMSOL predicts larger plasmonic shifts than MEM. One possible explanation is the concept of the local shell volume fraction excited by the intense local field near the bicone tips. This feature enhances the sensitivity of plasmonic bicones and bipyramids [40] to their local environment. However, the strong localization near the tips violates the assumption of a homogeneous internal field distribution, which questions the physical basis of MEM + DEM in this context.

To improve the MEM accuracy, we introduced [11] a new MEM + DEM version in which the MEM parameter $\varepsilon_1(AR)$ and the modal volume function $V_1(AR)$ are represented in factorized forms $\varepsilon_1(AR)f_\varepsilon(s)$ and

$V_1(AR)f_V(s)$ where functions $f_\varepsilon(s)$ and $f_V(s)$ depend on the shell thickness only and do not depend on the particle metal, size, and shape. In the original version of MEM [12], the modal volumes and eigenvalues were functions only of the generalized shape parameter and did not depend on the particle size or their material (gold or silver). Our proposed theory for coated plasmonic particles [11] used the dipole equivalence method we developed earlier [13] to relate the original MEM polarizability of a metal particle to the polarizability of a coated particle. Generally speaking, it was not obvious a priori that this approach would work for particles of arbitrary shape. However, comparison with numerical calculations showed that for all realistic particle models, including pentagonal bipyramids with vertex radii of 2–6 nm, the MEM + DEM method yields excellent results. An exception was found only for a model with a geometric feature – a bicone with a very small vertex radius of curvature. For this model, the influence of a very thin coating exhibits clear nonlinearity, and the analytical spectra disagree with the numerical ones regarding the coating-induced shift of the plasmon resonance. As we stated previously, the physical reason for this is related to the strong non-uniformity of the local field near the vertices. From a physical point of view, this means that the modal volumes and eigenvalues of the electrostatic problem should now depend not only on the shape but also on the coating thickness. In the general case, they should be functions of two variables. The idea of the factorization method is that these (unknown) functions of two variables can be represented as a product of shape functions and coating thickness functions. Here, the shape functions are the same universal parameters of the original MEM and do not depend on the particle size or shape material. On the other hand, the coating thickness functions should be equal to 1 at zero thickness. They also do not depend on the particle size and, to a first approximation, do not depend on the shape material.

We derived the factorized functions by comparing benchmark simulations with analytical spectra and introduced them to the calculation scheme. Figure 5 illustrates the excellent accuracy of the factorized MEM method in predicting the extinction and scattering cross-section spectra. Similar results were obtained for coated

gold and silver bicones with other aspect ratios from 2 to 6 and other diameters from 10 to 30 nm. Note that because of more complex factorized MEM+DEM parametrization, we used different MEM parameters for silver and gold bipyramids. In a sense, the universality of the factorized analytical method is somewhat limited, but this is compensated by higher accuracy.

By contrast to sharp-tipped bicones, for bipyramids with the rounding radii as given by Eq. (2) the MEM + DEM analytical method works almost perfectly without any additional factorized parametrization for the eigenvalues and modal volumes (Fig. 6). Figure 6 demonstrates excellent accuracy of MEM + DEM method for extinction spectra of gold and silver bipyramids with dielectric coating thickness ranging from zero (bare metal) to 30 nm. In this simulations, the width of bipyramids was set to be 30 nm and the aspect

ratio varied from 2 to 5, in agreement with most experimental observations [29, 41–44]. The shell thickness was varied from 0 to 30 nm although equally good agreement between MEM and COMSOL spectra was obtained for thicker shells (up to 60 nm) when the shell volume fraction was greater than 0.9. The main differences between MEM and COM spectra are observed for slightly elongated ($AR = 2$) coated ($s = 30$ nm) gold bipyramids. For example, the peak positions and amplitudes are: MEM – 564 nm, 4649 nm² (extinction), and 316 nm² (scattering); COM – 568 nm, 4832 nm² (extinction), and 365 nm² (scattering). Accordingly, the percentage difference between MEM and COM peak position, extinction and scattering are 0.7%, 3.8%, and 13.4%, respectively. Similar estimations hold also for silver bipyramids.

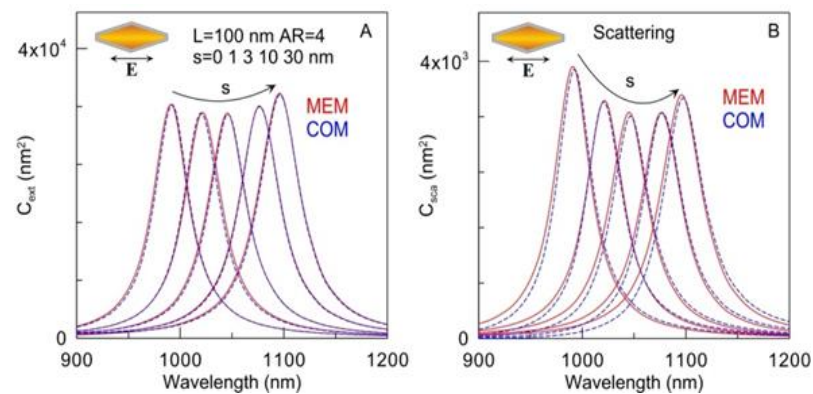


Fig. 5 Extinction (A) and scattering (B) spectra coated gold bicones excited along the symmetry axis $L=100$ nm. The aspect ratio is 4, and the coating thicknesses are $s=0, 1, 3, 10$, and 30 nm. Calculations by COMSOL (blue) and factorized MEM + DEM (red) methods. Note the extraordinary sensitivity of the LPR peak position to the local environment (thin 1-nm dielectric coating).

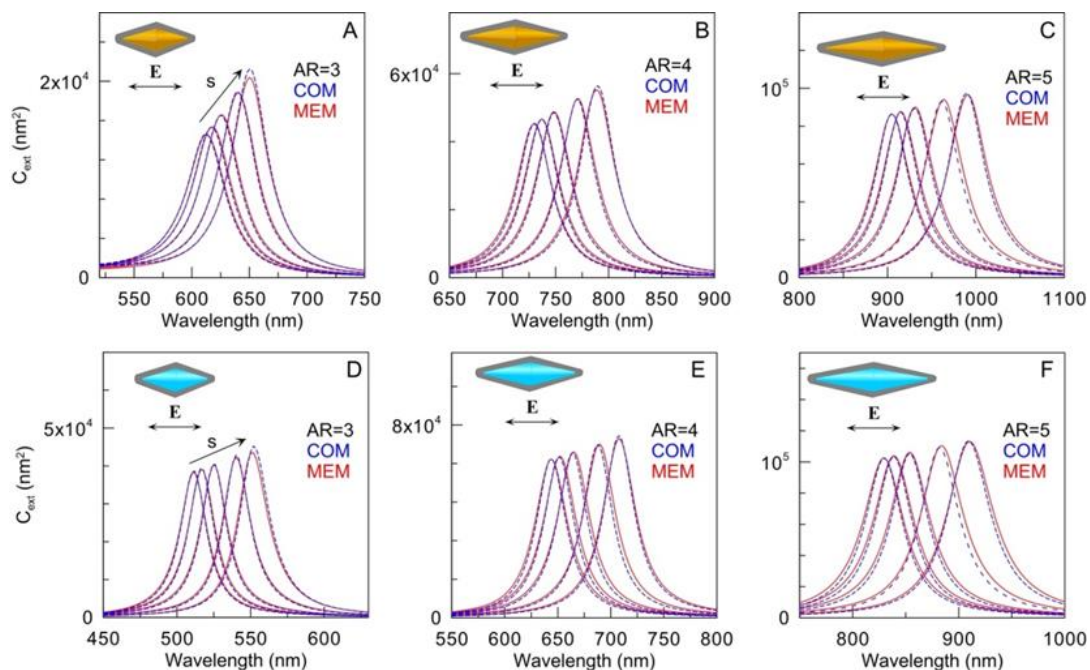


Fig. 6 Extinction spectra of gold (A-C) and silver (D-F) bipyramids calculated by MEM (red) and COMSOL (blue) for a constant width of 30 nm and aspect ratios 3, 4, and 5. The shell thicknesses are 0, 1, 3, 10, and 30 nm. The rounding radii are given by Eq. (2).

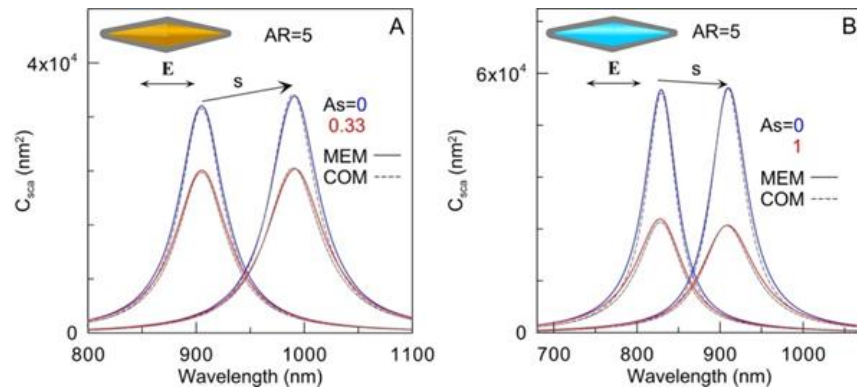


Fig. 7 Scattering spectra of (A) gold and (B) silver bare and coated ($s = 30$ nm) bipyramids with a fixed diameter of 30 nm, aspect ratios of 5, and rounding radii of tips and bases are determined by Eq. (2). Calculations by COMSOL (dashed lines) and MEM (solid lines) under in longitudinal excitation for bulk ($A_s = 0$, blue) and size-corrected optical constants ($A_s = 0.33$ for gold and $A_s = 1$ for silver, red).

Now, we briefly discuss the main differences between extinction and scattering spectra calculated using bulk and size-corrected optical constants for gold and silver (a more detailed discussion can be found elsewhere [45]). In practical simulations, selecting the electron scattering constant is the most challenging step. For literature data and discussion of this point, the readers are referred to papers [25, 26]. In this work, we used $A_s = 0.33$ [46, 47] for gold and $A_s = 1$ for silver [25]. We conducted extensive simulations for five particle models, two metals (gold and silver), and shell thicknesses ranging from 0 to 100 nm. These results will be discussed elsewhere. Here, we focus on a single example that exhibits the most pronounced size-correction effects.

Figure 7 shows scattering spectra of gold and silver bare ($s = 0$) and coated ($s = 30$ nm) bipyramids. Calculations were performed for bulk ($A_s = 0$, blue) and size-corrected ($A_s = 0.33$ for gold and 1 for silver; red) dielectric functions. First, we again note excellent agreement between analytical (MEM) and numerical (COMSOL) spectra. Second, there is significant reduction of resonance scattering amplitudes and peak broadening, especially for silver particles. Finally, we note somewhat unusual decrease in scattering peak for coated silver particles. Similar effects were described and explained previously [11].

4 Comparison of MEM + DEM with Other Analytical Approximations

The simplest analytical approach is the quasi-static (or electrostatic) approximation (EA), which is valid in the limit of small particle sizes. In particular, analytical solutions are known for two-layered spheres and spheroids [48]. However, we have previously shown [14] that for gold and silver spheroids with sizes on the order of 50–100 nm, the EA is not applicable for predicting either the position or the amplitude of the plasmon resonance, even for metallic particles, let alone coated ones.

Another physical approach is to treat a plasmonic particle with a single-layer or multi-layer dielectric coating as a homogeneous particle with some effective refractive index. Essentially, a similar idea underlies the Discrete

Dipole Approximation (DEM). There are many theoretical approaches of this type, collectively known as the family of effective medium approximations (EMA) [49]. The history of EMA goes back to pioneering works by Maxwell Garnett [50] and Bruggeman [51], with sequential development in many reports (see, e.g., Refs. [52] and [53]). Stroud and Pan [54] suggested a so-called extended EMA (EEMA) to go beyond the small-size limitation of classical EMA. For a very useful and detailed discussion of various EMA versions, the reader is referred to the book chapter by Chylek et al. [49]. All versions of EMA are computationally simple and offer analytical insight into the averaged behavior of various composites. They can describe phenomena such as the plasmonic redshift with increasing filling fraction. However, they have serious limitations because the key assumption of EMA is that inclusions are small, typically spherical in shape, isolated, and embedded in a homogeneous local field. In other words, arbitrary particle shapes and sizes are beyond the typical EMA framework, which works within the small-sphere limit. In contrast, the MEM + DEM approach is designed for plasmonic coated particles of arbitrary shape and typical core sizes under 150 nm, offering reasonable accuracy compared to more time-consuming numerical methods.

The quasinormal mode (QNM) method is a more sophisticated semi-analytical approach for open resonant systems, such as optical cavities, nanoparticles, and metamolecules [55]. Quasinormal modes are the complex-frequency eigenmodes of such systems with radiative losses. Once the QNMs are computed (typically via a numerical solver like FEM or finite difference time domain (FDTD)), the optical properties are represented as a sum over QNMs. Such expansions provide an efficient and physically intuitive approach to describe Purcell effect and Fano resonances. However, the accuracy of the expansion depends on the completeness of the QNM set, which makes the QNM approach more complex and computationally demanding compared to MEM + DEM. For complex systems like large-scale random colloidal aggregates, QNMs become impractical.

5 Conclusion

We have expanded the MEM approximation developed for metallic nanoparticles to the coated nanostructures. It should be emphasized that our MEM + DEM approach does not require any additional numerical simulations except for those needed for the original MEM. By comparing analytical spectra with benchmark COMSOL simulations, we have demonstrated excellent accuracy and shape universality of the MEM + DEM approach.

Based on extensive simulations (the full data are not shown here; see, for instance, related references [11, 14, 45]) we can recommend the MEM + DEM approach for various coated particle geometries, provided that their maximal size, coating thickness, and generalized aspect ratio do not exceed 150 nm, 60 nm, and 10, respectively. Moreover, MEM + DEM is applicable to particles with either bulk or size-corrected optical constants [45]. Finally, their minimal curvature radii should be greater than those indicated in the original MEM paper [12]. For typical experimental pentagonal bipyramids, MEM + DEM is applicable as is.

We hope this extension can find valuable applications in simulating various properties of coated plasmonic particles. In particular, it would help us understand how the particle coating modulates absorption and scattering spectra, plasmon quantum yield, SERS enhancement, and metal-enhanced fluorescence. In particular, the

MEM + DEM approach provides a simple yet accurate analytical tool for simulating the plasmonic properties of coated metal nanoparticles and for developing machine-learning models. Recent trends clearly indicate a significant growth in studies and publications that integrate state-of-the-art synthesis protocols with AI capabilities [56–60]. Two instructive examples applying machine learning to the synthesis of gold nanorods [61] and nanostars [62] were recently published. The developed MEM + DEM analytical models are believed to be useful for predicting optimal strategies to achieve precise optical control of both intermediate and final nanoparticle products.

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Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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High-Temperature Transformation of Graphite Coating on Silica Surface under Continuous Laser Radiation

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Abstract. The physicochemical transformation of a graphite coating on silica glass (fiber and plate), when heated in air by micrometer wave laser radiation with a power of several kW/cm² with temperature up to 2500 K at which optical transmission increases from zero to 10% (UV range) and up to 40% (IR range), was detected. Based on a complex of the conducted studies, it was concluded that, along with elimination of part of absorbing species, the graphite powder of the coating as well as silicon dioxide and atmospheric oxygen present in its composition transform to others, transparent compounds: silica, silicon carbide SiC, and presumably (tri)(di)carbonates transparent in UV-VIS up to ~5 eV. Until recently, (tri)(di) carbonates of silicon were known mainly from theoretical works. The high-temperature coating consists of residual graphite, which allows laser heating, and C-O-Si compounds, including carcinogenic SiC at a level of ~2 10–6 mg.

Keywords: laser scalpel; semiconductor laser; silica fiber; light absorbing coating; high temperature stabilization; physicochemical transformation of coating materials; silica carbonates.

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1 Introduction

The study of material properties at temperatures of about 2500 K and the development of methods for synthesizing new materials are still highly relevant. Indeed, phase transitions of many important materials such as silicon carbide, diamond, quartz, a number of nonlinear optical, laser materials occur in this temperature region. Information on key parameters in this critical region is neither accurate nor reliable [1]. The same concerns both, developing technology production of traditional materials (quartz silicon carbide, diamond) [2–6,7,8] and searching for methods of obtaining new materials, for example, silicon (tri)(di)carbonates [9–12] and carbon-nitride materials [13]. A number of issues associated with solar hydrogen energy and CO₂ reduction in the atmosphere are related to this field of research [9–12, 14]. A number of works on producing materials under shock high-temperature conditions using pulsed lasers appeared in the last decay [15–19].

Recently, this problem has attracted researcher attention due to the advent of laser sources capable of creating a high radiation density of up to several kW/cm² in continuous mode without preliminary focusing, for example, semiconductor lasers with a fiber-optic silica output. It was found that, when a light-absorbing coating is applied to the tip (diameter ~0.5 mm) of a fiber of a semiconductor laser with a fiber-optic output (laser scalpel) promising for use in medical practice [15], the tip is heated in air to high temperatures (up to ~2500 K initially with next stabilization to ~1800 K) [21, 22]. This opened up new opportunities for laser surgery, in particular, for the treatment of urolithiasis [23,24] and malignant tumors [25]. The maximum temperature of the new point tool is determined by the temperature stability of silica that is used as a fiber material.

Laser surgery mainly uses fiber optic lasers, the cleaned tip of the quartz fiber acts as the cutting element [26, 27], whereas obtaining newly emerging chemical compounds as a result of high temperature processes

require additional study, for example, in terms of cancerogenicity. Therefore, from the point of view of toxicology, the study of high-temperature interaction of a silica fiber with a carbonized biotissue is important, especially in connection with the use in laser surgery of highly absorbing coatings, graphite being one of their components. The possibility of producing high-temperature objects with high temperature and pressure gradients is apparently associated with the recent reports on microdiamonds synthesized by laser and other methods with similar physical parameters [18, 19], on synthesized silicon carbide, carbide based ceramics and oxide based ceramics in silicone resins [28]. It is very interesting to study in more detail the phenomena occurring in materials of a light-absorbing layer heated by absorbed laser radiation [21, 22], both for applications of such fibers in medical practice and, in a broader field of high-temperature physics, for the search for new high-temperature materials and the improvement of production technologies for materials such as diamond, silicon carbides, etc. using new laser devices as sources of high-power controlled local heating.

Animal experiments [25] demonstrated the potential of the new instrument. Experimental lithotripsy procedures on real patients also proved successful [23, 24]. However, concerns about the safety of the high-temperature instrument based on a graphite-silicone coating regarding its carcinogenic potential arose. Indeed, at high temperatures (up to ~2500 K) during the production of high-temperature coatings, the starting materials form new products whose carcinogenic properties should be studied. One such product may be SiC, which is classified as a hazard class 4 [29]. Considering the extreme conditions of obtaining a high-temperature coating, the possibility of the formation of other compounds cannot be ruled out. The goal of this work is to determine the chemical composition of the products and the chemical processes under extreme temperature and radiation loads occurring when the silica fiber tip in contact with a graphite coating containing organosilicon substances is exposed to high-power IR radiation. For this purpose, we use the methods of UV-VIS and IR spectroscopy, optical microscopy, X-ray spectroscopy (X-ray powder diffraction), scanning electron with X-ray microanalysis microscopy, and analytical balance.

2 Equipment and Methods

The following equipment was used in the study: Lakhta-MilonTM lasers (Russia, Saint-Petersburg) with fiber-optic output and wavelengths of 1.315; 0.97 and 0.81 μm ; Shimadzu UV-1800 spectrophotometer (Japanize, Tokio); FSM 1201 FT-IR spectrometer; Nikon Eclipse CI-1800 microscope; XRD diffractometers, including Shimadzu XRD-6100 diffractometer, CuK α 1 operating in 10–60 deg, 2 deg/min mode; scanning electron microscope JSM-IT300LV (JEOL, Japanize, Tokio) with electron beam diameter of about 5 nm at a probe current of less than 0.5 nm; elemental

X-ray microprobe analyzers with X-MaxN 20 detector (Oxford Instruments, USA); and Shirko VIBRA AJH-CE analytical balance, accuracy 1 mg.

The high color temperature of a laser heated object was determined from measurements of the emission spectrum of the heated coating with the Ocean Optics QE65Pro spectrometer (USA) in the range of 400–800 nm. Accuracy is approximately 20 K.

The object under study was a high-temperature graphite coating applied to the output tip of a silica fiber of a semiconductor laser, or to the surface of a silica-glass optical plate heated by absorbing IR radiation from the semiconductor laser (the effect is virtually independent of wavelength). The coating application technology, in brief, consists in applying a drop of graphite powder emulsion in silicon varnish to the distal end of the fiber or the optical silica plate. The varnish with graphite powder is dried at a temperature of several hundred degrees K and then heated by laser radiation up to ~1000 K. Note that the coating is colored black. Next, the laser power is increased (up to ≥ 1 W in the case of the fiber). The coating temperature rises sharply, the tip is visualized as flashes of white light, then within ~1 s the glow intensity decreases and the temperature self-stabilizes at ~1800 K. As a result, the coating structure changes and becomes grey after cooling [21, 22]. Clearly, this is connected with the processes in the coating and in the fiber material, as a result of which physicochemical transformations of the coating composition can occur.

In what will follow, we will present the results of physicochemical studies of the structure and composition of the obtained coating.

3 Results

3.1 Microscopy

Primary microscopic data on the structure of the coating immediately on the fiber tip (diameter of about 0.5 mm) show the change in its color from black to grey [21]. Since investigation of such a specific sample is rather difficult, a few optical studies were carried out on a model, analogue sample. A coating of the same composition was applied to an optical silica plate as ~9 cm² area spot; the model sample was exposed to the radiation from the same laser using a piecewise raster technique with a laser beam defocused by 1.5–2 times with a corresponding power increase (Fig. 1). Thus, the temperature regime for obtaining an analog coating on silica (ACS) remained almost the same as for the fiber. The dried, non-irradiated coating on the silica plate (spot 1) and after irradiation (spots 2 and 3) is shown in Fig. 1. The spot 2 was preheated at a laser power of 100 W/cm² ($T < 1000$ K, the spot remained black) and then annealed at a power of 400 W/cm² in the high-temperature glow mode ($T = (2500, 1800)$ K). The spot 3 was annealed at a power of 600 W/cm² without preheating. No ablated light-absorbing particles were detected during the high-temperature annealing ($T \approx (2500, 1800)$ K), but the mass of the coating decreased by 2–3 times.



Fig. 1 (1) Absorbing coating on silica plate. Dried (~ 300 K) drop; (2) dried, heated at 100 W/cm^2 ($T \sim 1000$ K) and annealed at 400 W/cm^2 ($T \sim 2000$ K); (3) dried at room temperature and annealed at 600 W/cm^2 ($T \sim 2000$ K).

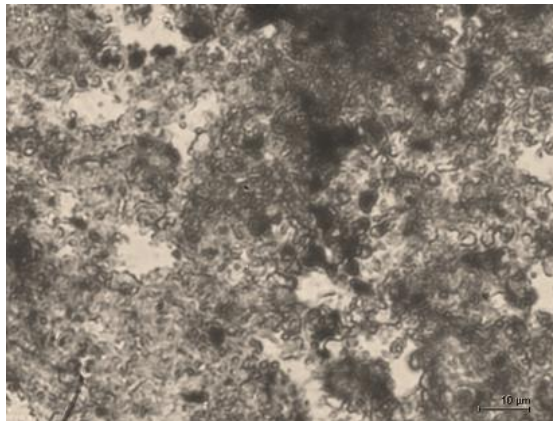


Fig. 2 ACS microstructure.

The ACS microstructure after annealing is shown in Fig. 2. The coating has a rather inhomogeneous transparency and density of microparticle components.

3.2 UV-VIS Spectroscopy

The UV-VIS transmission spectra obtained for the sample at different stages are presented in Fig. 3: 1 – the sample dried at room temperature, 2 – dried at room temperature and exposed to a temperature of ~ 1000 K, 3 – dried at room temperature and irradiated by a laser at a power of 6 W ($T \sim 2000$ K), 4 – dried at room temperature, irradiated by 1 W laser ($T \sim 1000$ K) plus exposed to 6 W radiation ($T \sim 2000$ K); 5 – transmission spectrum of dried silicon varnish.

Obviously, during high-temperature laser treatment the transmission increases from 0 to 40% at the laser wavelength and smoothly decreases to 15% in the ultraviolet region. The drop at absorption at a wavelength of $1 \mu\text{m}$ explains the temperature stabilization effect [21]. The drop at absorption in a wide region indicates ablation of most of the graphite from the irradiated region and the transition of the remaining graphite and other coating components to secondary chemical forms with band-gap energies of $3\text{--}5.5 \text{ eV}$. The residual absorption is apparently determined by the remains of graphite and uncontrolled impurities. The regular increase in losses with a decrease in wavelength is most likely due to light scattering on a polydisperse coating. Based on the available literature data [28], it was assumed that silicon dioxide SiO_2 and silicon carbide SiC can be formed as a result of decomposition of silicon varnish under high-temperature (up to $\sim 1000^\circ\text{C}$) conditions. On the other

hand, SiO_2 (the fiber and the glass substrate are also SiO_2) interacts with graphite C at temperatures of $1600\text{--}2500^\circ\text{C}$ following the known reaction $\text{SiO}_2 + 3\text{C} \rightarrow \text{SiC} + 2\text{CO}\uparrow$ to yield silicon carbide SiC . SiC microcrystals were observed, for example, in a silicon film irradiated with femtosecond laser pulses in the transparency band ($\lambda = 522 \text{ nm}$) [28]. The band gap of SiC is known to be $\sim 3 \text{ eV}$ [29–31]. The possibility of diamond formation under similar conditions was speculated in Refs. [18,19,28].

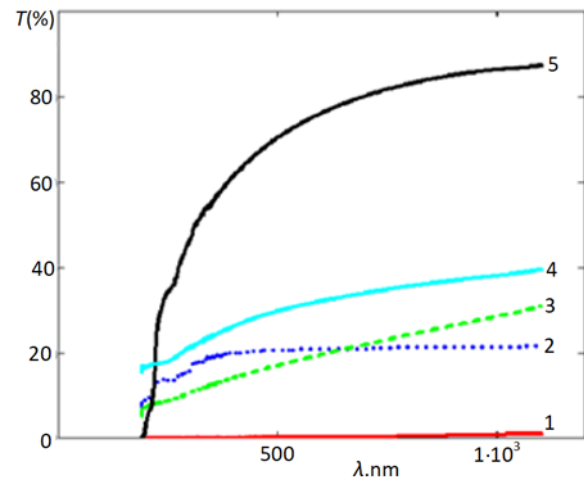


Fig. 3 Transmission spectra of silica plate with light absorbing layer: 1 – dried drop of graphite in silicon varnish, 2 – drop irradiated by 1 W laser, 3 – irradiated by 6 W laser; 4 – drop exposed to 1 W laser plus irradiated by 6 W laser; 5 – dried layer of silicon varnish.

Further studies (UV-VIS-IR spectral, X-ray structural, electron and optical microscopic) were carried out with ACS powder obtained by scraping the coating from a silica plate produced by the method described above. A typical micrograph of the powder with single transparent microcrystallites is shown in Fig. 4.

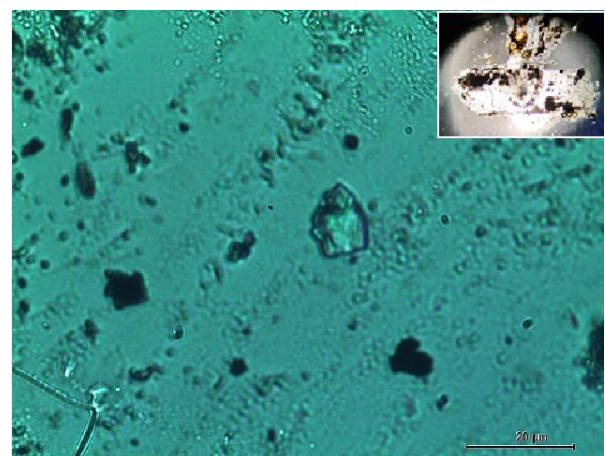


Fig. 4 Photomicrograph of microscraping on object-plate. Shadow mode. Inset: crystalline aggregate ($\sim 10 \mu\text{m}$ diameter).

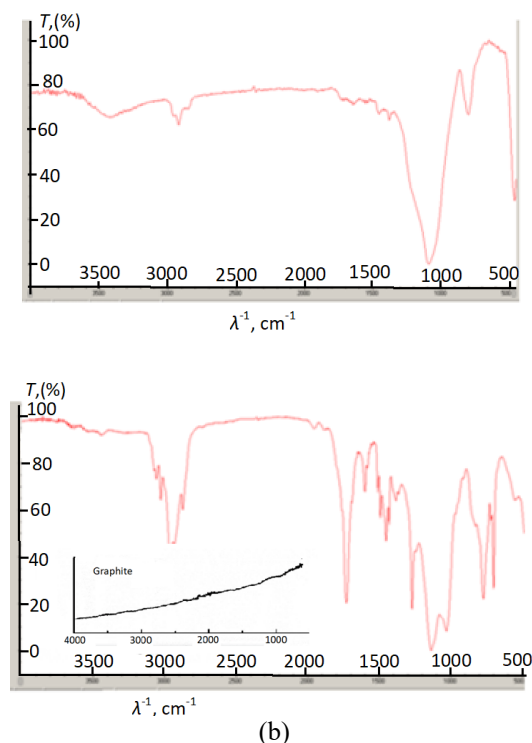


Fig. 5 IR transmission spectrum of ACS powder in KBr pellet (a) and of silicon varnish film on KBr window dried at 200 °C (b). Inset in Fig. 5b shows typical graphite IR spectrum [32].

3.3 IR Spectroscopy

An IR transmission spectrum obtained on ACS powder in a KBr tablet is shown in Fig. 5a. IR spectra of the

samples were measured on a FTIR “FSM 1201” spectrometer (Russia) in the 400–4000 cm^{-1} wavenumbers range at the resolution of 4 cm^{-1} using the KBr pellet. Peak wavenumber accuracy is 0.25 cm^{-1} . Figure 5b shows the spectra of raw materials: silicon varnish on the KBr window dried at $T = 200\text{ }^{\circ}\text{C}$ and graphite (Inset) [33].

The absorption bands at 3500 and 3000 cm^{-1} are due to the presence of impurities in the parent KBr powder (Fig. 5a), and the rest to the powder under study. According to the literature, the 1075, 750, and 480 cm^{-1} bands belong to the Si-O and C-O bonds and their associates [34, 35], including amorphous SiO_2 [35, 36]. The main characteristic absorption bands of SiC are also located in the 500 cm^{-1} and 800 cm^{-1} regions [7]. The analysis of the results of IR absorption spectra does not contradict the presence of silicon carbide SiC in the powder-scraping, but draws attention to the fact that the highest absorption intensity in the spectrum is observed in the 1050 cm^{-1} region, which corresponds to oxygen containing silicon and carbon compounds. Amorphous SiO_2 makes a significant contribution to the ACS powder IR spectra.

3.4 X-Ray Powder Diffraction

The obtained compounds were measured with a Shumadzu XRD-6100 diffractometer featuring $\text{CuK}\alpha 1$ rays in a scanning mode of 10–60°, 2°/min. The measurements were made in a 0.5 mm thick aluminum cuvette. The XRD spectra for two portions of ACS powder obtained in independent experiments are presented in Fig. 6 a, b. The used graphite was also measured (Fig. 6 c).

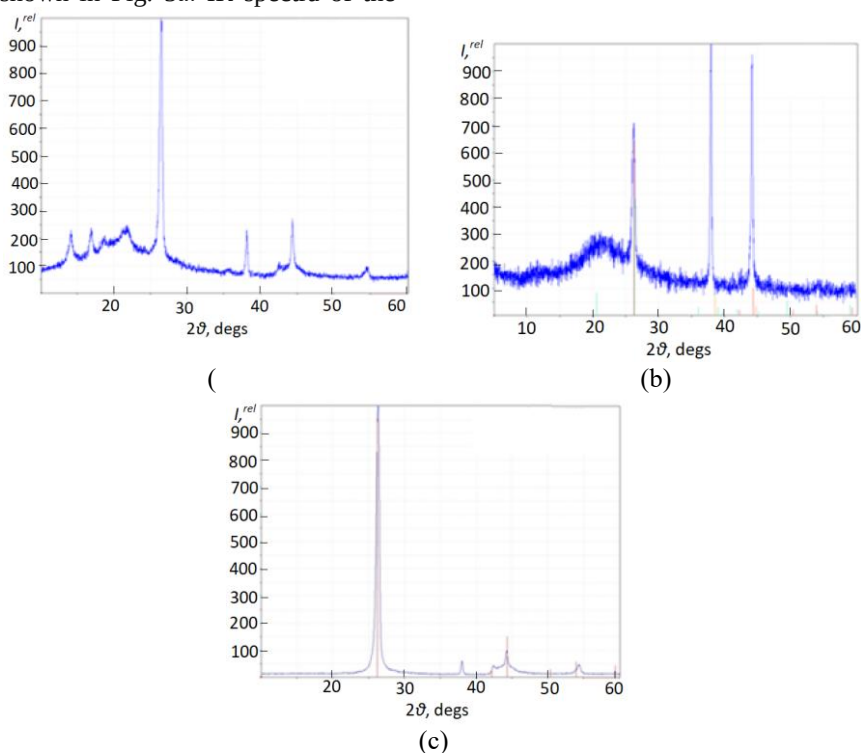


Fig. 6 XR diffractograms of (a, b) ACS product and of (c) graphite powder.

Graphite is present in all samples. In the first measurement (Fig. 6 a), the graphite lines in the powder demonstrate 100% coincidence with the graphite powder (c), both in the angle and qualitatively in the ratio of peak intensities. There is only a small hint to an additional line at about 38°. In Fig. 6 b, the main lines at first sight repeat Fig. 6 a. However, the ratio of the peaks is significantly different. The first peak at 26°, leading in the first measurement, becomes noticeably smaller than the second and the third ones, the ratio of the intensities of the 2-nd and 3-rd peaks also changes to the opposite. It can be concluded that in the second X-ray diffraction pattern, the proportion of graphite decreases significantly and there appears the contribution of another compound. This compound may be SiC, the XRD lines of which in the range of 35–60° are close to the corresponding ACS lines [8], and the intensity ratio corresponds to the observed differences.

A distinct band ($2\theta = 22^\circ$) corresponding to amorphous compounds with Si-O bonds is observed in all XRD spectra of the ACS powder [37]. Further, two reflexes at 14 and 17° are clearly visible in the first XRD. No explanation for these bands has been found in the literature. The discussion will be presented below.

3.5 Scanning Electron Microscopy Studies with X-ray Microanalysis

The images of the micropowder structure (Fig. 7 a–c) of the ACS product were obtained by scanning electron microscopy (SEM) with a JSM-IT300LV microscope (JEOL) having an electron beam diameter of about 5 nm

and a probe current below 0.5 nA. The surface topography of the samples was studied using low-energy secondary electrons and backscattered electrons. The elemental composition (Fig. 7 a–d, Table 1) of the samples was studied using X-ray microprobe analysis (XMP) with X-MaxN 20 detector (Oxford Instruments). The error depends on the element and its concentration in the mixture. For concentrations of 0.1–1 mass%, the absolute error is 0.03–0.25, for concentrations of 1–5 mass%, the absolute error is 0.02–0.75, for concentrations of 5–12 mass%, the absolute error is 0.5–1.2, and for concentrations above 12–15%, the absolute error is 1–3. For light elements—at similar concentrations, the error is 1.5–2 times higher.

An example of general picture with marked sites of elemental composition measurements is shown in Fig. 8 a, an example of carbon distribution in Fig. 8 b, a silicon distribution in Fig. 8c, and an example of oxygen distribution is illustrated in Fig. 8 d.

The comparison of the photomicrographs in Fig. 7 and in Fig. 2 shows the internal structure of the particle – a cellular, dendrite-type structure appeared in the photos in Fig. 8. It should be noted that the Si and O distributions coincide in the element distribution maps (Fig. 8 c, d).

Judging by the element distribution maps, the sample has a heterogeneous composition (Fig. 8a). Two distinctive areas can be distinguished:

- 1) high (predominant) carbon content (Fig. 8 b);
- 2) high silicon and oxygen content (Fig. 8 c and 8 d).

The composition of these phases was measured individually (Table 1).

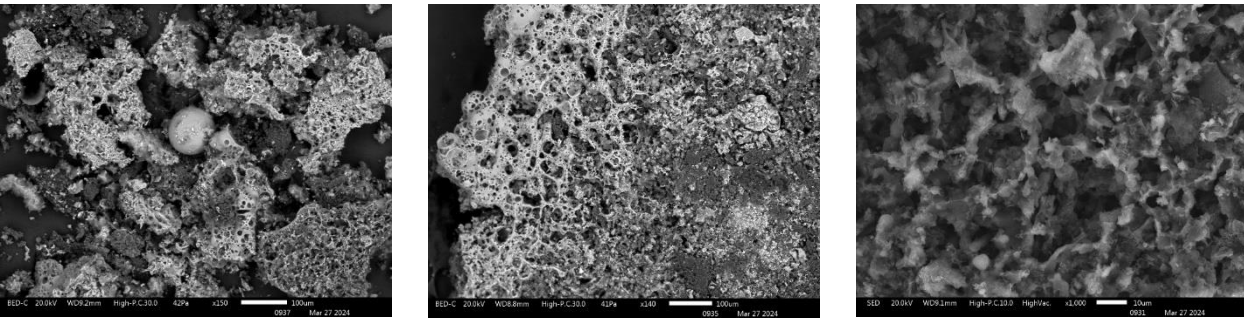


Fig. 7 Scanning electron photomicrographs of the surface of ACS powder particles: (a) separate particles, (b) dendrite-type, (c) pore-type structures.

Table 1 The elemental composition in atomic fractions at the points highlighted in the electron image in Figs. 2 (a–d).

Spectrum	C (atomic fraction)	O (atomic fraction)	Si (atomic fraction)	Area
Spectrum 281	4.132	2.369	0.446	(2)
Spectrum 282	4.518	2.239	0.354	
Spectrum 283	7.184	0.733	0.073	(1)
Spectrum 284	7.267	0.686	0.065	
Spectrum 285	7.151	0.752	0.077	

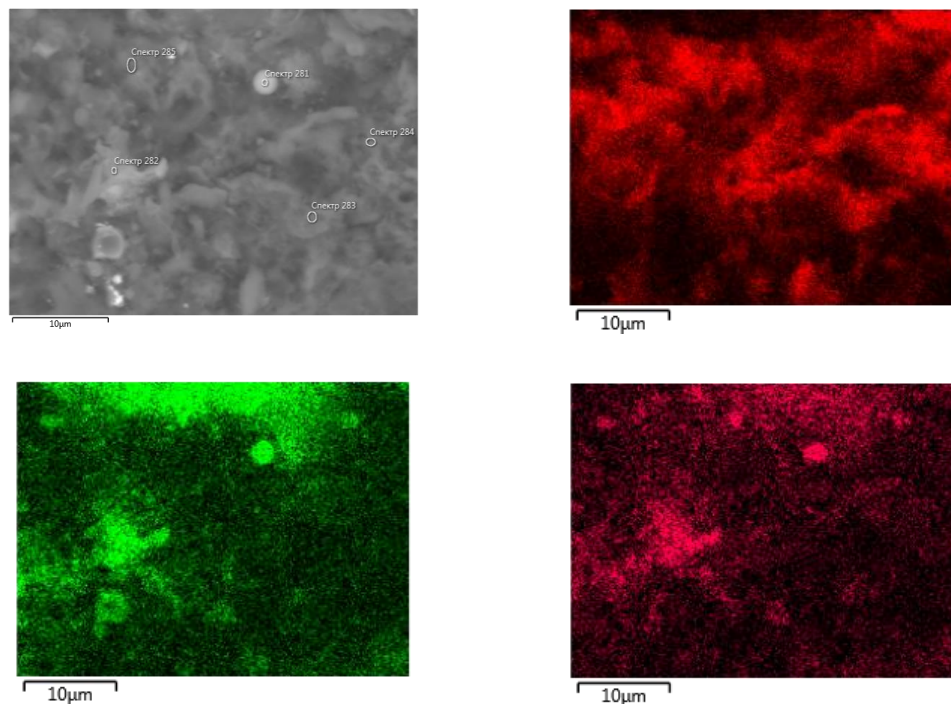


Fig. 8 Electron images of element distribution maps: (a) general, (b) carbon, (c) silicon, and (d) oxygen.

In area (2), the Si to O ratio does not correspond to silicon oxide SiO_2 , since there is much less silicon, while there is a noticeable amount of carbon. Carbon can be either part of the Si-O-C compound or a background signal from area (1).

In area (1), oxygen and silicon are detected besides carbon. The Si to O ratio does not correspond to silicon oxide SiO_2 either, since there is much less silicon. Here we have a phase of pure C or a combination of C and O.

The Si to C ratio in both areas does not correspond to silicon carbide SiC , there is much more carbon. No other impurities were detected within the method sensitivity (0.1 mass%). An increased error in determining the amount of carbon and oxygen (up to 5%) due to the low energy of their X-ray radiation was noted.

Measurements of the coating's atomic composition show that it consists primarily of carbon, silicon, and oxygen. Silicon, the presence of which determines the formation of the high-temperature solid Si-C-O compound, has the lowest concentration. Carbon, in the form of graphite, is introduced during the coating process and determines the residual optical absorption of laser pump radiation. Therefore, the silicon concentration can be used to estimate the potential mass of Si-C-O compounds in the coating. Based on the total mass of the scalpel tip coating of $\sim 2 \cdot 10^{-3}$ mg (according to micro-weighing of samples) and the relative concentration of silicon, it can be estimated that the tip mass of Si-C-O compounds is $\sim 2 \cdot 10^{-4}$ mg. The first result is that the seemingly obvious expectation of the presence of silicon carbide (SiC) is not met. If it is present, its tip content does not exceed a few percent of the silicon atom concentration, i.e., no more than $\sim 2 \cdot 10^{-6}$ mg. This result allays concerns about the hazards of SiC , but raises questions about the residual

mass of the coating. The very high oxygen concentration indicates the possible presence of compounds such as silicon carbonates SiO_2CO_2 , $\text{SiO}_2(\text{CO}_2)_2$, and $\text{SiO}_2(\text{CO}_2)_3$. Information on such compounds, and especially on their potential hazards, is currently extremely limited in the literature.

4 Conclusions

The physicochemical properties of the carbon – silicon coating of the laser scalpel tip in result of laser high temperature heating were studied. It was found that:

- As the temperature is stabilized, the UVI-VIS absorption of initial black carbon-silicon varnish layer drops sharply, the transmission at a wavelength of $1 \mu\text{m}$ increases from 0 to 40%. The transparency window from $\sim 0.2 \mu\text{m}$ to $\sim 1 \mu\text{m}$ is observed. It corresponds to the effective band gap of the coating materials of $\sim 3\text{--}5 \text{ eV}$.
- The high-temperature coating consists of residual graphite, which allows laser heating, amorphous SiO_2 , and C-O-Si compounds mainly. Significant amounts of oxygen have been shown to be present in the resulting lesion. Amount of carcinogenic SiC does not exceed of $\sim 2 \cdot 10^{-6}$ mg.
- We have hypothesized that high-temperature crystalline C-O-Si compounds with a band gap of up to 5 eV such as silicon (tri)(di)carbonates are present in the coating. The properties of such compounds, in particular their carcinogenicity, require further study.

Conflicts of Interest

The authors declare no conflict of interests.

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Highly Accurate Skin Cancer Diagnosis Using HMT-NET and Vision Transformer Models

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Abstract. Skin cancer, especially melanoma, is one of the most aggressive and fatal cancers, with rising global incidence driven largely by ultraviolet exposure. Early detection is critical, as visual similarities among lesions complicate diagnosis. Clinical methods include self-examination, dermoscopic, and biopsy, while computational approaches range from traditional Computer-aided design systems to deep learning models like convolutional neural network and Vision Transformers. Although these models enhance accuracy, they face limitations such as high data requirements, limited interpretability, and inconsistent image quality, emphasizing the need for scalable and explainable diagnostic systems in clinical settings. A Hybrid Multi-layer Transformer Network (HMT-NET) and Vision Transformer (ViT)-based model is proposed for accurate skin lesion segmentation and multiclass skin cancer classification. This study presents a hybrid HMT-NET and ViT framework for accurate skin lesion segmentation and classification. Evaluated on HAM10000 and ISIC2019 datasets, the model achieved high Dice score and Jaccard index and 98.75% classification accuracy, demonstrating superior performance and reliability for integration into automated dermatological diagnostic systems.

Keywords: skin cancer; melanoma; early detection; dermoscopic; biopsy; self-examination; computer-aided diagnosis; convolutional neural network; vision transformer; HMT-NET; image segmentation; lesion classification; deep learning; medical image analysis; explainable AI; scalable diagnostic systems.

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1 Introduction

Skin cancer, driven primarily by ultraviolet (UV) exposure, represents a major global health concern with rapidly rising incidence rates [1]. Melanoma, though less common, causes the majority of skin cancer deaths due to its aggressive nature. Early and accurate diagnosis is

crucial but remains challenging because of the visual similarity between benign and malignant lesions [2, 3]. Traditional methods such as dermoscopy and biopsy, while effective, are subjective and unsuitable for large-scale screenings [4]. Computational approaches, particularly deep learning-based systems, have

transformed dermatological diagnostics by automating lesion segmentation and classification [5, 6].

Early computer-aided diagnosis (CAD) systems relied on handcrafted features like color, texture, and asymmetry, analyzed through machine learning algorithms such as support vector machine (SVM) and k -nearest neighbors (KNN) [7, 8]. However, these models suffered from limited generalization and sensitivity to lighting conditions. The advent of convolutional neural networks (CNNs) and attention-based architectures significantly improved feature representation and classification accuracy [9, 10]. Hybrid models integrating CNNs and Transformers [11–13], such as BAT [14], LCAUnet [15], and Att-SwinU-Net [16], have achieved substantial gains in lesion segmentation by combining local detail extraction and global context modeling [17–19]. Vision Transformers (ViT) [20, 21] further enhance diagnostic performance by processing images as patch sequences, enabling the capture of long-range spatial dependencies crucial for skin lesion recognition [22].

Recent studies highlight the strength of combining CNNs and Transformers [23], as in HMT-Net [24] and DermViT, which demonstrated notable improvements across datasets like ISIC2019 and HAM10000. These approaches have motivated the current study's design of a hybrid HMT-NET and ViT framework for accurate, interpretable, and efficient skin cancer diagnosis.

2 Proposed Method

To address the challenges of accurate skin lesion segmentation and classification, a hybrid deep learning framework is proposed. This approach integrates a Transformer-enhanced segmentation network (HMT-NET) with a ViT-based classifier, aiming to enhance diagnostic precision by isolating lesion regions before classification. This design minimizes background interference and ensures that the model focuses on clinically relevant features for reliable diagnosis.

Figure 1 illustrates the workflow of the proposed hybrid framework for automated skin cancer classification, integrating HMT-NET for segmentation and ViT for classification. The process begins with the HAM10000 dataset, which provides dermoscopic skin lesion images and their corresponding binary masks. Initially, all images undergo preprocessing steps – resizing, contrast enhancement, and normalization – to ensure consistency and enhance feature clarity. These processed images and masks are then used to train HMT-NET, a hybrid architecture that combines Transformer and Multi-Layer Perceptron (MLP)-based mechanisms to capture both global contextual and local boundary information for precise lesion segmentation. Following training, the HMT-NET operates in two distinct phases: segmentation and classification training. During segmentation, unseen test images are preprocessed and passed through the trained HMT-NET to produce binary lesion masks. These masks are applied to the original images to generate region-of-

interest (ROI) patches, isolating lesions while excluding irrelevant background regions and artifacts.

In parallel, the classification training phase employs a ViT trained on dermoscopic images and their corresponding class labels [3]. Each image is first processed by the trained HMT-NET to emphasize lesion-specific regions before being fed into the ViT. Leveraging its global self-attention mechanism, ViT models spatial dependencies across image patches to obtain a rich and discriminative feature representation, leading to accurate multi-class classification. The ViT is trained to distinguish among eight skin lesion classes: actinic keratoses (AK), basal cell carcinoma (BCC), benign keratosis-like lesions (BKL), dermatofibroma (DF), melanoma (MEL), melanocytic nevi (NV), squamous cell carcinoma (SCC), and vascular lesions (VASC). During inference, each masked test image is passed through the trained ViT, which outputs the predicted lesion category. This integrated framework – combining segmentation precision with transformer-based classification – enhances diagnostic accuracy by suppressing irrelevant background data and focusing on lesion-specific features, demonstrating strong potential for real-world clinical deployment.

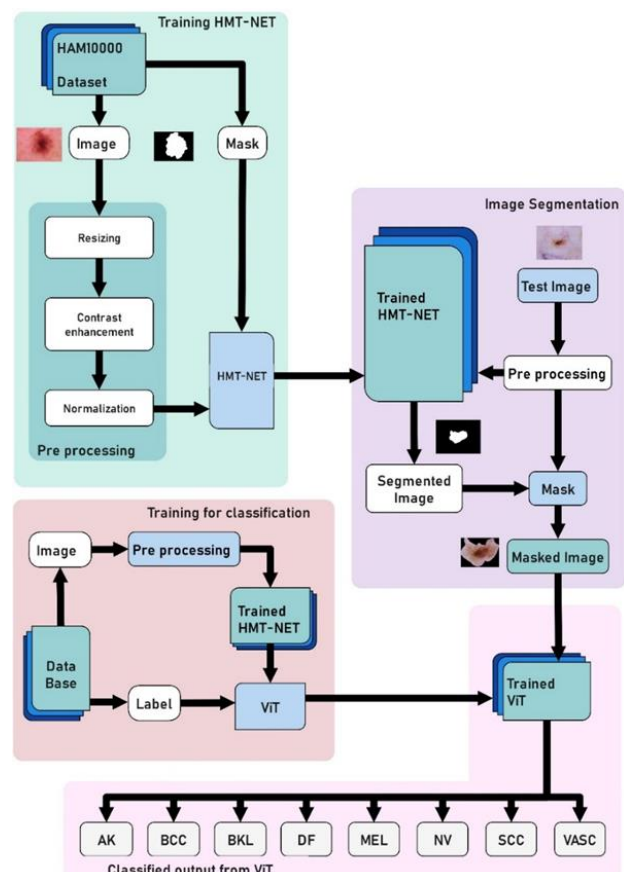


Fig. 1 Block diagram of the proposed skin cancer classification.

2.1 Preprocessing

The preprocessing stage includes three key steps – resizing, contrast enhancement, and normalization –

designed to standardize and optimize Dermoscopic images for model input.

Resizing ensures that all images conform to a fixed resolution compatible with the network architecture, minimizing variations caused by differing acquisition settings [25]. Contrast enhancement improves lesion visibility and boundary clarity, addressing challenges such as low contrast or uneven lighting common in dermoscopic images. Finally, normalization scales pixel values (typically within [0, 1] or standardized to zero mean and unit variance), reducing illumination variability and stabilizing model training. Together, these steps improve image consistency, highlight clinically relevant features, and enhance convergence stability for the subsequent deep learning stages.

2.2 HMT-NET

The HMT-NET architecture performs precise lesion segmentation by combining convolutional encoding, transformer-based global attention, and MLP-driven refinement. As shown in Fig. 2, it comprises three main components: a multi-stage encoder (G_1 – G_5), a dual-attention pathway (Convolutional Transformer (CTrans) and Token-Mixing Multi-Layer Perceptron (ToK-MLP)), and a decoder that reconstructs the final segmentation mask [26].

The encoder progressively extracts hierarchical features – ranging from shallow textures to deep semantic representations. The CTrans module applies multi-head self-attention to capture long-range dependencies, while the ToK-MLP module enhances edge precision and local boundary details [6]. The decoder then fuses these refined

features through up-sampling and skip connections to produce an accurate binary lesion mask. This hybrid design allows HMT-NET to effectively integrate global contextual understanding with local structural detail, resulting in highly accurate segmentation of complex skin lesions in Dermoscopic imagery.

2.3 ViT

The ViT is a deep learning architecture that adapts the Transformer model, originally developed for natural language processing, to image classification tasks. Unlike CNNs that capture local spatial hierarchies, ViT represents an image as a sequence of fixed-size patches, enabling the model to learn global contextual relationships across the entire image early in the processing pipeline.

Figure 3 illustrates the workflow of ViT used for skin lesion classification. The input dermoscopic image is divided into non-overlapping patches, each flattened and projected into an embedding vector. Positional encodings are added to preserve spatial information, and a special classification token ([CLS]) is prepended to aggregate image-level features. The sequence is then processed through multiple Transformer layers consisting of multi-head self-attention (MHSA) and feedforward MLP blocks, each followed by normalization and residual connections.

For classification, the final representation of the [CLS] token is passed through fully connected layers and a SoftMax activation to predict lesion categories. The model is trained to classify eight skin lesion types: AK, BCC, BKL, DF, MEL, NV, SCC, and VASC.

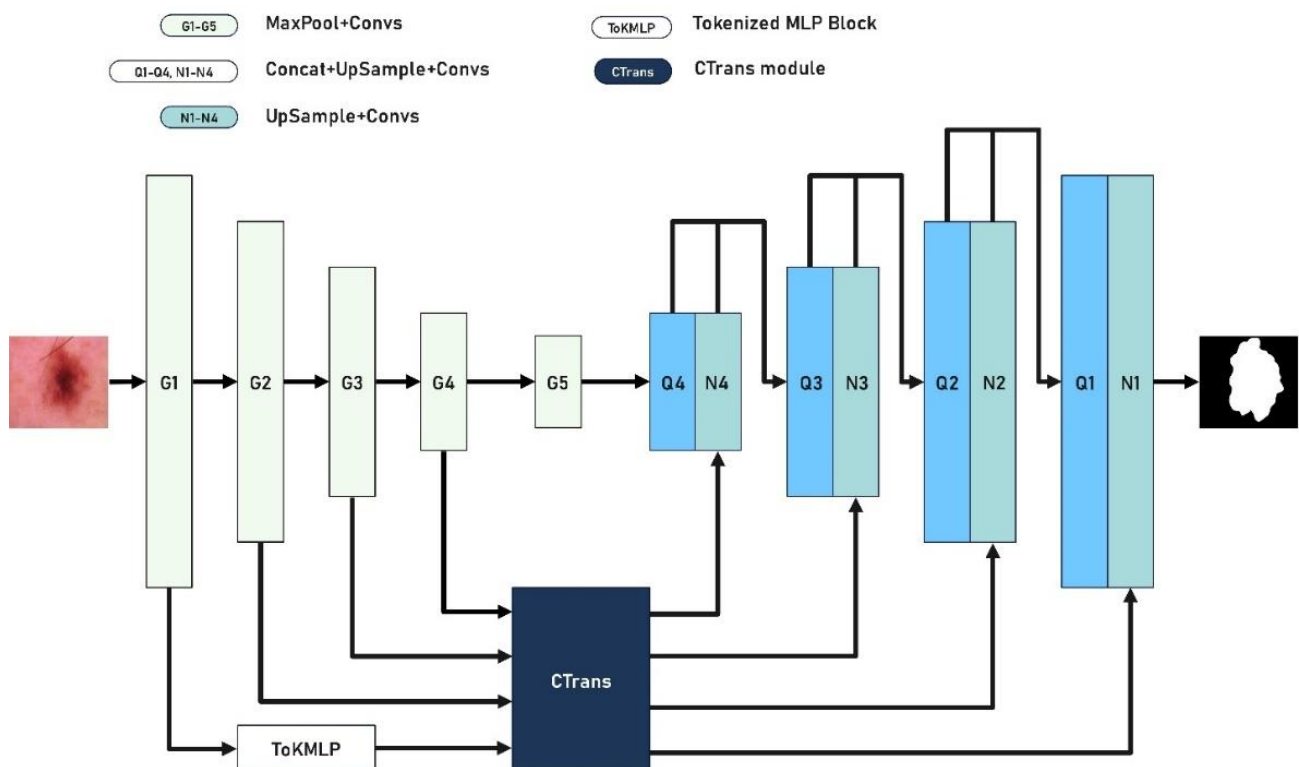


Fig. 2 Block diagram of the HMT-NET working.

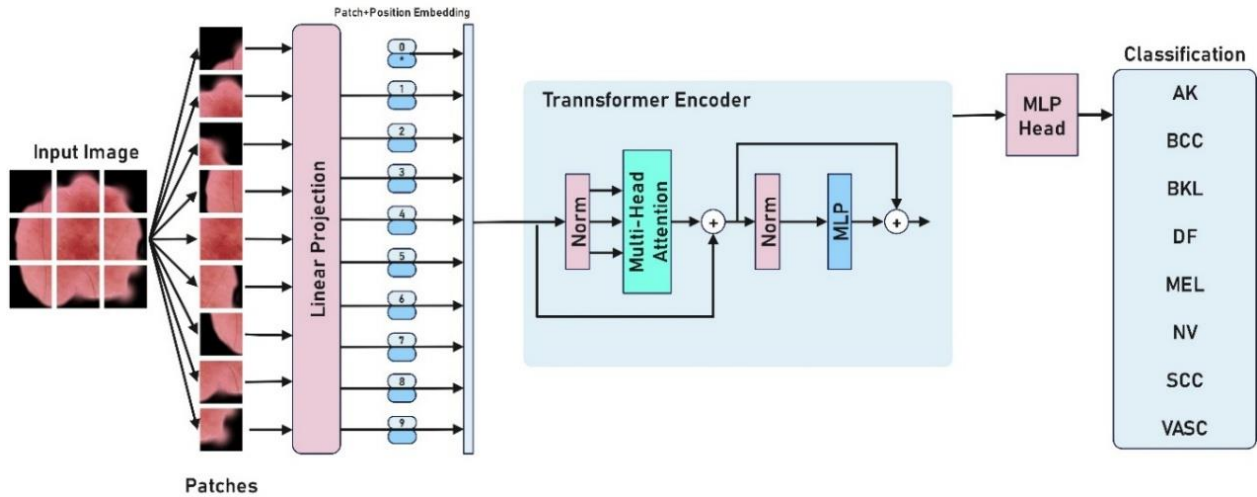


Fig. 3 Block diagram of Vision Transformer working.

3 Results and Discussion

This section summarizes the experimental results of the proposed HMT-NET + ViT framework for skin lesion segmentation and multi-class classification. Experiments were conducted on the HAM10000 and ISIC2019 datasets using a GPU-enabled Google Colab environment. Evaluation metrics included Dice Coefficient, Intersection over Union (IoU), accuracy, precision, recall, and F_1 -score, computed per image and averaged across the datasets for fair comparison.

3.1 Dataset

All images underwent preprocessing for resizing, contrast enhancement, and normalization. The HAM10000 dataset (seven lesion classes) was used for segmentation, while the ISIC2019 dataset (eight classes: AK, BCC, BKL, DF, MEL, NV, SCC, VASC) was employed for classification. Macro-averaging was applied to mitigate class imbalance.

HAM10000 Segmentation Performance

The HMT-NET achieved a Dice score of 91.85% and an IoU of 88.42%, surpassing baseline architectures such as U-Net, R2U-Net, and FAC-Net. This improvement arises from the CTrans attention for long-range feature capture and the tokenized MLP for precise boundary refinement. Qualitative outputs (Fig. 4) show accurate lesion delineation even under challenging conditions such as hair occlusion and low contrast. Table 1 shows the comparative performance of the proposed method HMT-NET.

ISIC2019 Classification

Using segmented ROIs from HMT-NET, the ViT-based classifier achieved 89.72% top-1 accuracy and a macro F_1 -score of 88.35%, outperforming ResNet-50, EfficientNet, and DenseNet. High F_1 -scores (> 90%) for NV and MEL classes confirm strong discrimination

between benign and malignant lesions. The segmentation-guided input significantly reduced false positives by eliminating irrelevant background, while ViT's self-attention effectively captured subtle inter-class variations. The confusion matrix (Fig. 5) illustrates consistently high class-wise accuracy, especially for MEL and SCC, validating the model's reliability in real-world diagnostic settings.

3.2 Performance Metrics

To assess the effectiveness of the proposed hybrid HMT-NET and ViT framework, a set of standard performance metrics was employed, focusing on both segmentation quality (HAM10000 dataset) and classification accuracy (ISIC2019 dataset).

Accuracy

Accuracy measures the proportion of correctly classified pixels (for segmentation) or samples (for classification) among the total.

$$Accuracy = \frac{TP+TN}{TP+TN+FP+FN}. \quad (1)$$

Dice Coefficient (DSC)

Also known as the F_1 -score in segmentation, Dice measures the overlap between predicted and ground truth masks.

$$DSC = \frac{2TP}{FP + 2TP + FN}. \quad (2)$$

Jaccard Index

Also known as IoU, this metric evaluates the intersection divided by the union of predicted and actual segmentations.

$$Jaccard = \frac{TP}{TP+FP+FN}. \quad (3)$$

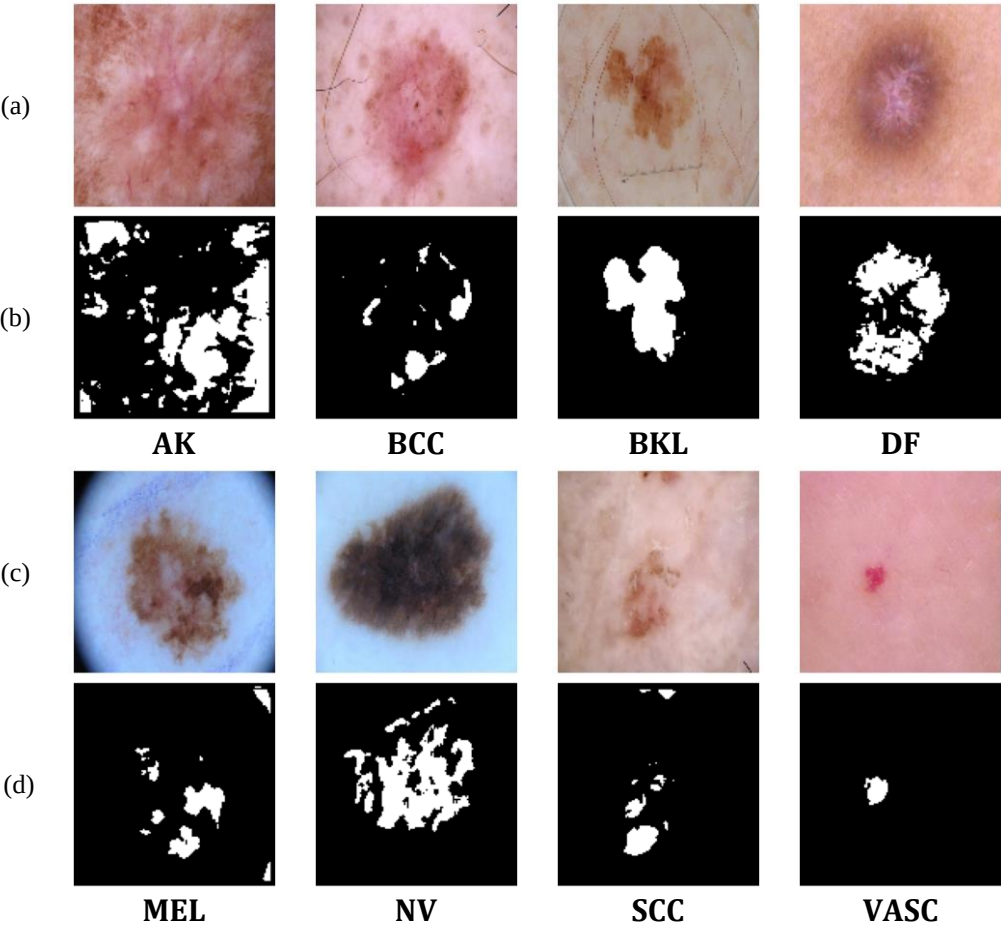


Fig. 4 Segmented output of classified image: (a) input image of HAM10000, (b) segmented image of HAM10000, (c) input image of ISIC2019, (d) segmented image of ISIC2019.

Table 1 Comparative performance of the proposed method HMT-NET.

Model	Accuracy (%)	Dice Coefficient (%)	Jaccard Index (%)	Sensitivity (%)	Specificity (%)
Detectron2 (Mask R-CNN)	98.40	94.00	86.90	–	–
Detectron2 (Mask R-CNN)	99.80	98.50	94.20	–	–
Detectron2 (Mask R-CNN)	98.90	96.10	91.50	–	–
Ultra-Light VM-U Net	96.46	90.91	83.34	90.53	97.9
Ultralight VM-U Net	95.58	89.40	80.56	86.80	97.81
Ultralight VM-U Net	95.21	92.65	86.31	93.45	96.06
CNN + Classifier	98.34	97.15	–	97.25	99.13
UCM-NetV2	96.82	91.54	–	90.85	98.22
UCM-NetV2	96.10	90.74	–	90.43	97.77
ESC-UNET	98.25	95.15	88.41	95.67	99.24
ESC-UNET	98.30	93.48	83.88	94.17	99.32
ESC-UNET	97.89	92.53	81.67	93.63	99.03
CCT-Net	–	93.21	87.70	–	–
CCT-Net	–	89.19	82.05	–	–
CCT-Net	–	92.72	86.81	–	–
CCT-Net	–	96.21	92.79	–	–

Table 1 Cont.

Ensemble-A	94.10	87.10	79.30	89.90	95.00
Ensemble-S	93.80	90.70	83.90	93.20	92.90
O-Net	94.71	87.04	80.36	89.70	96.30
O-Net	95.14	92.12	86.15	89.23	96.75
YOLOv4+ActiveContour	93.90	92.00	96.00	94.00	95.20
YOLOv4+ActiveContour	95.00	89.00	98.00	93.20	93.45
Used Method	98.00	91.00	98.00	93.60	93.88

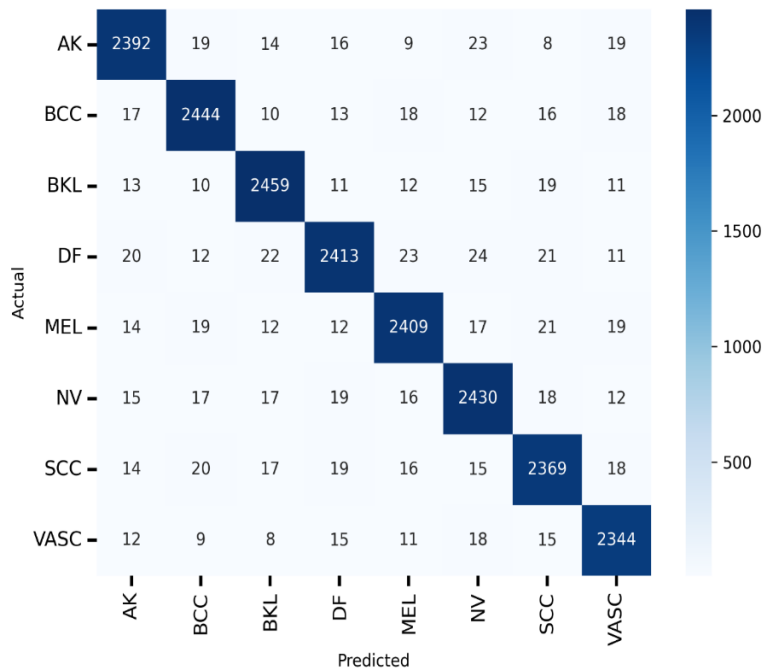


Fig. 5 Confusion matrix of classification.

Table 2 Comparative performance of the proposed method ViT.

Model Used	Accuracy	Precision	Recall	<i>F</i> ₁ -Score
IncepX + Ensemble (SVM, RF, KNN)	97.32%	96.80%	96.59%	96.60%
Class-weighted RL + ResNet50	91.62%	90.13%	91.72%	90.91%
YOLOv8 + Vision Transformer (ViT)	98.60%	98.10%	98.40%	98.20%
Generative AI + Reset Variant	95.40%	94.30%	94.90%	94.60%
Interpretable CNN + CBAM (Attention)	93.28%	92.91%	93.12%	93.00%
Generative AI + Deep CNN	95.40%	94.30%	94.90%	94.60%
Enhanced CNN + Grad-CAM Attention	93.70%	93.10%	92.80%	92.90%
Shuffle Net + Non-local Attention	96.12%	95.86%	95.90%	95.88%
Multi-Model Attentional Fusion Ensemble	98.40%	97.90%	98.10%	98.00%
Hybrid CNN + SVM (post-segmentation)	92.00%	—	—	—
SVM, RF on Gene Data	91.20%	90.60%	90.90%	90.70%
FCN-Based Dense Net Framework	94.31%	94.00%	94.35%	94.12%
Used Method	98.75%	96.37%	98.66%	98.90%

Recall (Sensitivity)

Specificity assesses the proportion of actual negatives correctly identified.

$$\text{Sensitivity} = \frac{TP}{TP+FN}. \quad (4)$$

Specificity

Specificity assesses the proportion of actual negatives correctly identified.

$$\text{Specificity} = \frac{TN}{TN+FP}. \quad (5)$$

Table 2 summarizes comparisons with state-of-the-art frameworks such as YOLOv8 + ViT, Multi-Model Attentional Fusion, and IncepX + SVM/RF/KNN.

While existing attention-based ensembles reach ~98% accuracy, the proposed HMT-NET + ViT achieved the highest overall performance – 98.75%

accuracy, 96.37% precision, 98.66% recall, and 98.90% F_1 -score – demonstrating superior diagnostic precision and generalization.

Class-wise metrics (Table 3) show uniformly strong results across all categories, with near-perfect accuracy for NV, BCC, and SCC, and significant improvement in MEL classification ($F_1 = 0.982$). This balanced performance highlights the framework's ability to handle data imbalance and complex lesion textures effectively.

Across both datasets, the hybrid HMT-NET + ViT pipeline consistently outperformed state-of-the-art methods in segmentation and classification. Its integration of transformer-based global attention with MLP-driven local refinement ensures fine boundary detection and robust contextual understanding. With high accuracy, balanced sensitivity/specificity, and efficient computation, the framework demonstrates strong potential for real-time, clinically interpretable skin cancer diagnosis. Fig. 6 shows the performance of the proposed method.

Table 3 Classification performance by classes.

Class	Accuracy	Precision	Recall	F_1 -Score
AK	0.987	0.961	0.984	0.987
BCC	0.988	0.965	0.987	0.989
BKL	0.986	0.957	0.988	0.986
DF	0.988	0.962	0.986	0.987
MEL	0.982	0.942	0.976	0.982
NV	0.992	0.981	0.992	0.991
SCC	0.989	0.966	0.986	0.988
VASC	0.991	0.969	0.987	0.989

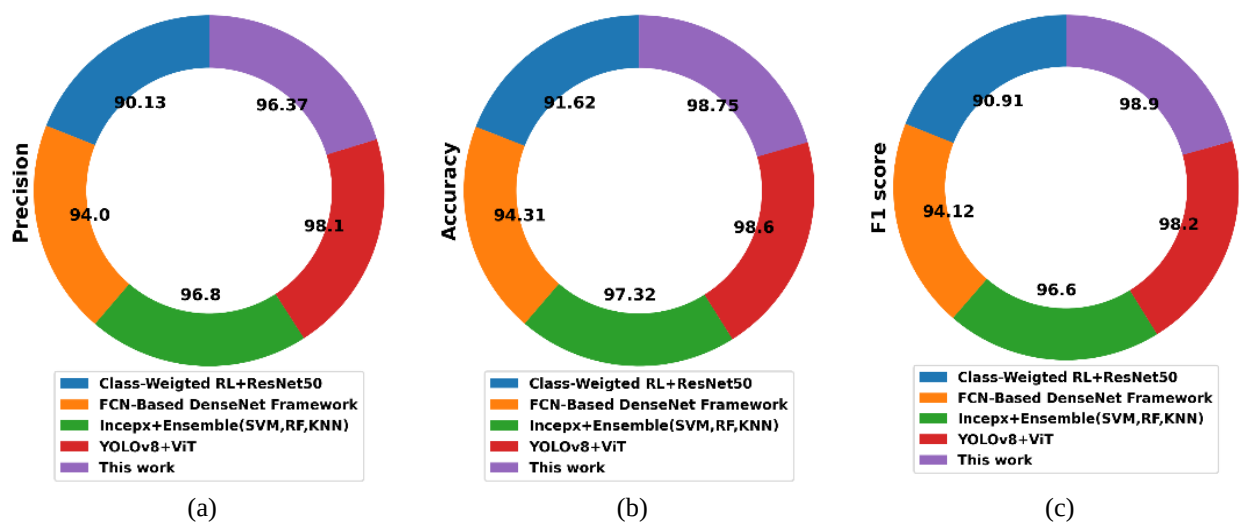


Fig. 6 Performance of the proposed method. (a) Performance of precision, (b) performance of accuracy, (c) performance of F_1 -score.

4 Conclusion

This study presents a hybrid diagnostic framework integrating the HMT-NET segmentation model with a Vision Transformer (ViT) classifier for effective skin cancer detection. The proposed pipeline was evaluated on the HAM10000 and ISIC2019 datasets using GPU-enabled platforms. Experimental results demonstrated that HMT-NET achieved a Dice coefficient of 91.85% and an IoU of 88.42%, outperforming established models such as U-Net, R2U-Net, and FAC-Net. These improvements are attributed to the use of tokenized MLP and attention-based components, which enhanced lesion boundary detection even under challenging conditions like hair occlusion and low contrast. For classification, the ViT model achieved a top-1 accuracy of 89.72% and a macro averaged F_1 -score of 88.35%. When compared

to models such as YOLOv8+ViT and IncepX-Ensemble, our method surpassed existing solutions with a final classification accuracy of 98.75% and F_1 -score of 98.90%. Class-wise results further confirmed consistent performance, particularly for high-risk lesions such as NV and SCC. These findings demonstrate the framework's robustness, scalability, and clinical relevance. Overall, the proposed method shows strong potential for integration into real-time, automated dermatological diagnostic systems, offering improved accuracy, explainability, and efficiency for multi-class skin lesion detection in practical healthcare applications.

Disclosures

All authors declare that there is no conflict of interest in this paper.

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An Ensemble Method for Analyzing Spectral and Clinical Data of Patients with Skin Neoplasms

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Abstract. The work is devoted to the application of the ensemble method for the analysis of spectral and clinical data of patients with skin neoplasms. In vivo Raman spectra of skin neoplasms are used as spectral data. To identify the features reflecting the relative concentrations of skin components, the multivariate curve resolution method was used. In addition to spectral signs, clinical data on patients (anamnesis) were used. Ensemble models based on stacking have been developed for three classification cases: benign versus malignant neoplasms, malignant melanoma versus pigmented nevus, malignant melanoma versus pigmented nevus, and seborrheic keratosis. The the area under the Receiver Operating Characteristic (ROC) curve (ROC AUC) of the developed models ranges from 0.81 ± 0.06 to 0.85 ± 0.11 , which confirms the effectiveness of the ensemble method and the anamnesis for the identification of skin neoplasms.

Keywords: skin neoplasms; Raman spectroscopy; multivariate curve resolution method; ensemble algorithm; stacking; medical history; clinical data.

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1 Introduction

Skin neoplasms are one of the most common groups of diseases in the world [1]. Among them, there are benign neoplasms that are not life-threatening, as well as extremely malignant ones, for example, malignant melanoma. That is why accurate and rapid diagnosis of skin neoplasms is an urgent task of modern medicine [2].

The “gold standard” for the diagnosis of skin neoplasms is histological analysis. However, this method has a number of disadvantages, such as invasiveness and the length of waiting for results. Optical diagnostic methods, in particular, Raman spectroscopy, are gaining increasing popularity [2]. Compared to histological analysis, Raman spectroscopy is faster and cheaper. In addition, Raman spectroscopy can make the diagnosis of skin diseases completely non-invasive.

Various studies demonstrate that Raman spectroscopy can be used to make diagnostic decisions, that is, to determine the disease or type of neoplasm [1]. However, despite advances in technical equipment, the analysis of experimental Raman spectra remains a difficult task. The reason for this is that the Raman spectra of light contain a large amount of information

about all the chemicals contained in the skin, and may also contain spurious signals caused by the optical system [3].

In this regard, an urgent task is to find a new method for interpreting the Raman spectra of the skin.

As part of the research, the task of classifying Raman skin spectra was performed using a number of machine learning algorithms. The multidimensional curve resolution (MCR) method was used to reduce the initial spectral data. A comparative analysis of the classification efficiency was carried out on sets of features formed with a different number of decomposition components: 8, 10, 15 and 30. It was found that the highest classification accuracy is achieved when using a set of 30 components, which indicates a more complete description of the spectral variability of biological samples [4–6].

It is assumed that taking into account the clinical data of patients (that is, their medical history) provides additional information for a more accurate classification and interpretation of spectra [7].

Medical history plays an important role in the diagnosis of skin diseases. Information about past illnesses, allergies, heredity, and lifestyle helps the doctor create a complete picture of the patient's

condition. To make a correct diagnosis, it is necessary to know when the disease began, how it developed, and what symptoms are bothering the patient. In addition, it is necessary to take into account the presence of concomitant diseases, place of residence and risk factors in the workplace [8].

Taking an anamnesis is the first step in making a diagnosis. It is based on finding out: the duration of the disease; the severity of its onset; localization; the prevalence of the process; symptoms of the disease; family history; profession; previous treatment.

The purpose of collecting medical history is also to clarify the etiological factors contributing to the occurrence of dermatosis. By tradition, the medical history begins to be collected before the general examination.

An important part of the diagnostic process is clarifying the life history. When collecting it, one should strive to identify possible etiological factors, including: alcohol consumption; taking medications; conditions of professional activity; peculiarities of everyday life; family history.

When collecting a life history, information about past illnesses is important.

Skin diseases are often a manifestation of internal pathology. Thus, diabetes mellitus can cause candidiasis of the skin, and malignant neoplasms of internal organs can metastasize to the skin [9].

The course of some skin diseases depends on the season [10]. Diseases associated with the adverse effects of ultraviolet rays (photodermatoses) are more common in spring and summer. Erythema multiforme and pink lichen – in spring and autumn. Dermatoses caused by impaired capillary circulation (cold urticaria) are common in the cold season.

Thus, the integration of clinical and spectral data is not just a technical improvement, but a methodological necessity for the transfer of spectroscopic methods from the research plane into real clinical practice.

The vast majority of research devoted specifically to spectral methods focuses exclusively on spectra. If clinical data is used, it is at the stage of sample formation or for post-analytical interpretation of the results, but it is not directly integrated into the model at the feature level [11–14].

Thus, the novelty of our method lies in the purposeful and systematic combination of two modalities – biochemical (Raman spectra) and phenotypic (clinical data) – into a single analytical model. This allows us to create a model that simultaneously takes into account both the molecular composition of the tissue and the individual characteristics of the patient, which leads to the creation of a more reliable and clinically applicable decision support tool.

Based on the above, the aim of the work was to implement an ensemble algorithm for the identification of skin growths, taking into account spectral data and the patient's medical history.

2 Methods and Materials

2.1 Raman Spectra

The Raman spectra of neoplasms were recorded using a portable spectroscopic setup with a spectral resolution of 0.2 nm in the range from 860 to 922 nm with a signal accumulation time of 60 seconds when excited at a wavelength of 785 nm.

The spectra were preprocessed, including cropping in the range from 860 to 920 nm, baseline removal, and smoothing using the Savitsky-Golay method.

The *in vivo* study was conducted at the Samara Regional Clinical Oncology Dispensary. More than 500 patients participated in the study. For each patient, the spectrum from the skin area with the disease was recorded. In addition to the Raman spectra, information about the actual diagnoses for each patient was obtained. These diagnoses were made by doctors of the Samara Regional Clinical Oncological Dispensary based on the results of histological analysis. Table 1 shows information about the set of registered Raman spectra.

Table 1 Raman spectra.

Nosology	Number of registered Raman spectra
Dermatofibroma	26
Hemangioma	40
Sebrrheic keratosis	113
Basal cell carcinoma	122
Squamous cell carcinoma	12
Malignant melanoma	70
Pigmented nevus	170
Papilloma	62

2.2 Clinical Data

It can be argued that the clinical picture is extremely important for making a correct diagnosis of skin diseases. Therefore, in addition to spectral data, clinical data and patient anamnesis were collected. The study involved 178 men and 437 women.

At the first appointment, the oncologist collected the patient's medical history and potential risk factors for skin cancer: gender, age, place of residence, genetic predisposition, eye color, harmful profession, indoor work, concomitant diseases, the presence of skin diseases in the past, the presence of sunburn, tumor localization, age spot size, height spots, bleeding, dropouts, and localization. All data has been encoded (Table 2).

All 15 risk factors were later used as classification parameters.

Table 2 Encoding of the collected clinical data.

Sign	Encoding
Gender	0 – female 1 – male
Age	number of years from 20 to 88
Place of residence	0 – city 1 – village 0 – no
Genetic predisposition	1 – Father and mother 2 – Brother and sister 3 – Grandma and Grandpa 4 – distant relatives
Eye color	1 – gray 2 – blue 3 – green 4 – brown
Harmfulness of the profession	0 – no 1 – yes
Working indoors for more than 15 years	0 – yes 1 – no 0 – no
Concomitant diseases	1 – malignant tumors 2 – cardiovascular diseases 3 – diabetes mellitus 4 – thyroid diseases 0 – no
The presence of skin diseases in the past	1 – benign neoplasms 2 – malignant neoplasms 0 – never been
The presence of sunburn	1 – has been 1-2 times 2 – occur quite often 1 – head and neck 2 – the torso 3 – upper limb 4 – lower limb
Localization of the tumor	1 – from 0 to 5 mm 2 – from 6 to 20 mm 3 – 21 mm
The size of the pigment spot	0 – yes 1 – no
Spot growth	0 – yes 1 – no
Bleeding	0 – yes 1 – no
The appearance of dropouts and localization	0 – yes 1 – no

2.3 The Multivariate Curve Resolution Method

To reduce the amount of spectral data, the multidimensional curve resolution (MCR) method was applied. It is a method of analyzing multidimensional data that allows you to separate complex mixtures into their constituent components. It is based on the use of linear algebra to extract information about components from multidimensional data.

Each pure component has its own spectrum, which can be represented as a column vector s_i of dimension J , where J is the number of values in each spectrum (corresponding to the number of wavelengths or wave numbers).

If you mix the components into one mixture and remove its spectrum, then the resulting spectrum will simply be a linear combination of the spectra of the pure components. This can be expressed by the equation:

$$D = CS^T, \quad (1)$$

where C is the matrix of concentrations of pure components in the mixture; S is the matrix of spectra of pure components.

The task of MCR methods is to find the matrices C and S , knowing D . This is not a trivial task, since the specified expression does not have a single solution.

The main advantage of MCR analysis is the ability to physically interpret the components into which the original Raman spectra are divided. The analysis results in:

- the spectra of the substances that make up the sample;
- their contribution to the Raman spectrum (which is directly related to the actual concentrations of substances) [15, 16].

In addition, unlike, for example, the methods of Principal Component Analysis (PCA) and Partial Least Squares Discriminant Analysis (PLS AD), this method is more resistant to outliers in the data.

Some researchers divide such spectra into 8–10 components to identify specific substances [17]. Such a goal was not set in this work: it was important to preserve the maximum possible amount of valuable information for classifying the Raman spectra of the skin.

After completing the MCR analysis, compressed spectral data is obtained in the form of 30 components and their concentrations in each sample of biological tissue. The data on the concentrations of the obtained substances are further used as classification criteria. The MCR ALS GUI v4c program developed by Dr. Andras Gorjas (Sweden) was used to implement the MCR analysis [18].

Basically, the resulting components are different mixtures of lipids and proteins. But there are also those that are purer substances, some of them are shown in the Fig. 1. Components 1, 4 have identical shapes, they have peaks at 1161 cm^{-1} and 1182 cm^{-1} , respectively, and are ceramides. Components 6 and 9 correspond to the optical contribution of the system. In component 7, the most intense peaks are at 1450 and 1662 cm^{-1} , these peaks correspond to collagen. In component 17, the highest peak is at 1652 cm^{-1} , which corresponds to the peak in the water spectrum. Component 24 contains 1654 cm^{-1} water, 1372 and 1564 cm^{-1} melanin, and various proteins. In component 30, the most pronounced peaks at 1290 , 1450 , and 1657 cm^{-1} correspond to C-H bending proteins and may be due to keratin, collagen, and elastin. In addition, the peak at 1450 cm^{-1} may also correspond to the contribution of urocanic acid [19].

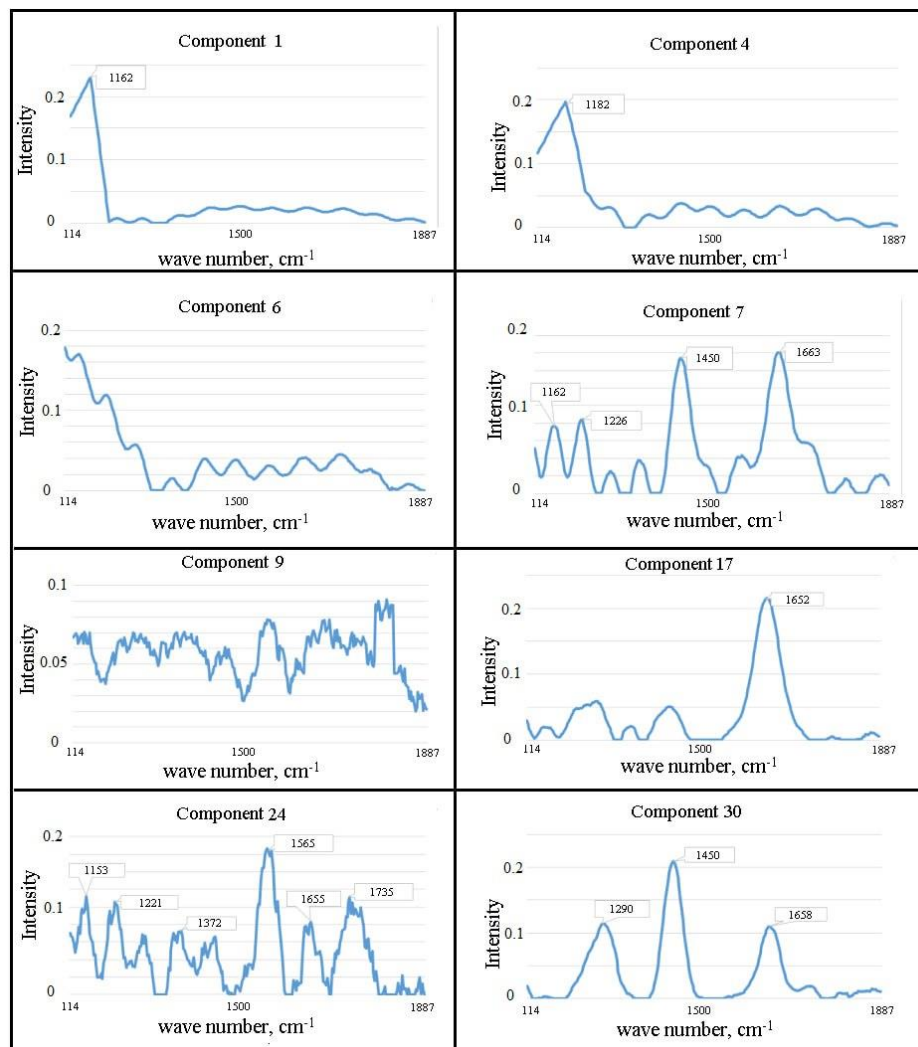


Fig. 1 Spectra of some of the obtained components.

The choice of exactly 30 components was not accidental. Several additional studies were conducted to determine the optimal number of components in order to reduce the amount of data without losing valuable information [4, 5].

2.4 The Ensemble Algorithm

The ensemble algorithm was developed for three cases:

- benign neoplasms versus malignant neoplasms (case 1);
- malignant melanoma versus pigmented nevus (case 2);
- malignant melanoma versus pigmented nevus and seborrheic keratosis (case 3).

The paper examines the application of an ensemble algorithm that takes into account the solutions of several independently operating machine learning algorithms: random forest, gradient boosting and multilayer perceptron. The algorithms were implemented in the Python programming language using the scikit-learn and LightGBM libraries.

First, a study was conducted to find the optimal hyperparameters for each individual model included in the ensemble (random forest, gradient boosting, and multilayer perceptron). The Grid Search method was used.

For a random forest, such hyperparameters were selected as `n_estimators` – the number of “trees”, `max_depth` – the maximum depth of the “trees”, `min_samples_split` – the minimum number of objects required to split a node, `min_samples_leaf` – the minimum number of objects in the “leaves”. The values of the obtained hyperparameters for a random forest are shown in Table 3.

Table 3 Hyperparameters of a random forest for different cases.

Hyperparameter	Case 1	Case 2	Case 3
<code>n_estimators</code>	100	100	775
<code>max_depth</code>	25	13	25
<code>min_samples_split</code>	2	26	2
<code>min_samples_leaf</code>	2	2	2

Table 4 Hyperparameters of gradient boosting for different cases.

Hyperparameter	Case 1	Case 2	Case 3
n_estimators	100	1050	100
max_depth	1	1	1
num_leaves	2	2	2
learning_rate	0.0001	0.1	0.0001

Table 5 Hyperparameters of a multilayer perceptron for different cases.

Hyperparameter	Case 1	Case 2	Case 3
hidden_layer_sizes	100, 50	100	100
activation	logistic	logistic	logistic
max_iter	400	300	300
solver	sgd	adam	sgd

Such hyperparameters as n_estimators, the number of “trees”, max_depth, the maximum depth of the “trees”, num_leaves, the number of “leaves” in the “tree”, and

learning_rate, the learning rate of the model, were chosen as the hyperparameters of gradient boosting. The results of executing the GridSearch command for gradient boosting are shown in Table 4.

To develop the multilayer, hyperparameters were used: hidden_layer_sizes – the number of neurons in each hidden layer, activation – the activation function for hidden layers, max_iter – the maximum number of training iterations, solver – an algorithm for optimizing weights. Table 5 shows the hyperparameters found for a multilayer perceptron.

Next, models with optimal hyperparameters were combined into an ensemble using the stacking method, when the predicted values of the basic individual models are used as input data to the overall meta-model. Logistic regression was chosen as a meta-model.

3 Results and Discussion

To prove the effectiveness of the ensemble algorithm models using patient clinical data, Figs. 2, 3 and 4 show ROC curves for different classification cases, where only the concentrations of the substances obtained and a data set with concentrations of substances and clinical data of patients were used as parameters.

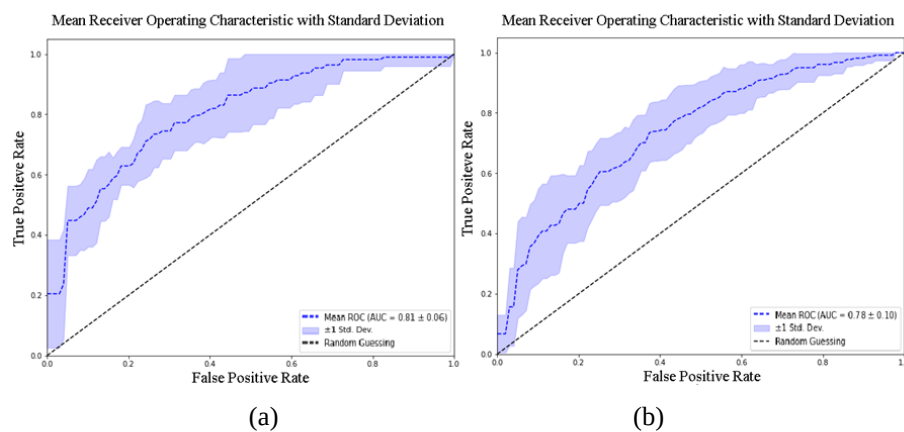


Fig. 2 ROC curves for the case of benign versus malignant neoplasms: (a) an ensemble algorithm with clinical data, (b) an ensemble algorithm without clinical data.

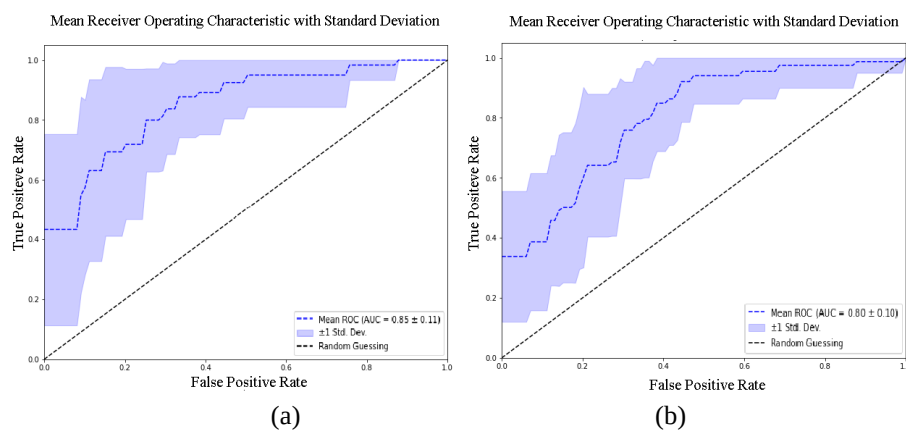


Fig. 3 ROC curves for malignant melanoma versus pigmented nevus: (a) an ensemble algorithm with clinical data, (b) an ensemble algorithm without clinical data.

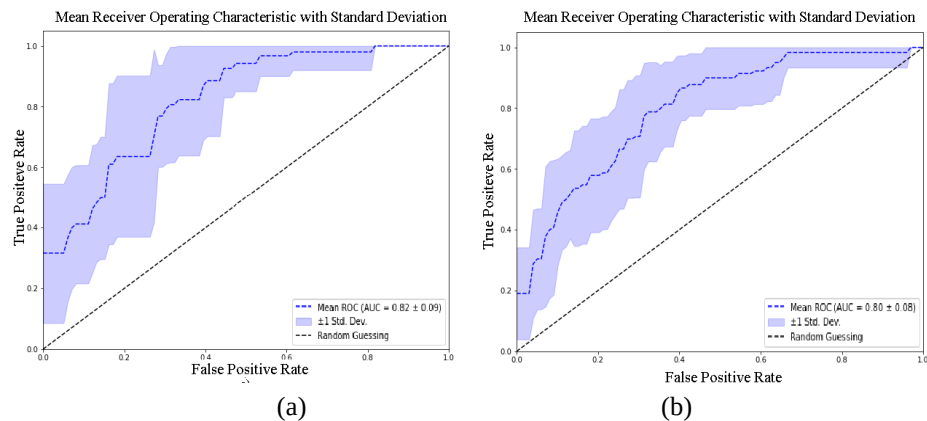


Fig. 4 ROC curves for the case of malignant melanoma versus pigmented nevus and seborrheic keratosis: (a) an ensemble algorithm with clinical data, (b) an ensemble algorithm without clinical data.

Table 6 Metrics of classification models.

The classification case	ROC AUC (no medical history)	ROC AUC (with medical history)
Benign neoplasms vs. malignant neoplasms	0.78±0.10	0.81±0.06
Malignant melanoma vs. pigmented nevus	0.80±0.10	0.85±0.11
Malignant melanoma vs. (pigmented nevus + seborrheic keratosis)	0.80±0.08	0.82±0.09

Table 6 shows the average values of ROC AUC with the standard deviation calculated for all folds. One column contains information for models where only spectral information about the neoplasm was used as signs, and the other column takes into account both spectral signs and clinical patient data (anamnesis).

The quality of skin cancer diagnosis by visual examination largely depends on the level of qualification and professional experience of the doctor. For example, a doctor correctly identifies malignant melanoma only in 40–80% of cases (depending on qualifications) [20, 21].

Based on this, the ROC AUC threshold of 0.8 was chosen for a decent primary diagnosis of skin diseases by the method being developed.

The development results fully satisfy the task. In all cases, the ROC AUC exceeds the threshold of 0.8.

In the case of benign neoplasms against malignant ones, the ROC AUC increased by 0.03, from 0.78 ± 0.10 to 0.81 ± 0.06 . The ROC AUC for the case of malignant melanoma against pigmented nevus was 0.85 ± 0.1 , increased by 0.05 compared with the ensemble without clinical data, and in the case of malignant melanoma against pigmented nevus and seborrheic keratosis increased by 0.02 and it was 0.82 ± 0.09 .

To understand whether the increase in ROC AUC is statistically significant, a paired *t*-test was performed. The *p*-value for benign versus malignant neoplasms was

0.279, for malignant melanoma versus pigmented nevus – 0.165, and for malignant melanoma versus pigmented nevus and seborrheic keratosis – 0.477. Statistical analysis shows that the observed improvements in ROC AUC with the addition of clinical data are not statistically significant at the generally accepted level of $p < 0.05$, although it shows an improvement trend.

It can be seen from this study that the data on the relative concentrations in the studied samples is sufficient for automated diagnostics. However, interpretability of data is important for medicine, so clinical data can be included in the model so that the model makes predictions not only based on spectral data, but also on the basis of signs that the doctor understands – clinical data. That is why both approaches should be used to identify the Raman spectra of the skin.

In addition to ROC AUC, specificity and sensitivity were calculated for the models, and their values are shown in the Table 7.

Table 7 Specificity and sensitivity of the model for different cases.

no medical history			
	Case 1	Case 2	Case 3
Specificity	0.55	0.52	0.38
Sensitivity	0.86	0.82	0.88
with medical history			
	Case 1	Case 2	Case 3
Specificity	0.60	0.57	0.43
Sensitivity	0.81	0.82	0.87

The addition of clinical data to the ensemble classification model of Raman skin spectra allowed us to create a more balanced diagnostic tool with significantly increased specificity while maintaining very high sensitivity. This shows that combining spectroscopy data and clinical information gives a better result than each method individually.

It can be said that the method of Raman spectroscopy is comparable to the diagnosis by doctors during dermatoscopic examination. Thus, in the study by Haenssle et al. [20], the average sensitivity and specificity of 58 dermatologists for the classification of 100 skin lesions was 86.6% ($\pm 9.3\%$) and 71.3% ($\pm 11.2\%$), which corresponds to the diagnostic accuracy (the proportion of correctly determined diagnoses) of 79% ($\pm 0.06\%$).

4 Conclusion

As a result of the work, an ensemble stacking-based method was developed for the classification of Raman spectra of skin neoplasms using clinical patient data.

In addition to the high accuracy of the classification of skin diseases, due to the use of the MCR analysis method to reduce the amount of data, the developed

algorithm makes it possible to identify real chemicals in the test sample.

In each case, the accuracy of the classification is comparable to the accuracy of the identification of the disease by an experienced oncologist.

The considered method of Raman spectroscopy in combination with intelligent analysis and taking into account the medical history of patients can be used for population screening as a fast and fairly accurate method of analyzing skin neoplasms. It can also be implemented in hospitals where there is a lack of highly qualified personnel and technical equipment.

Disclosures

All authors declare that there is no conflict of interest in this paper.

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