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Application of Raman spectroscopy and SERS for the detection of fungi-destructors capable of biodegradation of cultural heritage at the State Tretyakov Gallery

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Abstract. We explored the possibility of using Raman spectroscopy and SERS for the analysis of molds using the example of strains collected from exhibits of the State Tretyakov Gallery. The fungi contained in the samples were cultivated on the surface of aluminum oxide substrates containing plasmonic nanostructures based on silver and gold nanoparticles. The mapping of samples using Raman spectroscopy made it possible to visualize the distribution of organic substances contained in mold fungi. The purpose of the study is to develop a methodology for the identification of fungi, including those that destroy cultural heritage.

Keywords: nanoparticles, silver, gold, fungi, plasmonic nanostructures, Raman spectroscopy, SERS, mapping, diagnostics of biodeterioration of cultural heritage

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Материалы конференции
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Применение рамановской спектроскопии и SERS для обнаружения грибов-деструкторов, способствующих биодеградации объектов культурного наследия в Государственной Третьяковской галерее

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Аннотация. Мы исследовали возможность использования рамановской спектроскопии и SERS для анализа плесневых грибов на примере штаммов, собранных с экспонатов Государственной Третьяковской галереи. Грибы, обнаруженные на образцах, культивировались на поверхности подложек из оксида алюминия, содержащих плазмонные наноструктуры на основе наночастиц серебра и золота. Картирование образцов с помощью рамановской спектроскопии позволило визуализировать распределение органических веществ, содержащихся в плесневых грибах. Цель исследования – разработка методики идентификации грибов, в том числе разрушающих культурное наследие.

Ключевые слова: наночастицы, серебро, золото, грибы, плазмонные наноструктуры, рамановская спектроскопия, SERS, картирование, диагностика биоповреждений объектов культурного наследия

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Introduction

Ancient tempera paintings, such as icons, consist of various organic and inorganic components that provide favorable conditions for microbial growth. The timely detection of microbial infections is an imperative task in the conservation of art objects [1]. Continuous monitoring is necessary to prevent irreversible damage to these precious artifacts. When studying cultural heritage objects, highly sensitive non-invasive methods are used to establish the necessary measures for their conservation and/or restoration [1]. Raman spectroscopy is a valuable technique for the identification of a various biological samples. It offers several benefits that make it a useful tool in mold identification, such as: allows for in situ and non-destructive analysis with minimal sample preparation, often without the need for staining or labeling, requires very small sample amounts [2]. Raman spectrum acts as a molecular fingerprint, allowing for the identification of specific mold species [3]. The technique is not limited to specific types of molds and can be used for the identification of fungi, spores, and other microorganisms [4]. Raman spectroscopy also has some limitations that should be considered. Many organic compounds, including some molds, exhibit fluorescence during spectroscopy, making it difficult to extract the desired Raman spectrum [5]. Water interference is particularly relevant when analyzing mold samples in aqueous environments or high humidity conditions [6]. Raman scattering is low for molecules with small interaction cross-section, which requires the use of bulk samples or concentrated solutions for research [3, 7]. Also samples collected from artworks are characterized by high inhomogeneity from the presence of multiple fungi species, different growth stages, or heterogeneous distribution of mold within a sample [1, 8, 9]. This research aimed to address the aforementioned challenges by exploring and implementing diverse approaches to sample preparation. We also applied Raman spectroscopy with surface-enhanced signal (SERS), works on the principle of enhancing Raman signals by metallic nanostructures, such as gold or silver nanoparticles. These nanoparticles act as amplifiers, greatly enhancing the weak Raman signals emitted by the target molecules of the pathogenic fungi. This enhancement enables the detection of even trace amounts of fungal biomolecules, providing valuable insights into their composition and structure [2, 3]. The main advantages of SERS are its non-invasiveness, *in situ* applicability, speed, and reliable spectral response [7].

Materials and Methods

In the course of the study of the microbiome of the State Tretyakov Gallery (Moscow, Lavrushinsky lane, 10), more than 100 microbiological samples were collected from exhibits and surfaces within the halls of ancient Russian painting [1]. The microorganisms in the samples were cultivated, with a particular focus to fungi-destructors of tempera paintings, such as: *Aspergillus versicolor* STG-25G, *Mucor circinelloides* STG-30, *Ulocladium* sp. AAZ-2020a STG-36, *Cladosporium halotolerans* STG-52B, *Simplicillium lamellicola* STG-96, *Aspergillus protuberus* STG-106, *Penicillium chrysogenum* STG-117 and others. To explore the possibility of

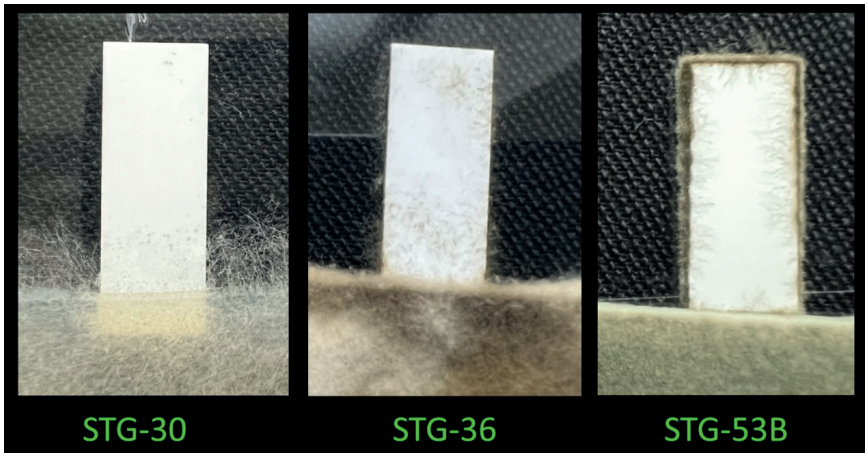

Fig. 1. Cultivation of fungi on Petri dishes, the edge of the plates is partially immersed in agar. Mycelium and spores of fungal strains STG-36, STG-30, STG-56B are visible

using Raman spectroscopy to analyze fungal strains we developed a method for cultivating fungi on the Aluminum oxide (Al_2O_3) surface (Fig. 1).

The studied objects are freeze-dried mold, which are part of various morphological forms, such as mycelial hyphae, sporangia, conidiospores. Based on the literature, it is evident that the composition of organic components in different fungal structures varies [9].

We also cultivated fungal samples on a Al_2O_3 substrate coated with a film of gold nanoparticles to obtain SERS signal amplification. Nanoparticles are synthesized by spark ablation of gold electrodes with a material purity of 99.99% in a flow of nitrogen N_2 with a purity of 99.9999% and transported to a coaxial nozzle with a Q_a flow of 1 L/min for further deposition on the substrate. The deposition process was carried out using a coaxial nozzle with an outlet diameter of 300 μm, positioned at a distance of 4 mm from the substrate. The substrate, fixed on a coordinate table, was moved relative to the coaxial nozzle at an optimized speed of 7 mm/s, which facilitated the formation of plasmonic nanostructures. The desired thickness of the nanostructure was achieved by adjusting the printing time while maintaining a constant speed. The control over the thickness was achieved by setting a specific number of repetitions for the movement of the coordinate table.

The Raman spectra were recorded on a Thermo Scientific™ DXR. The measurement parameters were selected based on the results and using published studies [3, 9]. The primary challenge encountered in achieving a satisfactory signal-to-noise ratio was the potential destruction and carbonization of organic samples upon laser exposure. Furthermore, instrumental inaccuracies prevented accurate determination of the precise location within the sample from which a single spectrum was obtained, a deviation of 2–3 microns was critical. To address this challenge, sample mapping was employed, which not only resolved this issue but also enabled visualization of substance distribution on the surface that may not be readily observable under microscopy [8].

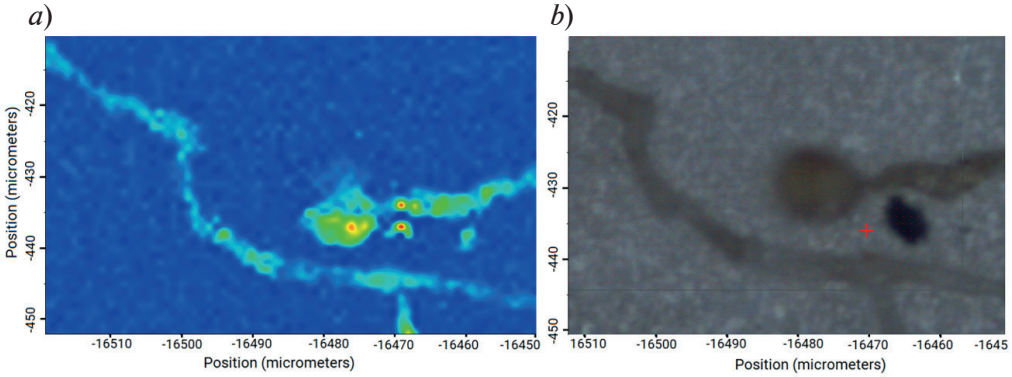

Fig. 2. Mapping of a Ulocladium sp. AAZ-2020a STG-36 grown on the surface of silver nanoparticles: (*a*) – contrast map showing the intensity distribution of the Raman signal at 1100 cm^{-1}; (*b*) is the corresponding optical image on an aluminum oxide plate with gold nanostructures

The implementation of mapping significantly broadened the scope of the research, facilitating the study of the sample in its original state with distribution of organic matter in situ. In the automatic mapping mode (1 μm step), two-dimensional contrast maps of samples (up to 100×100 μm size) were obtained. Mapping reflects the distribution of the intensity of specific lines in the Raman spectra (Fig. 2).

In summary, gold nanoparticles were deposited onto aluminum oxide substrates using a coaxial nozzle system. The deposition process was carefully controlled to achieve the desired thickness of the plasmonic nanostructures, ensuring optimal conditions for subsequent SERS signal amplification. The average gold particle size was 9.5 nanometers.

Results and Discussion

Mapping of molds using Raman spectroscopy made it possible to visualize areas with the highest content of organic compounds on the site. As an illustration, a heterogeneous distribution of the beta-carotene metabolite was observed on a sample of Mucor circinelloides STG-30, as evidenced by the recorded spectrum (Fig. 3).

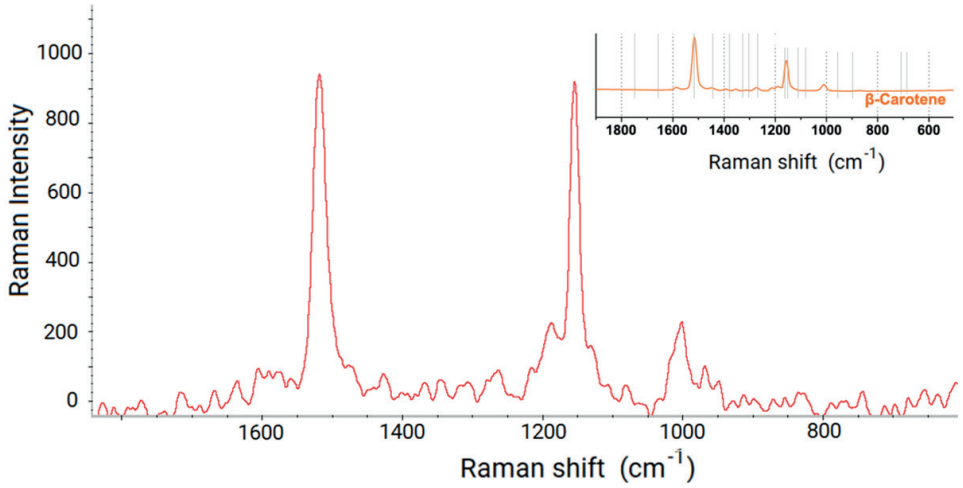

Fig. 3. Example of a signal obtained from Mucor circinelloides STG-30, the spectrum corresponds to β-carotene [9] (shown at upper right)

Conclusion

We did not observe a significant difference in the signal intensity between samples on plasmonic particles and those on a substrate without particles. This may be due to insufficient contact between sample and nanoparticles. The resulting spectra indicate that laser radiation caused carbonization of the organics in the compounds under study, which makes it difficult to identify these substances.

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