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Metal enhanced fluorescence using nanostructures on silver formed with Ti:Saphire femtosecond pulsed laser

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ABSTRACT

Metal enhanced fluorescence is a phenomenon that occurs when a fluorophore is positioned near a conductive structure. Structures can be formed by the interaction of high-energy laser with a sample, on which the femtosecond laser pulses interact with the surface without heat effects. This work aims to study nanostructure formation in polished bulk silver, in order to amplify signals of fluorescence. A Titanium-sapphire femtosecond laser was used to mark silver surfaces. SEM images shows nanostructures formed in chaotic agglomerate of nanospheres with size of 50-800nm. Using Protoporphyrin-IX, the fluorescence amplification was around 300 times compared to a surface without the nanostructures.

Keywords: Metal enhanced fluorescence, Femtosecond laser, Nanostructures

1. INTRODUCTION

In recent years, the use of sensors has been increasingly abundant, and the development of more accurate forms has become necessary. The use of fluorescence spectroscopy technique is presented as a practical tool for use in diagnosis, environmental monitoring, food industry and other applications such as genetic, molecular and physical research, among other areas. For the identification of these molecules, it is desirable to develop technologies with higher resolution and more specificity, in order to amplify the signal of fluorophores that are not naturally very intense. In this context, Metal Enhanced Fluorescence (MEF) has great applicability for the development of sensors for analysis of molecules in low concentration.[1]

When excited by external radiation, metal surfaces interacting with substances can provide an increase in the electric field perceived by these molecules. These surfaces can be created using high intensity laser. A femtosecond pulsed laser can create Laser Induced Periodic Superficial Structures (LIPSS) or non-regular structures on surfaces, so that, silver being a high electrical conductivity metal, can generate MEF-compatible nanostructures. The surfaces required for this purpose rely on the excitation of surface plasmons, which increase the intensity of the local electric field, causing the fluorescence signal of the molecules to increase.[2] LIPPS formation in the material can be described, with certain limitations, by Sipe's theory. When there is a roughness on the surface of the material smaller than wavelength gerenerated by a high energy radiation that strikes it, the interaction between the incident electric field and the surface generates inhomogeneous energy absorption just below the rough region. The surface is then polarized with frequency similar to that of the incident field and generates an interference pattern when this radiation is close to the damage threshold.[3]

The interaction between a fluorophore and the metal with the increase of the fluorescence signal can be described by the concentration of the local electromagnetic field. The metal structures act as antennas that concentrate local fields where

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the fluorophores are, which causes the increase of fluorescence, that is described by solving Maxwell's equations around the metal structure. In the Drude model for metals, they are treated as a positively charged matrix with an electron gas moving through these ions, which when subjected to an external electromagnetic field move coherently through the material. In this model, the restorative effects of Coulomb's law cause the charge density to oscillate across the metal. As it is a localized effect, this oscillation is called resonance of localized surface plasmons. This resonance in two or more nanostructures can be combined with the highly amplified electric field between the particles.[4]

This work has an innovative character and aims to generate silver nanostructures capable of increasing the fluorescence signal of molecules near them, using a femtosecond pulsed laser. For this, we prepared the metallic surface (silver) to eliminate scratches, in order to perform femtosecond pulsed laser marking and observe created structures, analyze the formation of nanostructures and the fluorescence increase due to the technique.

2. METHODS

2.1 Silver samples preparation

125x125x1mm samples were prepared from silver plates. Every piece was embed on bakelite with a BUEHLER SIMPLIMET XPS1 embedder for easier handling and polishing.

All the samples were polished. Polishing procedure started with granulation sandpaper from 800 to 2500, until to obtain homogeneity of all the surface. Then, the process of final polishing was done using diamond (9-3 µm) and alumina (1-0,05 µm) suspensions. The polishing process is essential for removing scratches that may interfere with the formation of nanostructures on the surface.

After polishing, the samples were cleaned using ultrasonic bath in absolute ethanol to remove residues that could influence its marking. For cleaning was used 5 minutes of sonication at 40 ºC.

2.2 Femtosecond marking process

The process of marking proceeded using Laser Libra Ti:Sapphire from Coherent, 450 mW, 1 KHz of frequency and wavelength of 850 nm.

The varied parameters in the marking process were the distance between the sample and the laser focal point from 0 to 5 mm, laser scan speed from 1 mm/s to 8000 mm/s, distance between consecutive marking lines (0,1 µm to 0,1 mm) and loop number. The combination of these parameters indicates the number of laser pulses on a specific region of the sample.

Using the combination of longer distances between the sample and laser focus for higher marking speed and distance between lines, less energy was transmitted to the surface. On the other hand, with the focus on the sample, low scan speed and short line distance, more energy was transmitted to the surface.

2.3 Structure analysis

Analysis of all samples was obtained by optical microscopy, to observe the macroscopic changes and checking for the presence or absence of scratches after polishing. Atomic Force Microscopy (AFM) was used to measure the amplitude

generated in the nanostructures formed on the surface. Scanning Electronic Microscopy (SEM), allowed visualization and characterization at high resolution nanometer or micrometer scales, enabling bidimensional surface images.

2.4 Fluorophore deposition

We used Protoporphyrin IX (PpIX) for analysis of the fluorescence signal increase. For all analyzes the concentration of 0,5mg/ml of PpIX in ethanol was used. Fluorophore deposition was made by the addition of a drop directly onto the surface and analysis after drying, or by spin coating at 3000 rpm and 60s.

2.5 Fluorescence analysis

Samples were analyzed using a photon excitation Zeiss confocal fluorescence microscope (inverted model LSM 780) (LASER Diode 405 nm; LASER Argon: 458/488/514 nm; LASER HeNe: 543/594/633 nm) or two photons (LASER Ti: Sapphire (emission: 690-1050 nm) from the Confocal Microscopy Laboratory (IFSC / USP).

3. RESULTS AND DISCUSSIONS

3.1 Sample polishing

The analysis of the silver plates already polished, realized by SEM and AFM, presented risks below 350 nm, indicating that they will not affect the creation of nanostructures of interest. Figure 1 shows the results of SEM (a) and AFM (b) analysis of silver surfaces after polishing, indicating some of the larger scratches found.

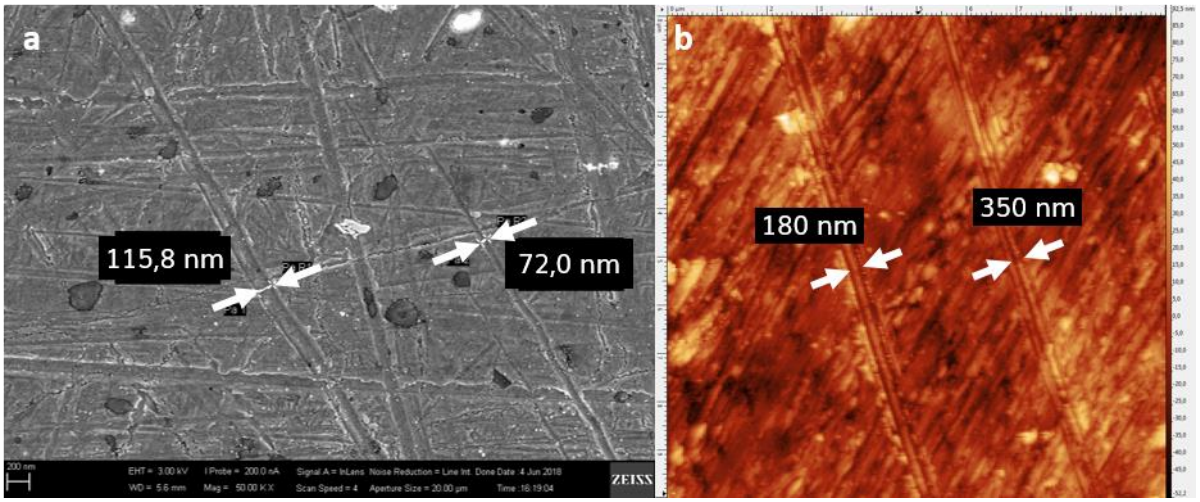

Figure 1. SEM (a) and AFM (b) analysis of polished silver plates, indicating the scratches and their respective sizes.

3.2 Nanostructure formation

Using parameters of greater distance between sample and laser focus, greater distance between marking lines and lower scanning speed, there was the formation of periodic structures in the metal, which can be observed directly by staining the marked region. On this region the ambient light is diffracted due to the presence of nanostructures. These nanostructures could be observed in the SEM and AFM analyzes, indicating the presence of periodic nanostructures ranging from 450 to 550nm. We can see in Figure 2 the periodicity of the structures formed when these parameters were used.

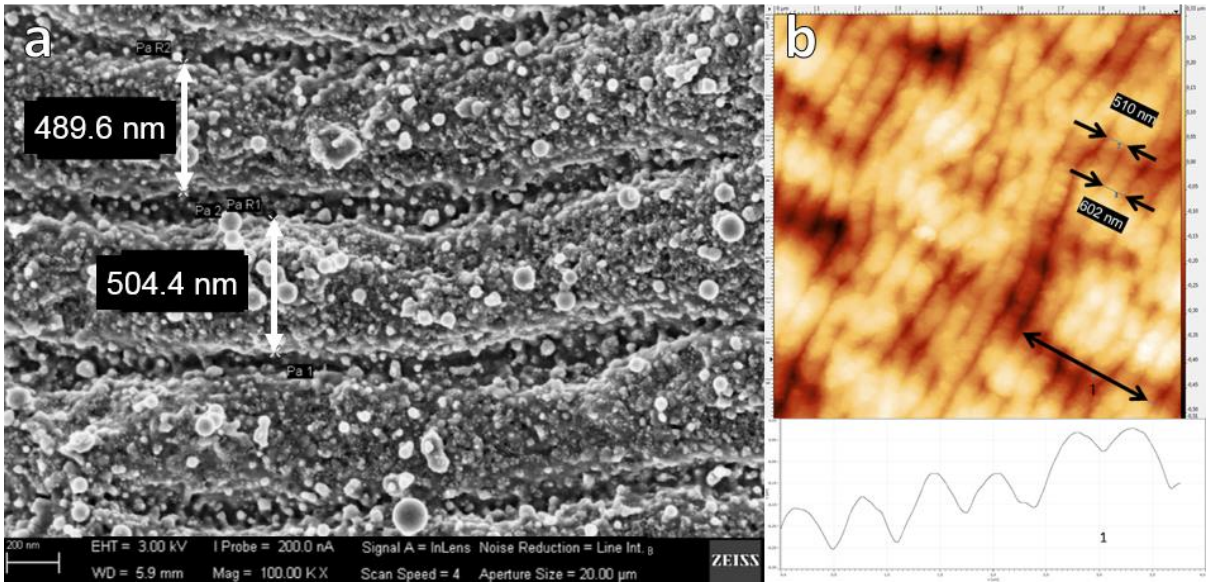

Figure 2. SEM (a) and AFM (b) images of periodic structures formed by laser marking with low intensity parameters.

When high intensity parameters were used, more energy was transmitted to the surface. These parameters were defined by locating the sample over the laser focus, as well as the marking speed and line distance are smaller. Using these parameters, the surface presents a very different profile from the previous ones, formed by chaotic clusters of nanometric spheres. These spheres have sizes ranging from 10 to 500 nm. AFM analysis on this type of structure was not possible due to surface irregularity. Figure 3 shows a region marked with these parameters and the formation of spheres in various sizes (a). Highlight (b) shows one of these spheres.

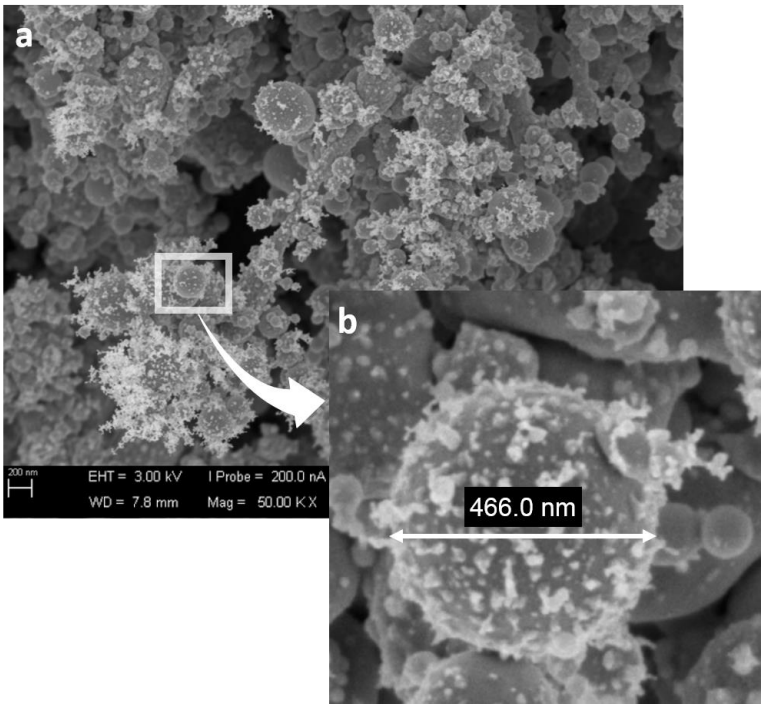

Figure 3. SEM images of structures formed by laser marking with high intensity parameters as chaotic agglomerate of nanospheres.

3.3 Fluorescence enhancement

The increase in fluorescence signal was observed only when the most chaotic structures was formed (markings with higher intensity parameters). The periodic structures obtained did not show significant levels of signal increase. By using excitation at 405 nm, an increase in fluorescence signal was obtained with the presence of ethanol-diluted Protoporphyrin IX in direct deposition, when comparing a labeled to unlabelled region of the plate. Comparison of spectra indicates an increase of approximately 25 times. In Figure 4, we see the emission spectrum of PpIX with ethanol marked in the nanostructured region in red related to the external region marked in green. Also can be observed the region marked with fluorophore on the plate (a) and the nanostructures present (b).

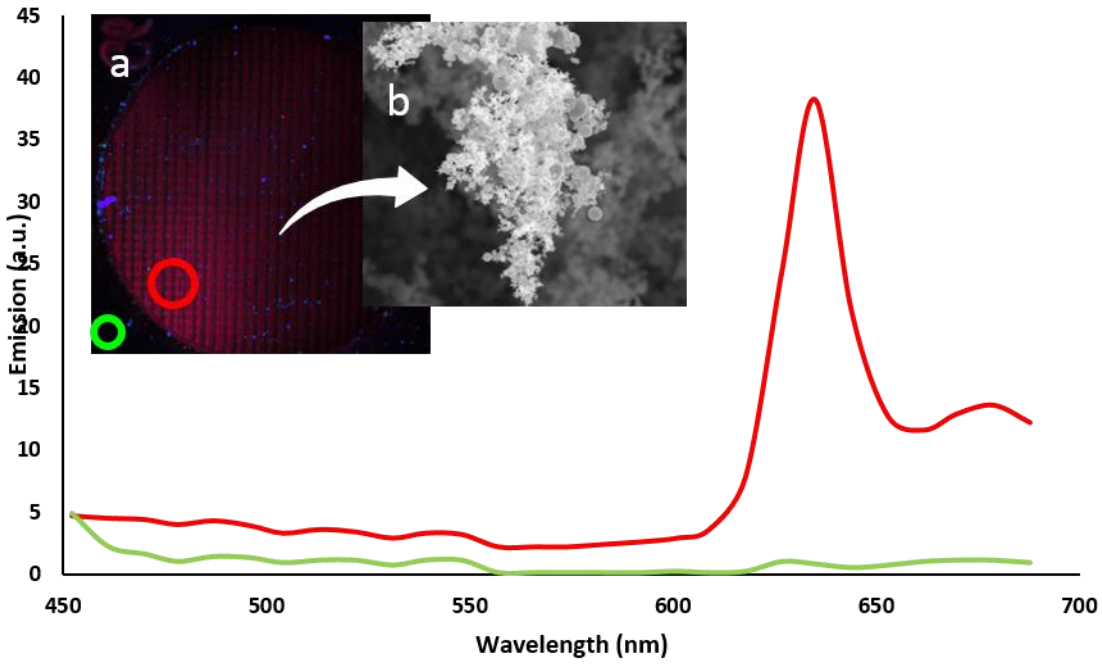

Figure 4. PpIX emission spectrum comparing the region with nanostructures (red) and without nanostructures (green). (a) Region of the laser-marked silver plate. (b) SEM image of the nanostructures present.

By analyzing the deposition by spin coating of PpIX in ethanol on the surface, it was found a significant increase of the signal in relation to the unmarked region. The porphyrin emission signal, for the excitation of two photons (excitation at 800 nm) over the region with nanostructures related to the one, that does not present them, presents an increase value of approximately 300 times. Figure 5 shows the increase in fluorescence emission of from PpIX when compared to the marked region (red) with regions near the without nanostructures (blue and green). (a) Indicates the regions of acquisition of the spectra and (b) the nanostructures present in the region.

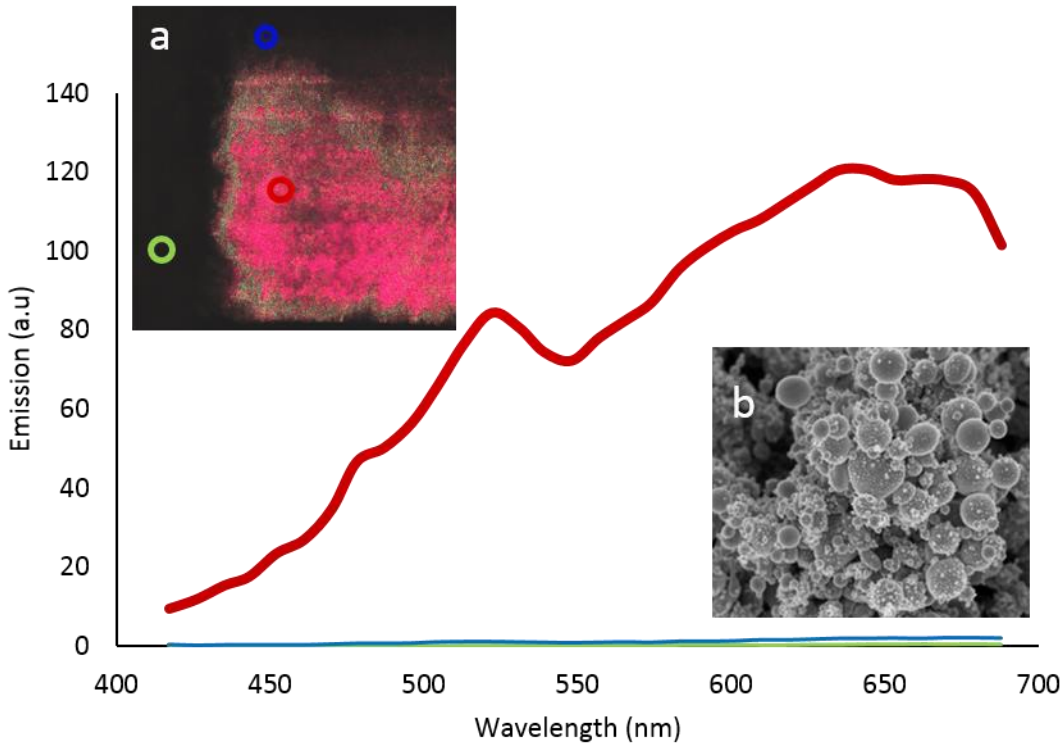

Figure 5. PpIX emission spectrum comparing the region with nanostructures (red) and without nanostructures (green/blue). (a) Region of the laser-marked silver plate. (b) SEM image of the nanostructures present.

4. CONCLUSION

Using polishing techniques suitable for soft metals such as silver, with the aid of specific equipment, it was possible to obtain surfaces with small scratches that do not interfere with the nanostructures creation process. Risk elimination is necessary, when working with structures formed on the surface is highly dependent on the surface condition. The presence of scratches affects the laser's interaction with the material as it is dependent on the distance between the surface and the laser's focal point.

Laser marking with the various parameters provided different structures according to the parameters used in each sample. LIPSS structures were formed when the marking parameters were softer, while chaotic structures with higher number of nanostructures were formed with higher intensity marking parameters. The formed structures showed specific optical properties. Periodic structures act as diffraction grids, and the color observed on the surface changes with the angle of incidence. Meanwhile, the accumulation of creep due to the repeated pulses of greater intensity on these regions causes it to change significantly, accumulating changes in the outermost layer of the material, which generates chaotic and disordered structures on the nanometer scale. The effect of the generation of these LIPSS is due to the interaction of the femtosecond laser electric field with the surface, and the results obtained are similar to those of Vorobyev and Guo (2018)[5], who use the silver nanostructures to alter the optical properties of the metal, for possible applications in photonics, plasma, optoelectronics and other areas.

The fluorescence signal from marked surfaces, when PpIX is deposited by spin coating and analyzed at 405 nm excitation, shows a 25-fold increase in signal. When fluorophore is added directly to the surface and the two-photon

excitation analysis (800 nm) is performed, a 300-fold increase in signal is observed. This increase is due to the high proximity between the formed nanostructures and the added fluorophores. The presence of chaotic structures, which provide spaces for the molecules of interest to enter and be located very close to the metal, has made the goal of increasing the fluorescence signal successful. Shtoyko et al.[6] used fractal structures made of silver to increase the fluorescence signal and obtained about 100-fold increase for the rhodamine-labeled conjugated antigen associated rabbit IgG antigen. The increase was due to the proximity of the fluorescent molecule to the fractal metal structures.

In summary, using the combination of polished surfaces, and a labeling process that generates nanostructures capable of significantly increasing the fluorescence signal of fluorescent molecules at micromolar concentrations, it can be concluded that the technique proves to be appropriate for fluorescence analysis of diluted samples. The interaction between the fluorophore and how it is located on the metal is still uncertain, but future studies may propose ways to verify their adhesion on the nanostructures and the coupling forms between them.

5. ACKNOWLEDGMENTS

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