INVITED PAPER

Multi photon excitation fluorescence imaging microscopy for the precise characterization of corrosion layers in silver-based artifacts

**F. Faraldi · G.J. Tserevelakis · G. Filippidis ·
G.M. Ingo · C. Riccucci · C. Fotakis**

Abstract In this study, we present results of the implementation of multi photon excitation fluorescence (MPEF) imaging measurements to silver-based artifacts for the identification of the corrosion layers (generally composed by chlorargyrite, AgCl). We employ as an excitation source a compact femtosecond (fs) laser operating at 1028 nm. Silver-based reference alloys, artificially aged, were used as samples. Two and three dimensional images of the corrosion layers, detected in the reflection mode, are depicted. MPEF imaging technique was proved to be an ideal, diagnostic tool for the precise (axial resolution of ∼1 μm) thickness determination of silver chloride layers. Moreover, this technique has the potential to provide complementary information about the physical and chemical stability of silver-based artifacts, without affecting the art objects. The non-destructive nature of the MPEF modality, constitutes a certain advantage in comparison to the conventional techniques used for these kind of measurements.

1 Introduction

Metals were widely used in the ancient times for functional and economic reasons and many metal artifacts represent the ability of ancient metallurgists to modify the structure of metals and create, in many cases, objects manufactured at a high level of technological competence. Unfortunately, metals are affected by corrosion, which leads to a chemical and structural modification of the original artifacts and the consequent weakening of their mechanical properties. Different factors of the material or the environment where the artifact remained for long time (soil or atmosphere), can determinate the development of the corrosion and the formation of stratified structures, characterized by a certain chemical composition.

The conservation of metal artifacts is a crucial issue for experts in the field of Cultural Heritage in order to preserve the witness of the ancient metallurgical art. Therefore, the constant development of novel accurate diagnostic and cleaning methods is highly necessary for the acquisition of the material information without affecting the artifacts.

Laser spectroscopic technologies have become an important tool for Cultural Heritage studies [1–3]. These diagnostic techniques can assist laser-based cleaning applications, however, they lack of an adequate axial resolution that is essential for the precise determination of different layers.

Nonlinear imaging techniques (multiphoton excitation fluorescence (MPEF), second and third harmonic generation (SHG, THG)) have been extensively used as diagnostic tools for biological studies [4–6]. Lately, these high resolution non-destructive imaging modalities have been employed as analytical tools in the service of Cultural Heritage. Specifically, MPEF microscopy measurements have been used to recover lost information in the area of archeology [7]. Additionally, SHG imaging measurements have been applied for the evaluation of corrosion of painted metals [8]. Higher harmonic generation (SHG–THG) spot measurements were implemented for the extraction of information related to the thickness determination and composition discrimination of

F. Faraldi · G.J. Tserevelakis · G. Filippidis (✉) · C. Fotakis
Institute of Electronic Structure and Laser, Foundation for
Research and Technology Hellas, P.O. Box 1527, 71110,
Heraklion, Greece
e-mail: filip@iesl.forth.gr

F. Faraldi · G.M. Ingo · C. Riccucci
Institute for the Study of Nanostructured Materials—CNR,
Research Area of RM 1—Montelibretti, via Salaria km 29.3,
00015, Monterotondo, Rome, Italy

G.J. Tserevelakis · C. Fotakis
Department of Physics, University of Crete, Heraklion, Greece

various types of natural and synthetic glue used for lining of painted artifacts [9]. In addition, recently, two photon excitation fluorescence (2PEF) and SHG imaging measurements have been used for wood characterization and historical coating analysis [10]. Moreover, these nonlinear modalities have been employed as non invasive tools for the accurate 3-D delineation of the bulk of the protective layers of model multilayer painting artworks [11, 12]. The precise thickness determination of protective layers (via THG imaging) and the identification of the chemical composition of the artifacts (via MPEF measurements) have been achieved.

In this work, the degradation phenomena of silver-based artifacts by means of the MPEF imaging technique have been studied. Silver is one of the metals known since ancient times, and it was considered, as well as gold, as a precious metal. Silver-based artifacts gained a significant artistic, historical and economical role over the time and they were used as currency or medium of exchange, jewels, artistic items and means of saving. Studies performed on several samples of silver-based artifacts coming from different countries of the Mediterranean basin demonstrated that the main corrosion product on silver objects is silver-chloride (chlorargyrite, AgCl) [13–15]. Here we propose a new non-destructive method for the investigation of the silver-chloride corrosion layer via the detection of MPEF signals. This is the first time, to the best of our knowledge, that multi photon imaging techniques are employed for the analysis of metal-based artifacts.

By employing tightly focused femtosecond (fs) laser pulses, the photon density is high enough to induce multi-photon absorption within the focal volume in the material. Fluorophores whose excitation maximum is in the ultra violet (UV) or in the visible (VIS) parts of the electromagnetic spectrum can be excited by two, three or four infrared (IR) photons. Since nonlinear absorption and thus induced fluorescence occurs solely at the focal volume of the laser beam, a high axial resolution and consequently the three dimensional (3-D) imaging capability of confocal microscopy can be attained without the use of a confocal aperture. Furthermore, there is no interfering fluorescence coming from the surrounding structures and "out of focal plane" photobleaching can be significantly reduced. Additionally, the use of IR light permits a high penetration depth into the sample due to the low absorption and scattering by the studied object. Therefore, the MPEF technique constitutes a potential indicator concerning the in depth chemical composition of the examined material.

Through the analysis of MPEF signal of the chlorargyrite compound, not only the thickness of the corrosion layer can be measured with accuracy in the order of 1 μm, but also the respective density can be evaluated without the necessity of taking samples from the artifacts or cutting them, in contrast

to the conventional techniques (Optical or Scan Electron Microscopy) used for these kind of measurements. The non-invasive nature of the proposed method, makes it a powerful tool for the in situ discrimination and characterization of the corrosion layer in silver-based artifacts, improving thus significantly the accuracy of the cleaning procedure. In general, this modality has the potential to be implemented for the exact determination of corrosion layers both for Cultural Heritage studies and diverse industrial applications (e.g. electronic systems).

2 Experimental apparatus—sample preparation

In our study, an Amplitude systems t-pulse laser (1028 nm, 50 MHz, 1 W, 200 fs) has been used as an excitation source. The fs laser beam was directed into a modified Nikon upright microscope (Nikon Eclipse ME600D). The average laser power on the specimen was 30 mW. Thus, the use of fs lasers enables high peak powers at the sample plane (∼3 KW) for efficient nonlinear (multi-photon) excitation, but at low enough energies (0.6 nJ per pulse) so that the examined cultural heritage objects are not damaged.

A 20x, 0.80 numerical aperture (NA) objective lens (Carl Zeis, Plan Apochromat) was employed for the tight focusing of the laser beam onto the sample. A telescope system which expands the beam has been used to fill the back aperture of the objective lens. The scanning procedure (xy direction) was performed with a pair of galvanometric mirrors (Cambridge Tech. 6210H). The samples were fitted into a motorized xyz translation stage (Standa 8MT167-100) and the focal plane was selected with this stage (1 μm resolution). Lab View interface controlled both scanning and data acquisition procedures. A CCD camera (PixeLINK PL A662) was used for observation of the objects.

MPEF signals were collected in the backward direction using a photomultiplier tube (PMT Hamamatsu R4220). The photomultiplier tube was attached at the position of the microscope eye-piece. A short pass filter (SPF 450 nm CVI) was placed at the photomultiplier input to cut off the reflected laser light and solely detect the violet and blue fluorescence from the sample (silver chloride).

For the verification of the obtained MPEF results, Scanning Electron Microscopy (SEM) measurements on the same silver-based alloy samples were performed (surface and cross section analysis).

The investigation of silver-chloride layer with MPEF microscopy was performed on reference silver-based alloys produced by the ISMN-CNR (Rome-Italy). The artificial corrosion layer was obtained by an accelerated degradation procedure and its properties have been experimentally proved to be comparable with those of the alteration layer of real archaeological artifacts [16]. A number of samples has been tested.

3 Results

The employed in multi photon microscopy excitation wavelengths of ~1 μm are ideal for providing in depth information of the sample since silver chloride is transparent in the visible and near infrared (IR) spectral regions of the electromagnetic spectrum. AgCl compound absorbs in the ultraviolet (UV) region (predominately below 260 nm) [17]. The minimal absorption of chlorargyrite in the near IR, visible and near UV regions indicates that the main contribution to fluorescence processes is probably due to four photon excitation. Specifically, by exciting the sample with 257 nm line (wavelength for four photon absorption in our case) the emission spectrum is located in the violet and blue regions (Fig. 1). When exciting at 514 nm (corresponding to two photon absorption) and 343 nm (for the three photon absorption case) no emission was detected, confirming that the origin of the fluorescence processes is mostly due to four-photon excitation. For the realization of the absorption and the emission measurements from silver chloride, a UV/Vis Spectrometer (Rerkin Elmer-Lambda 950) and a fluorometer (Jobin Yvon Horiba FluoroMax-P) were employed, respectively.
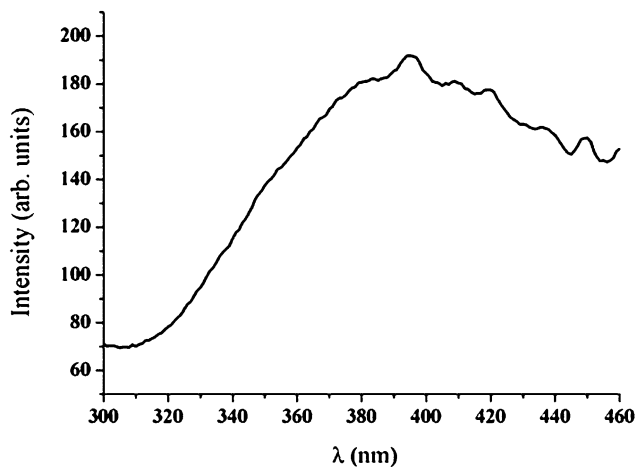
Fig. 1 Emission spectrum of the chlorargyrite (AgCl) layer following excitation at 257 nm
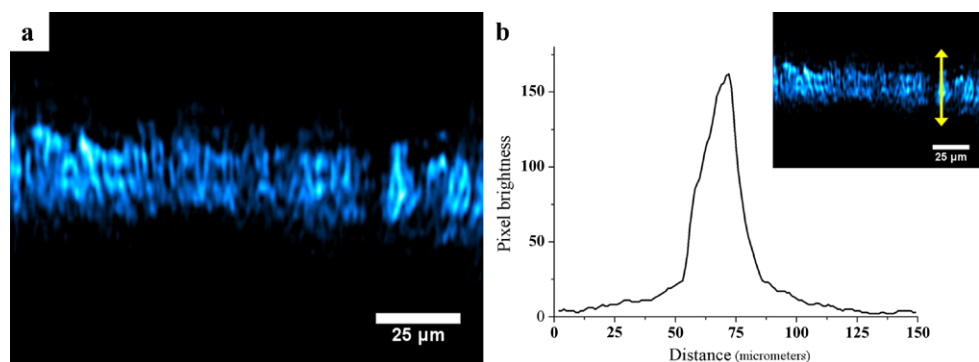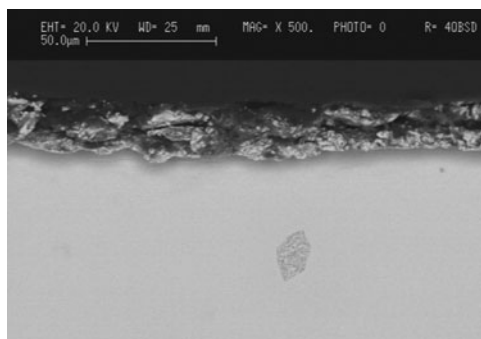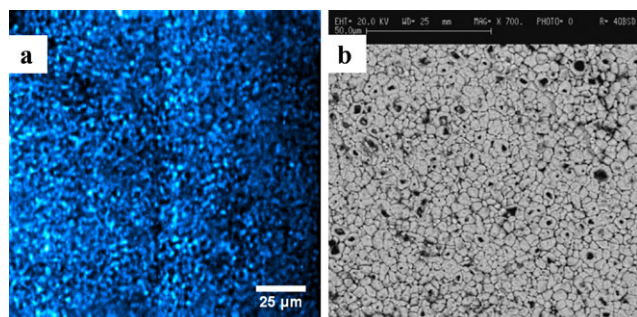
Figure 2a represents the cross section of the AgCl corrosion layer through the in depth detection of its respective MPEF signal. The distribution of the fluorescence intensity arising from the layer appears to be well defined, in contrast to the total absence of signal of the silver-based alloy which is totally reflective to the employed near IR excitation wavelength. The above different properties between the corrosion layer and the alloy, result into a high contrast image, which in combination to the intrinsic resolution (~1 μm) of the proposed technique, provides a detailed mapping of the degradation phenomena. For obtaining a quantitative view of the signal distribution, the graph of the pixel brightness across a vertical line passing through the high signal region was constructed (Fig. 2b). In this way, the thickness of the corrosion layer in this specific point was measured by calculating the full width at half maximum (FWHM) of the resulting curve and was found to be 19 μm. The thickness of the corrosion layer, due to inhomogeneity of the sample, was ranging from 15 to 22 μm. The results of MPEF were confirmed by the thickness measurement of the silver chloride layer obtained by SEM (Fig. 3), thus validating the reliability of the nonlinear technique.

Consequently, chlorargyrite corrosion layers depths of silver-based artifacts can be determined at a high axial resolution by employing the MPEF imaging technique. During cleaning procedure, either by mechanical, chemical or laser methods, it is crucial for conservator scientists to have an estimation of the corrosion layer thickness in order to detect the limit of cleaning and to stop the procedure at the point where the silver original surface is revealed. These critical information, related to the corrosion layers thicknesses, can be acquired via the realization of MPEF imaging measurements. Furthermore, a significant number of metal artifacts can be imaged via the implementation of MPEF technique, for the precise non-destructive determination of the corrosion layers based on their absorption and emission spectral features.

The corrosion layer thickness of a metal artifact can vary according to the local differences in the burial or exposure conditions (e.g. humidity, oxygen levels, pH etc.). The

Fig. 2 (**a**) Cross section image of the corrosion layer in an artificially aged silver-based alloy, obtained via the in depth collection of MPEF signals; (**b**) plot of the pixel brightness distribution across a vertical line, for the image (**a**)

**Fig. 3** SEM image of the cross sectioned artificially degraded silver alloy

Fig. 4 (**a**) Maximum *z*-projection MPEF image of the chlorargyrite layer surface. (**b**) The respective SEM image showing the AgCl crystals morphology

MPEF technique is capable to detect this variability in thickness, therefore it can provide an estimation of the alteration phenomenon degree for silver-based artifacts, as well as information concerning the physical and the chemical stability of these art objects. The silver chloride compound is thermodynamically stable; as a result, a compact layer of chlorargyrite can preserve the silver from any further alteration process.

Differences in the density of the chlorargyrite layer are represented by the intensity variations of the MPEF signal (Fig. 4a). More specifically, a high intensity of the MPEF signal indicates a greater layer density, while the weak signal regions can give information about the areas characterized by lack or low density of crystals, preferentially affected by alteration phenomena.

Moreover, the respective three dimensional (3D) reconstruction of the corrosion layer volume via the collection of MPEF signals from 50 sequential optical planes separated by a distance of 1 µm (Fig. 5), shows the spatial distribution and the morphology of the chlorargyrite crystals in space. Through the observation of the chlorargyrite SEM images (Fig. 4b), the crystalline structure of the silver chloride can be verified and the accuracy of the spatial information obtained via the MPEF signal analysis is clearly demonstrated.
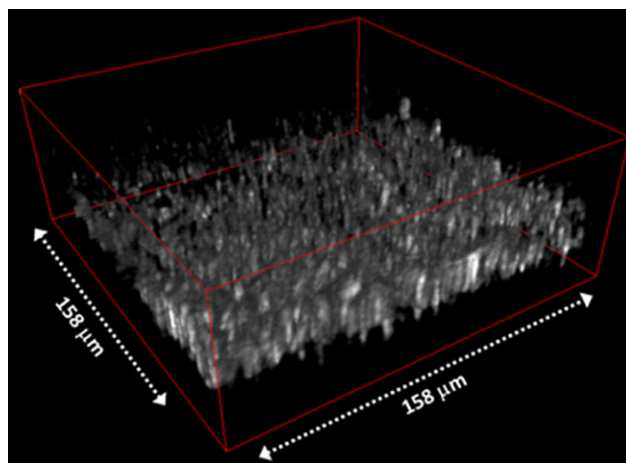
Fig. 5 3D reconstruction of the recorded MPEF signal, of the AgCl layer volume, generated through the combination of 50 sequential optical planes, separated by a distance of 1 µm

4 Conclusions

In the present study, MPEF imaging technique is reported as a powerful, non destructive, high resolution tool for the identification of the thickness of silver chloride layer without affecting the silver-based samples, while at the same time obtaining useful information about artifacts stability. Additionally, via the 3D reconstruction of MPEF signals the morphology and the density of the chlorargyrite crystals can be verified.

Since the MPEF signal detection took place in reflection mode for the examined silver-based artifacts, we anticipate that this non-invasive technique has the potential to be applied on the evaluation of original artifacts, facilitating the control of any cleaning interventions. Moreover, the reduced time of data acquisition (∼2 minutes for a cross sectional scanning) makes this technique ideal for in situ applications.

The perspective for the future is the development of a portable reliable system which will have the capability to analyze the silver chloride layer, provide precise information for the thickness of the corrosion layer and monitor the chemical and physical stability in artifacts of various sizes. The development of compact, user-friendly femtosecond laser sources, helps the widespread adoption of nonlinear imaging microscopy for Cultural Heritage studies. Multiphoton microscopes are now commercially available and constitute flexible and relatively easy to use systems, since a single femtosecond laser beam is required for the realization of the measurements. Therefore, we believe that nonlinear optical imaging, and especially the MPEF modality, has the potential to be extensively used as a novel non-destructive diagnostic tool for in situ analysis providing valuable information for Cultural Heritage studies and more general for industrial applications.

References

1. D. Anglos, Appl. Spectrosc. **55**, 186 (2001)
2. A. Nevin, S. Cather, D. Anglos, C. Fotakis, Anal. Chim. Acta **573**, 341 (2006)
3. P. Targowski, B. Rouba, M. Góra, L. Tymińska-Widmer, J. Marczak, A. Kowalczyk, Appl. Phys. A **92**, 1 (2008)
4. W.R. Zipfel, R.M. Williams, W.W. Webb, Nat. Biotechnol. **21**, 1369 (2003)
5. P.J. Campagnola, L.M. Loew, Nat. Biotechnol. **21**, 1356 (2003)
6. G.J. Tserevelakis, G. Filippidis, E.V. Megalou, C. Fotakis, N. Tavernarakis, J. Biomed. Opt. **16**, 046019 (2011)
7. G. Cormack, P. Losa-Alvarez, L. Sarrado, S. Tomas, I. Amat-Roldan, L. Torner, D. Artigas, J. Guitart, J. Pera, J. Ros, J. Archaeol. Sci. **34**, 1594 (2007)
8. J. Ying, F. Liu, P.P. Ho, R.R. Alfano, Opt. Lett. **25**, 1189 (2000)
9. G. Filippidis, K. Melessanaki, C. Fotakis, Anal. Bioanal. Chem. **395**, 2161 (2009)
10. G. Latour, J.P. Echard, M. Didier, M.C. Schanne-Klein, Opt. Express **20**, 24623 (2012)
11. G. Filippidis, E.J. Gualda, K. Melessanaki, C. Fotakis, Opt. Lett. **33**, 240 (2008)
12. G. Filippidis, M. Massaouti, A. Selimis, E.J. Gualda, J.M. Manceau, S. Tzortzakis, Appl. Phys. A **106**, 257 (2012)
13. E. Angelini, T. De Caro, A. Mezzi, C. Riccucci, F. Faraldi, S. Grassini, Surf. Interface Anal. **44**, 947 (2012)
14. A. Mezzi, T. De Caro, C. Riccucci, E. Angelini, F. Faraldi, S. Grassini, Surf. Interface Anal. **44**, 972 (2012)
15. G.M. Ingo, S. Balbi, T. De Caro, I. Fragalà, E. Angelini, G. Bultrini, Appl. Phys. A **83**, 493 (2006)
16. M.P. Casaletto, G.M. Ingo, C. Riccucci, F. Faraldi, Appl. Phys. A **100**, 937 (2010)
17. F. Moser, F. Urbach, Phys. Rev. **102**, 1519 (1956)