Early Fungal Contamination Tracking

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***Abstract*—**This paper presents a complex portable photonic setup that exploits LIF advantages for biological contamination control of a surface and a case study that validates the investigation procedure. Based on LIF method the necessary information for microbiological species discrimination is collected from distance in real-time, without mechanical contact and preserving surface reliefs. On the basis of the gained know-how, a compact fluorescence scanning apparatus has been designed and realized in the laboratory in order to perform advanced field measurements and investigations.

***Keywords*—** fluorescence, laser, microbiology, scanning device, spectroscopy

I. INTRODUCTION

FUNGAL contamination has been a threat ever since in our day by day existence, and especially in all the domains that implies organic materials preservation, such as food industry or cultural heritage conservation. Early detection of such contaminations is a must, in order to prevent further spreading of the biological agents that could endanger the health security of the living beings as well as the integrity security of the evidences of our past and history inherited from our predecessors.

Beyond the naked eye observation, the accurate optical investigation and bio-chemical analysis of non-contact and non-invasive devices provides improved warranty in surface contamination control. In order to develop such devices, a fast, accurate and a safe analyzing technique is needed.

In the last decades a lot of studies in the field of laser interaction with the matter were made. Among the most important challenges in various applications, investigation and analysis techniques of the surfaces based on the laser interactions on materials faced serious requests in all biological applications, mainly due to the laser beams high precision and its non-invasive character. Therefore there have been developed various laser based analyzing techniques.

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Two of the best investigation methods that are using the laser beam as the surface composition detection processes inductor are the laser induced breakdown spectroscopy (LIBS) and laser induced fluorescence spectroscopy (LIF). Within these two methods, a laser radiation is applied on a surface producing interaction processes that are monitored, analyzed and interpreted by the means of dedicated detection devices and software applications.

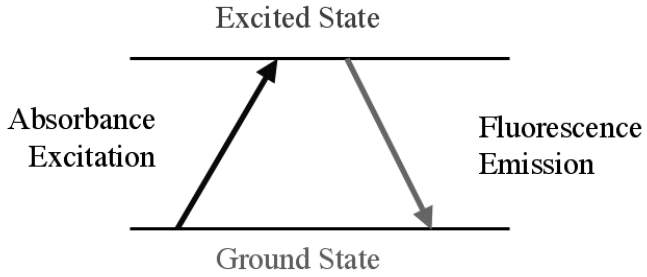


Fig.1 Basic principle of fluorescence emission

This applied method is a molecular emission spectroscopic technique based on the absorption of light emitted by the laser. [1]

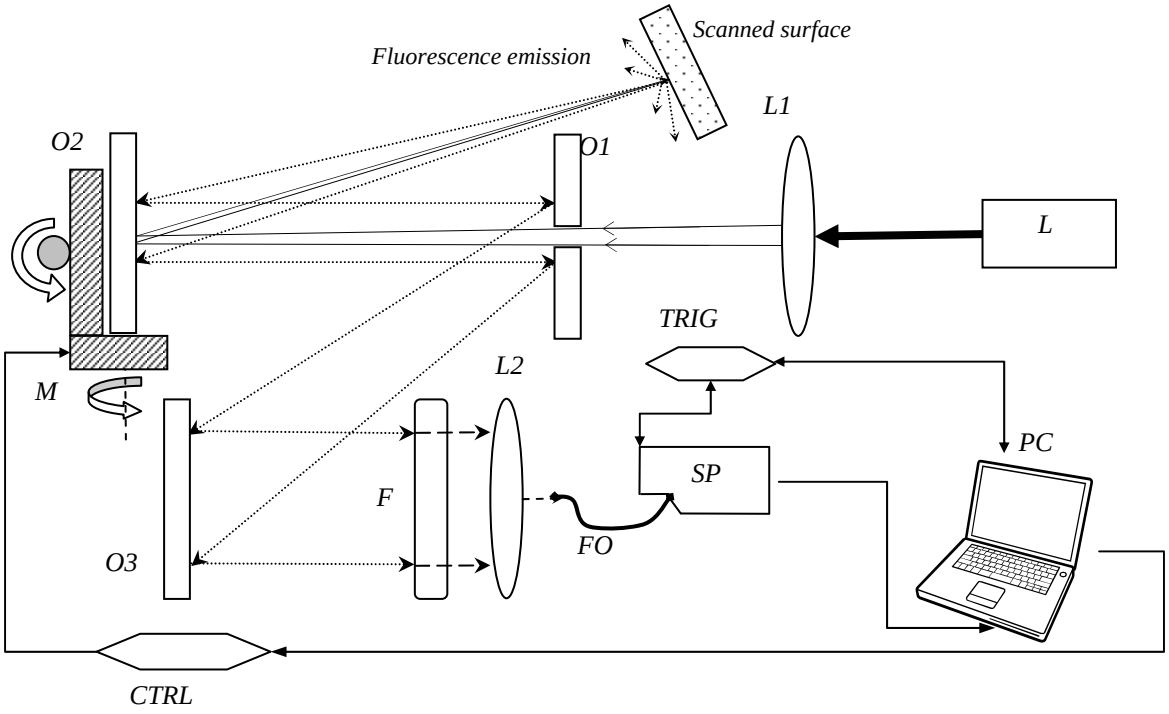
In Cultural Heritage this tehnique is used already used in material identification of artefacts. It is well-known that each material has its own characteristic electromagnetic absorption and emission properties. As a laser induced technique, the LIF method speculates these properties being the incoherent emission of photons from an unstable energy level populated by the absorbtion if photons from the laser. So, depending on the surface's molecular composition and the incident laser parameters, the absorbed and emitted photon wavelengths are different. Not only materials, but even the biological layers have their own fluorescence emission characteristic.

The emission spectrum, obtained using the LIF tehnicque, provides valuable informations directly related to the molecular structure of materials on the irradiated area**.** Besides the molecules in a molecular substrate, or some impurities or crystal defects into solids, the microbiological species are also responsible of specific fluorescence emissions. Pulsed UV lasers are succesfully and often used for inducing the fluorescence due to the solid substances' broad absorption bands in the UV region.

Due to the laser design field development, pulsed UV lasers of small dimensions are now available, so, portable LIF systems are now reachable. Such devices can assure on site high resolution identification of small traces of substances, chemical compounds or, even, incipient biocontaminations. It is useful to add that LIF results can be associated with on site high-resolution thermography, and high-resolution 3D models obtained by laser scanning, resulting in diagnostics of complex structures at one moment. [2]

Laser scanning of an area implies the redirection of the laser beam, emitted by the source, to each spot of that area (respectively with the input resolution) following a well-defined algorithm.

Figure 2 depicts the schematics of such a device that allows the LIF scanning of a defined area on a surface. The generated laser beam is optically deflected by a mirror mounted on an ensemble of two stage motors, controlled by a computer that allows the high precision rotation on the two central axes of the



This paper presents a complex portable photonic setup that exploits the LIF tehnique advantages for biological contamination control of a surface and a case study that validates the investigation procedure. There is large range of applications, of LIF investigations, from dermatology, food [4] and pharmaceutical industries to cultural heritage conservation [5].

II. PORTABLE LIF SCANNING DEVICE SETUP

Using the LIF technique, the necessary information for the contamination discrimination can be picked up from distance in real-time, without mechanical contact and preserving surface relief, using a proper setup.

Punctual interrogations of a surface can provide useful information on that point's surface quality. But a point by point interrogation of an entire area offers a much detailed data about that surface quality. Therefore an automated device that could actually scan a defined area of that surface based on a certain algorithm using an acquisition technique that can provide us with the desired data has been developed.

mirror. The interaction of the laser beam with the surface there will be induced a fluorescence emission of the molecules, consisting the surfaces' material, that will propagate in all directions. Partly, the emitted fluorescence falls on the stage motors mounted mirror and is transmitted through an optical ensemble further to a detection device. The optical ensemble is constituted by a perforated mirror, which allows the laser beam to propagate through its aperture but reflects the fluorescence emission that comes from the redirecting mirror, ensuring this way an coaxial geometry of the system; another redirecting mirror that reflects the fluorescence emission through-out an UV filter (which eliminates the second and third diffraction order and prevents the acquisition of the excitation energy) up to the optical collector; optical fibre for information transmission to the spectrometer.

The irradiation source ***L*** is a solid state passive Q-switched diode pumped laser that emits at 266 nm with a maximum repetition rate of 3 KHz at 1,25 μJ of energy per pulse. The ***L1*** lens converges the laser beam.

The redirecting mirror's rotational ensemble device is constituted by two stage step motors. These motors can proceed at high precision rotations, with a minimum step of

0.0005˚ with a maximum speed of 80˚/s. Both motors are connected to controller ***CTRL*** to communicate with the computer through a serial port. A software application designed under the LabView platform ensures the full control of the motors operation. The optical collector ***L2*** is a 25.4 mm diameter collimating lens. The collected data is transmitted to the spectrometer ***SP*** through the optical fibre ***FO***. In front of the collector it is mounted the UV filter ***F*** to avoid the acquisition of the incident laser beam reflection and to eliminate the 2nd and 3rd diffraction maximums. As for the detection it is used a low wavelength high sensitive spectrometer, best suited for fluorescence acquisition, detecting light radiation in the 200-1100 nm bandwidth with a 90% quantum efficiency. To synchronize the irradiation spot's position changes with the fluorescence emission acquisition, it was used a device that allows the external software triggering. The ***TRIG*** break-out box is the communication link between the spectrometer and the computer or other triggering devices.

Being designed as a portable and out-door deployable setup, the whole ensemble is constructed on a solid platform, all the components being arranged and fixed on it as the Figure 2 shows. [3]

Before any experiment should be taken, it is verified the alignment of the optical elements using a 640 nm laser diode.

Critical aspects that must be faced are regarding the acquisition timing synchronization with the laser spot repositioning. LIF emission takes place in a short interval after the initial light absorption event, in around 10^{-15} seconds. The fluorescence lifetime, the total time spent in the excited electronic state, is of the order of few nanoseconds to microseconds.

In Fig. 3, it is displayed the timescale of a scanning step, from the positioning of the laser beam at one point, on the surface, until it's reposition to the next one. The scanning software interface waits T_w ms. This time must allow the motors to move the laser beam to the new position (T_p duration), the system to send a triggering command (signal) to the spectrometer for a new reading (T_t duration), the acquisition interface to "read" the emission of fluorescence from the irradiated surface (T_{ac} duration. The acquisition time [T_{ac}] represents the duration of time while the surface is being irradiated for a single acquisition, and can be set from several milliseconds to a few seconds. The waiting time [T_w] is set within the scanning software interface and should cover the duration of all processes during one scan step: repositioning, triggering and acquisition.

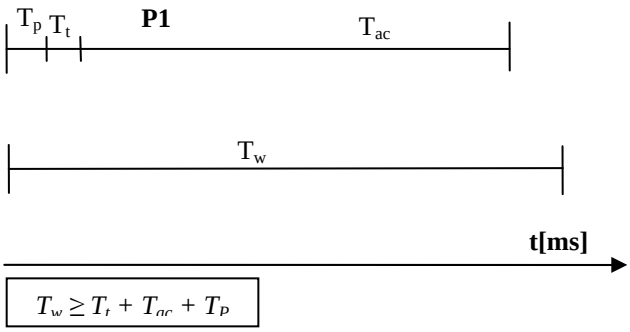


Fig. 3 Acquisition processes timescale

Therefore, it's value must be greater than the sum of all of these three parameters' values. This is a very important condition to have a precise data of the scanned area. If the T_w value is set lower than the sum of the other three, then the interface would order the motors to move to the next scan position before the acquisition interface should have finished the data acquiring. Of course this would spoil the whole experiment and the data obtained will not be synchronized with the scanned areas.

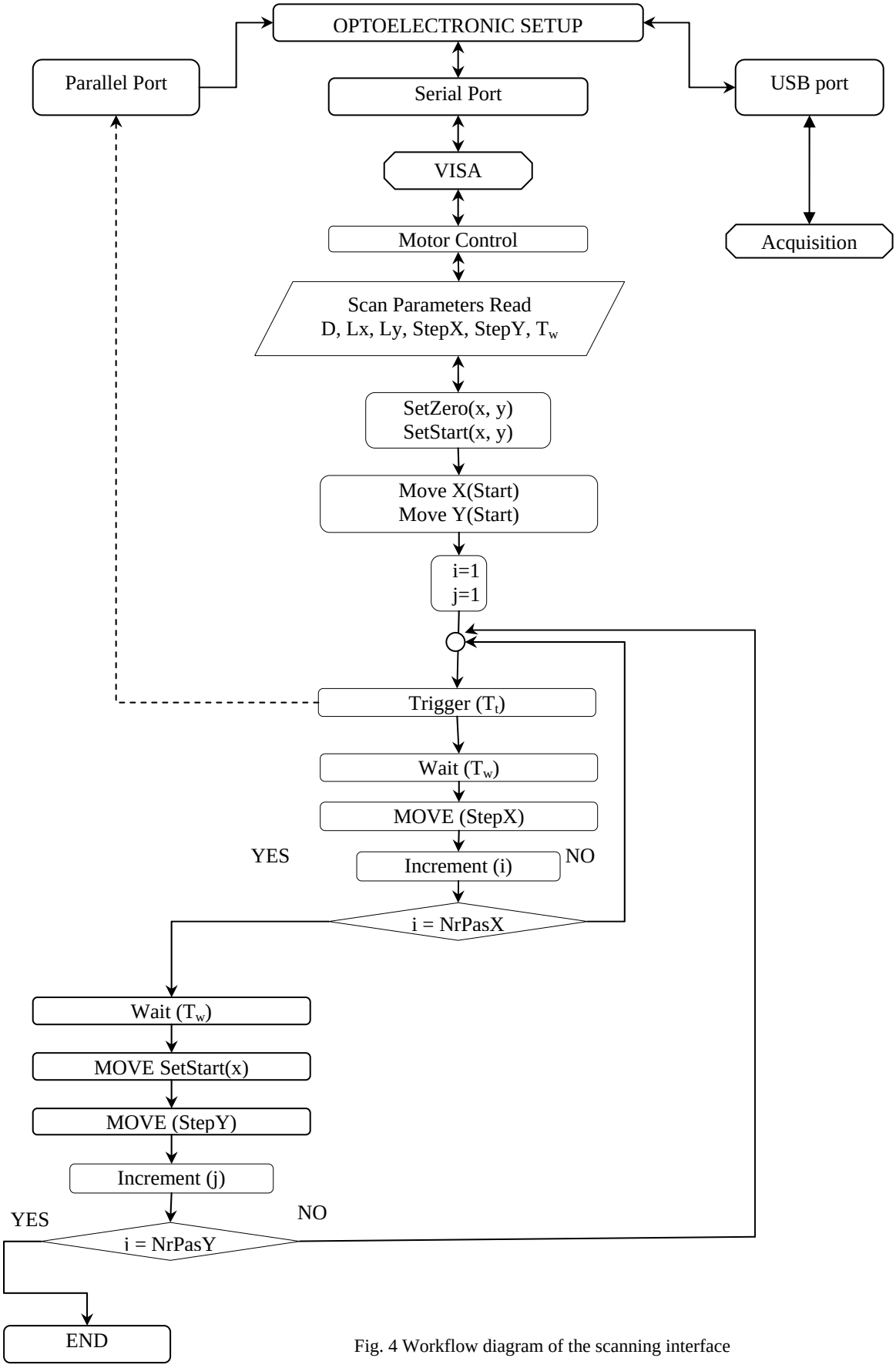


Fig. 4 Workflow diagram of the scanning interface

In the Fig. 4 diagram there are depicted the workflow relations between the scanning device's elements. The instructions within the command blocks have informational role and represents the major steps within the system workflow. It also depicts the communication channels between the hardware devices and the computer, or the optoelectronic setup, through serial, parallel and USB ports.

The scanning of the surface is realised by repositioning the laser beam on the surface on each spot, using the motor mounted mirror, which is controlled via serial port by a computer software interface. This interface is specially designed to make an easier scanning setup and operation. We cannot communicate directly with the motors. For that it is needed some devices to tell the motors what we actually want from them. For that there are two controllers (one for each motor) that receives our commands from the computer, using the VISA interfacing language on a LabView software support. The communication with these controllers is established with the serial port.

The scanning algorithm follows the mathematical model for a rectangular matrix crossing pattern for the step by step laser spot repositioning. It needs the starting coordinates, representing the upper left corner of the desired rectangular area to be scanned. Knowing the lengths and step dimensions, it automatically calculates the number of the rows on vertical axe and the number of steps to be made on each row.

The software interface also permits the input and modification of all the scanning parameters. Using rotational stage motors to scan a planar surface we need several parameters to set before starting a proper scan. So it needs to be specified the distance between the sample surface and the motor mounted mirror, and the starting coordinates, in order to calculate each step rotation angle so that the laser beam will be repositioned on equal step lengths each time. After inputting the distance between the sample surface and the optical collector, and choosing the desired starting point (the upper left corner of the area) using the direction controls, there must be specified the vertical and horizontal lengths and the step dimensions to create a matrix model of the area that has to be scanned. Each element of the virtual matrix represents a repositioning step, with it's own coordinates. Each step length is calculated depending on the distance value and represents the angular rotation parameter for the motors, so that the laser spot is to be repositioned at a constant planar step length on the sample's surface. As for synchronizing the acquisition with the laser beam positioning on each spot, this software interface also allows the operator to adjust the total waiting time T_w of the controller before telling the motors to change the laser beam position. As seen above, the T_w duration time must be set with a greater value than the sum of the values of triggering time T_t and the duration time of each motor move, T_p, and acquisition time T_{ac}.

The acquisition parameters are set within a dedicated software interface. This application can record either a single spectrum from the spectrometer or can use one of its options, namely Strip Chart, that allows the user to set a number of specific bandwidths from the whole spectrum to be recorded, thus reducing considerably the processing time and making the spectra interpretation much faster and easier. The recording can be software triggered, which means that the spectrometer receives triggering signals from another application via the parallel port. The resulted data are automatically written in text files, for each selected bandwidth. The format of these files contains a column with the average intensities of that bandwidth in scanning position.

The resulted files are processed with another LabView application specially designed to create maps of the scanned areas for each spectrum bandwidth. These maps display the intensity distribution of the desired characteristic fluorescence emissions (bandwidths) over the scanned area. To ease the processing, the operator must create a file that contains the data columns for all the recorded bandwidths for a specific area. This way, it is possible to view the intensity distribution map of a single channel using an adjustable white/blue gradient, with the brightest areas representing the highest fluorescence emission intensity of the selected channel; or to combine three different bandwidths in the RGB code, one bandwidth for each R (red), G (green) and B (blue) colour channels. The resulted image illustrates a coloured intensity distribution map of the scanned area. This latter combination can provide excellent observation of the given bandwidths intensity distribution. These resulted images can be saved and used in further discussions, presentations or for documentation.

The main goal of these mapping interpretations is to detect a specific known fluorescence characteristic such as pigments or biological attacks over the artworks surfaces, helping the conservators and artwork restorers to have a better knowledge of the objects surface pigment real distribution and to prevent the further negative effects of the biological attacks.

III. CASE STUDY

The case study validates the described device efficiency and proper functioning, performing a high resolution scan on an old wood painted Orthodox icon (dated from 1867) belonging to open air Village Museum „Dimitrie Gusti" from Bucharest, and aiming to detect and identify microbial contaminations based on different fluorescence emissions. To demonstrate biunique relationship between recorded fluorescence signals and cultivation methods results, the experiment was developed in both ways.

As it can be seen in Fig. 5, the 353x295 mm area of the icon's back side surface containing degraded and stained areas, dirt deposition, was selected .

Using a laser diode that emits in the visible spectrum, the operator adjusts the optical collector focus point right on the surface that has to be scanned. To identify each characteristic spectrum of the surface's contaminated areas

– as a restorers request – there have been made LIF analysis at different interrogation duration times.

Fig. 5. a - The XIXth century icon (front side)

Fig. 5.b The XIXth century icon (back side)

To obtain a good spectrum shape the interrogation time for the acquisition was set to 500 ms.

The acquisition application was set to record only the average intensity of several 10 nm bandwidths of the characteristic spectrum, depicted in Fig. 6-f , (445-455 nm, 495-505 nm, 550-555 nm, 595-605 nm, 655-665 nm) to fasten the acquisition process and to ease the interpretation work.

For this discussion, the attention is focussed on a 10x10 mm area. This scanned area can be seen in Fig. 6-a.

Fig. 6 a. Macro mode photo of Z2 area

The scanning resolution was set at 0.2 mm per step (on horizontal and vertical axes) taking advantage of the high precision laser spot positioning. This means a total 50x50 points to be interrogated. The distance between the repositioning mirror and the scanned surface was 1 m.

Acquisition time T_{ac} was set at 500 ms and the waiting time T_w at 550 ms.

After choosing the starting point and with the other scan parameters all set, the scanning process is started and the laser spot is repositioned each time for a new acquisition, following the pattern provided by the programmed algorithm.

After intense investigations and discussions there were selected several bandwidths for recording during the scan, corresponding to the spectrum peaks at: 450 nm, 500 nm, 555 nm, 655 nm and for the background bandwidth we choose 390 nm.

In the following images we present the resulted fluorescence intensity distributions maps for the 10x10 mm area, Z2 (on the verso).

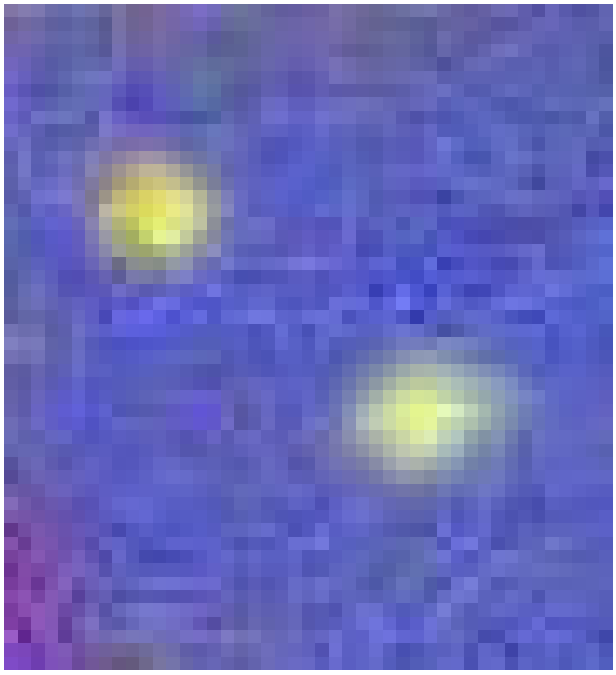


Fig.6 b. RGB bandwidth composition [555 500 450]

Each point of these images represents a fluorescence signal. So depending on the scanning resolution, on the amount of points interrogated we can have high or low resolution images. A 10x10 mm area with 1 mm scan resolution will result in 10x10 pixels images. With a 0.2 resolution we can obtain 50x50 pixels images. So, the more pixels, the better quality for the resulted images.

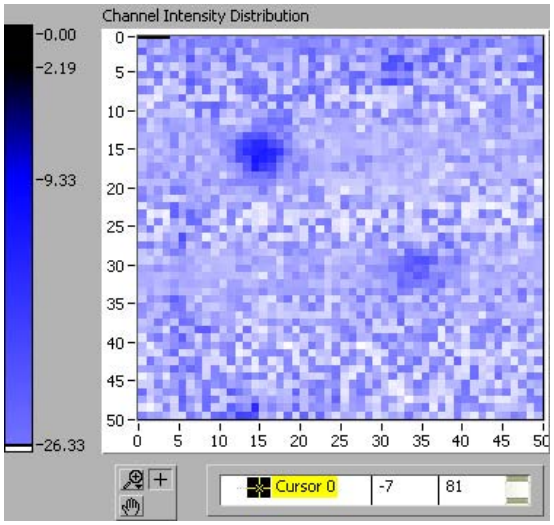


Fig.6 c. Intensity distribution map for the 450 nm bandwidth

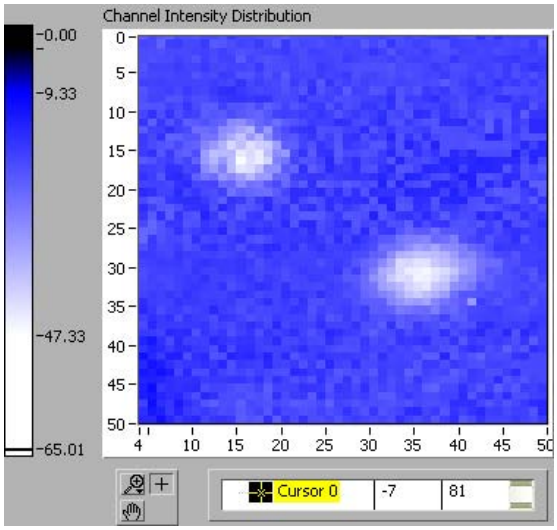


Fig.6d. Intensity distribution map for the 500 nm bandwidth

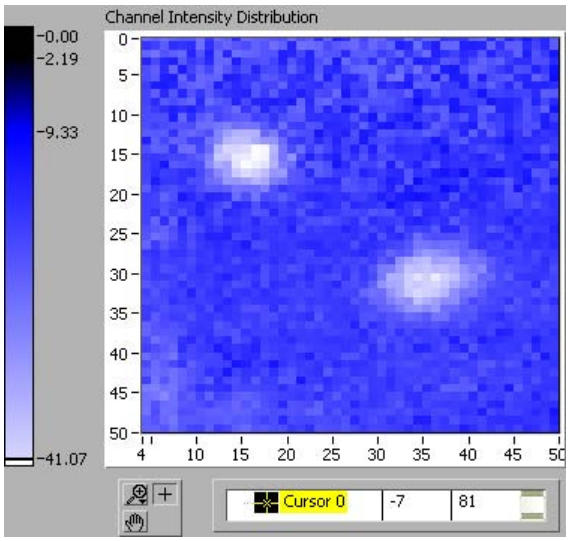


Fig.6e. Intensity distribution map for the 555 nm bandwidth

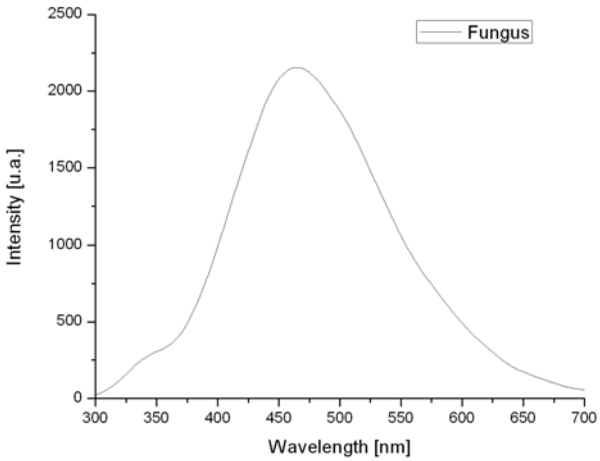


Fig.6 -f. LIF characteristic of the fungus collony

In above images there are represented information regarding the selected area:

Fig 6 a – displays a detailed image (macro) of the chosen area (Z2). There can be seen the fungi mycelia on wooden surface.

Fig.6 b – displays the RGB composition of the recorded data, constituted from three different bandwidth channels: Red for 555 nm, Green for 500 nm and Blue for 450 nm.

Fig.6 c to e – depicts the intensity distribution map of the scanned data for each bandwidth of interest: 450 nm, 500 nm, and 555 nm. This representation uses a three colour gradient (white-blue-black) to illustrate the distribution map of the intensities of each bandwidth of the emitted fluorescence characteristic as follows: the brightest areas represents the highest intensities, while the bluer, darker colour areas represents the regions with lower or the lowest fluorescence emission.

Fig.6 f – displays the LIF characteristic of the fungus colony.

This characteristic is influenced in small measure by the underlying material, the wood. This spectrum will be compared with the spectrum of the cultivated sample.

Part of the numerous selected points that responds to the laser stimulation by fluorescence emission having a large enough development have been detected and captured by high magnification optical microscopy (up to 500 x). Fig. 6 emphasized presents hyphae and mycelium (Fig.7 a- from Z2 area, and FIG.7 b- from X1 area).

From these specific points, classical microbiological sample have been collected and processed. The sampling areas were established taking in consideration the macroscopic deterioration of wood and specific morphology of fungal growth on the wood surface.

Samples had been taken with a sterilized cotton swab and with a sterile tweezers and introduced in 9ml of distilled water containing 0,2 mg/ml-1streptomycine. Then each 1ml of suspension was put in Petri dishes and poured nutrient medium (malt extract-agar). Finally, those 9 Petri dishes had been incubated at 28°C for 14 days. Fungal genera had been identified using pure culture and analytical keys.

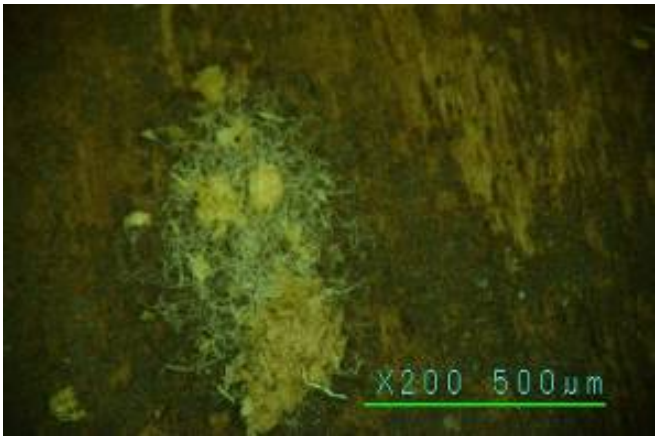


Fig. 7a – Z2 area

Fig. 7b – X1 area

Using cultivation methods for samples took from X1 area it was isolated *Acremonium sp* with white mycelium, slender; conidiophore is simple upright bearing conidia hyaline, 1 celled borne singly.

Using the same method for samples took from Z2 area were isolated 3 fungal strains: *Sclerotium sp., Penicillium sp 1 and Penicillium sp 2. (Fig.8 a)*

Sclerotium sp has white mycelium and spores lacking; sclerotia are olive to brown to blackglobose or irregular

Penicillium sp 1 and Penicillium sp 2 have fluffy mycelium with conidiophores branched near the apex to form a shape like brush; phialides bear conidia in dry chains; conidia are hyaline or brightly colour mostly blue-green, globes or ovoid.

In Fig.8. a-e are displayed the results of the cultivation method.

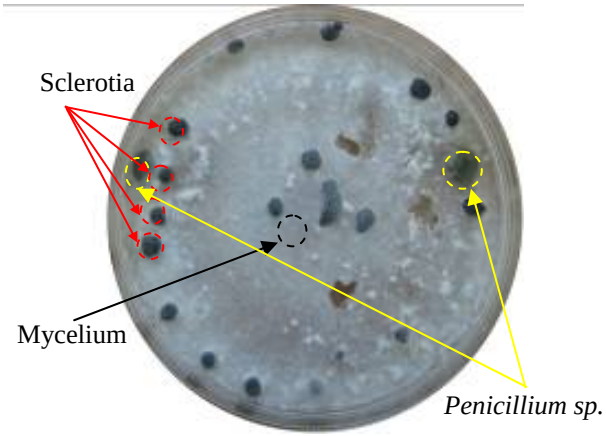


Fig. 8 a.

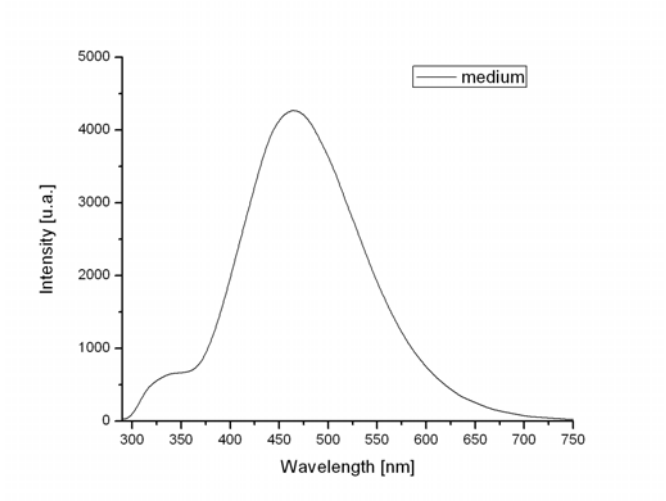


Fig. 8b

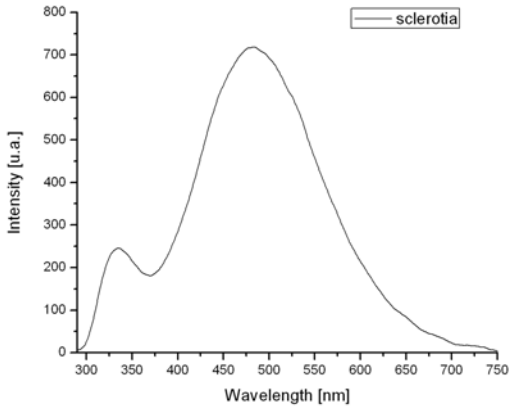


Fig. 8 c

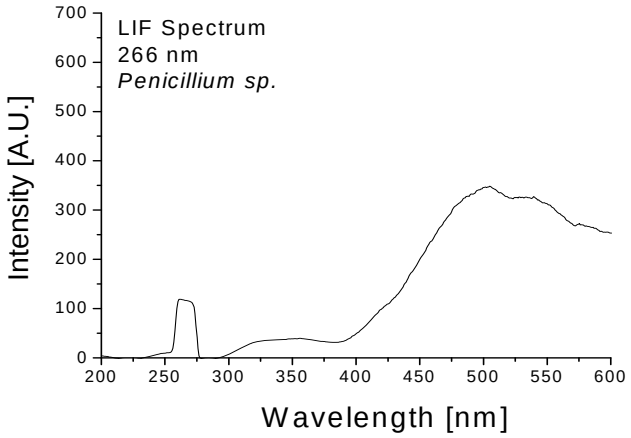


Fig. 8 d

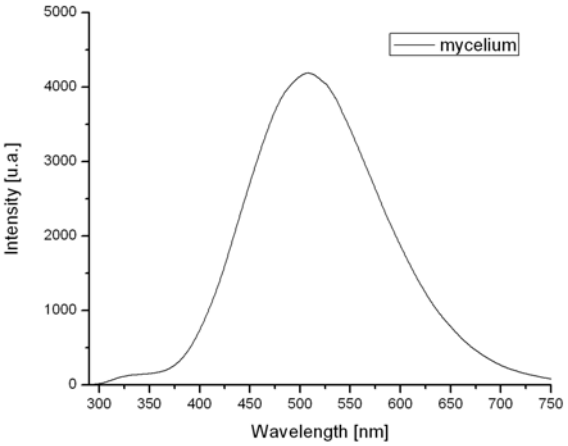


Fig. 8 e

Fig. 8a displays the photo of the cultivated colonies. There are outlined the *Sclerotium*, *Mycelium* and *Penicillium Sp* colonies. Fig 8b displays the laser induced fluorescence spectrum of the medium in which the colonies were cultivated. Fig 8c displays the LIF spectrum of the *Sclerotium* colony. Fig. 8d displays the LIF spectrum of the *Penicillium sp* and Fig. 8d displays the LIF spectrum of the *Mycelium*.

In spite of the evident fluorescence signals that were obtained between 450 and 555 nm from the scanned surface of the object, the punctually LIF spectra on the cultivated samples in laboratory were necessary for biunique relationship between record fluorescence signals.

The same experimental condition have been used for the analysed isolated species (Fig.8 a).

The significant peaks for each isolated species are found on the same spectral range of 450-550 nm.

IV. CONCLUSION

In summary, the use of LIF method is an extremely useful tool for microbiological contamination control. Mounted in a light case, this device becomes an important portable equipment, for any restorer or conserver, that can provide on-site valuable data on the inquired surfaces. More than that, it can be applied on long ranged hard reachable surface, like the ceilings of churches or other indoor sites that needs to be investigated.

As a non-contact and non-invasive method, it can be used especially for fragile, deteriorated, or dangerous surfaces inspection. Its low energy laser pulses, does not affect the integrity, aspect or chemical composition of the irradiated areas.

One of the great advantages of this optoelectronic setup is the automation and synchronization of the laser induced fluorescence and data acquisition processes. This provides a greatly increased speed and amount of the data acquisition.

Another proven advantage of this method is the short time of diagnosis and investigation of the presumed incipient bio-contamination. In this particular study case, the scan durations were between 5-10 minutes, depending on the resolution, acquisition time, waiting time on each step.

Over all mentioned advantages and in spite of the limited efficiency in case of the materials with low fluorescence response, the presented case study proves the detection accuracy of contaminated areas shape, and the possibility to have qualitative information about the existing species.

The presented setup is part of the mobile laboratory based on photonic techniques for artwork investigation and diagnosis. Future developments of the spectra databases will increase the diagnosis efficiency. An exhaustive database will lead to significant investigation time winning.

V. ACKNOWLEDGMENT

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