# APPLICATIONS OF LASER SPECTROSCOPY IN BIOMEDICINE AND PRESERVATION OF CULTURAL HERITAGE

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## Contents



## Summary

The use of laser spectroscopy in the diagnosis of various diseases has been demonstrated during both laboratory and clinical studies. The two main clinical areas of interest are the early diagnosis of various malignant lesions and the discrimination between normal and atherosclerotic tissue. Researchers have been using various analytical techniques such as Laser Induced Fluorescence Spectroscopy and try to correlate variations of the spectral signature of various tissues with the presence of chromophores related to the disease.

The same rationale has been recently used in art conservation. Here, the goal is to use non-catastrophic optical techniques for *in-situ* analyses or diagnostics of the surface of artworks and especially the environmentally altered surface layers. With proper use these techniques may replace the traditionally applied methods of analysis, owing to the sampling requirements and the non *in-situ* capabilities of the latter. Laser Induced Fluorescence Spectroscopy (LIFS) and Laser Induced Breakdown Spectroscopy (LIBS)

are two such techniques that have been proved to be exceptional tools in the surface analysis of various artifacts. Their capabilities in on-line monitoring during laser cleaning are also unique, allowing the precise removal of surface layers through the synchronous recording of the laser induced fluorescence or the emission spectra.

## 1. Introduction

Optical spectroscopy has the potential of being selective as well as sensitive with respect to the detection of various molecular species and it can be used for semi- or non-invasive medical diagnostics. Laser induced fluorescence spectroscopy (LIFS) in particular may prove useful in the early detection of diseased tissue. This relies on the fact that the tissue's chromophore content varies depending on the state of the disease.

Nowadays, various studies on both neoplastic and atherosclerotic tissue are performed. In these studies, LIFS is applied to both the natural occurring and the so-called fluorochromization induced fluorescence. In the first case, the detection - discrimination algorithms are based on the varying concentration of biological chromophores whereas in the second case the detection of the diseased tissue relies on the fact that the exogenous chemical probe has the ability to accumulate selectively in the diseased areas.

Numerous models and experimental techniques have been suggested regarding the most effective acquisition and processing of LIF signals. The main disadvantages of these techniques arise from the fact that both natural and exogenous chromophores posses broad and partly overlapping absorption and emission spectra, making quantitative measurements an almost impossible task. Furthermore, scattering and absorption complicate the acquired signal in the detection of solely superficial tissue layers. In this case the goal of the LIFS technique application is the correct interpretation of spectral variations, which are linked to the change of the relative concentrations of chromophores between normal and diseased tissue.

Modern technology is now playing an increasingly important role in medicine and the preservation of cultural heritage. Amongst the most promising candidates, laser-based techniques have been investigated in a variety of medical applications as well as artwork conservation applications. Besides laser spectroscopy, which has been extensively used in various biomedical areas for composition analysis, there is a number of other techniques that range from the use of holographic interferometry for damage assessment and diagnosis of environmental influences, to the removal of surface encrustations from marble sculptures and stonework or aged varnish from paintings. Current trends consider the need for scientific approaches that may compete with, or even replace, some of the traditional methods applied in art conservation.

For pigment analysis LIFS can be applied with remarkable results, as in the case of medical applications. Fluorescent pigments give characteristic LIF spectra and therefore can be easily distinguished without the need of a destructive analysis. Laser Induced Breakdown Spectroscopy (LIBS) is another laser-based analytical technique also used for elemental analysis of pigments. Here, the advantage over traditional analytical methods is the *in situ* type of sampling, where the consumption of material is of the order of nanograms (ng) compared to the traditional sampling (micro grams order of magnitude). Another important advantage is the in-depth analysis capability of LIBS in steps of sub-micrometers, which is extremely important in multiplayer systems. In this latter case a comparable resolution is impossible even when looking in cross-sections via advanced imaging techniques. Finally, spectral analysis of the ablation plume is the tool for the on-line monitoring and control at all stages of laser cleaning. In all previously mentioned studies there is a standard experimental set-up, which can be used in order to acquire LIF Spectra, LIB Spectra or both. In the following schematic a general experimental set-up is depicted where both LIFS and LIBS are possible. In principle using this set-up one can alternatively use the proper laser excitation source and acquire the corresponding spectra.

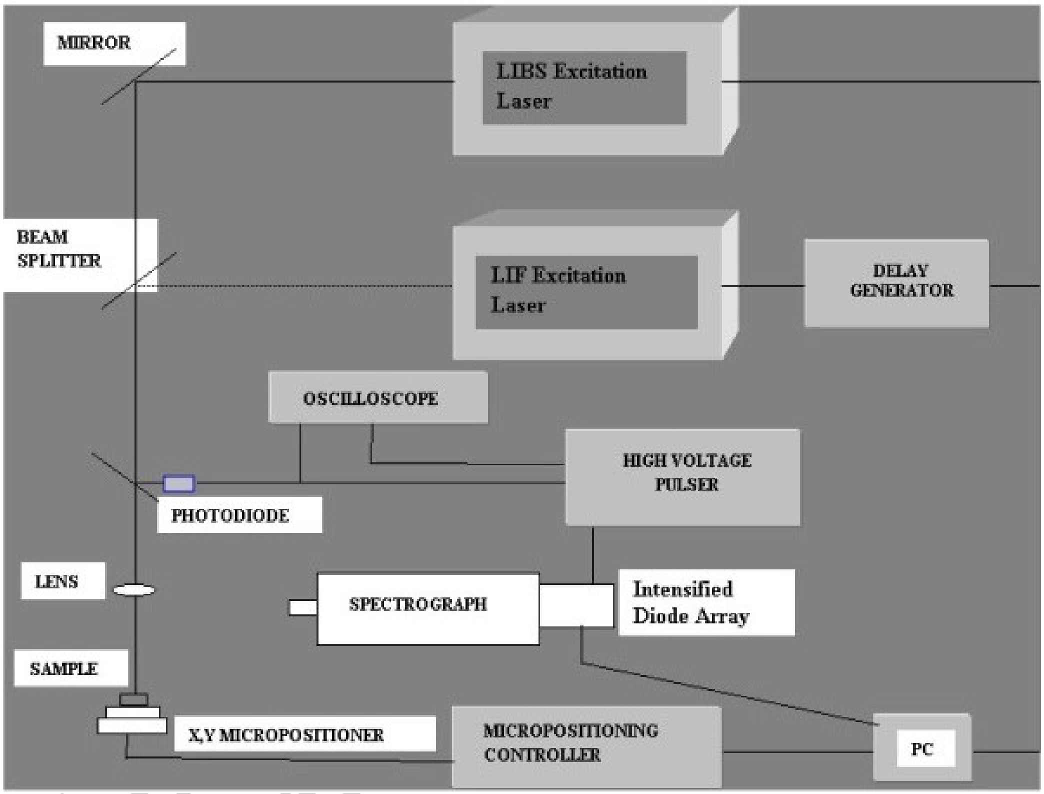


Figure 1. Typical experimental set up of a LIFS/LIBS system used in medical and conservation related studies

## 2. Tumor Detection and Localization

### 2.1. Clinical Studies - Present Trends

Technological improvements in the clinical instrumentation and the simultaneous introduction of lasers in clinical practice gave a new boost to LIFS-based diagnostic work. The result of the research effort of the Mayo Clinic in the late 1950's and early 1960's was reproduced and improved by various groups. The Profio et al. group played an important role during this period, focusing on lung carcinoma *in situ* (CIS) detection. This particular type of malignancy is hardly detectable by X ray or conventional white light bronchoscopy. The introduced methodology included the use of $Kr^+$ laser for excitation, the administration of HpD and the use of intensified video camera detection. The target in these studies was the detection-monitoring of the characteristic dual peak fluorescence intensity of HpD at 630 nm and 690 nm. There were other groups, mainly in Europe, which targeted on other types of tumors, and clinical groups shifting towards the use of δ-ALA and other chromophores. In Baumgartner's study, δ-ALA has been successfully used in the detection of CIS and pre-cancerous types of hyperplasia or dysplasia in the bladder (mucosal lesions). The acquisition of the produced Protoporphyrin IX laser induced fluorescence ($Kr^+$ laser excitation) revealed the power of this technique by detecting precancerous or malignant lesions, which were not recognized during routine cystoscopy. Keeping the rate of false positive diagnosis to low levels (16%) and not exhibiting any false negative results these studies open new perspectives in bladder diagnosis and treatment. Recently, Lam's group has also presented impressive results (94% specificity, 72.5% sensitivity) in the application of LIF imaging in bronchoscopy indicating that, at least, in the case of lung cancer, this method is slowly becoming a candidate for routine clinical use.

## 2.2. Laboratory Work - Future Trends

It is evident that the future of LIF based detection techniques will depend firstly on the improvement of the instrumentation and signal analysis algorithms and, secondly, on the development of new photosensitizing agents with higher selectivity/emission bands and lack of side effects, such as skin photosensitization.

The improvement of endoscopic instrumentation has created a tendency in research groups to try to combine LIF and 2-d imaging.

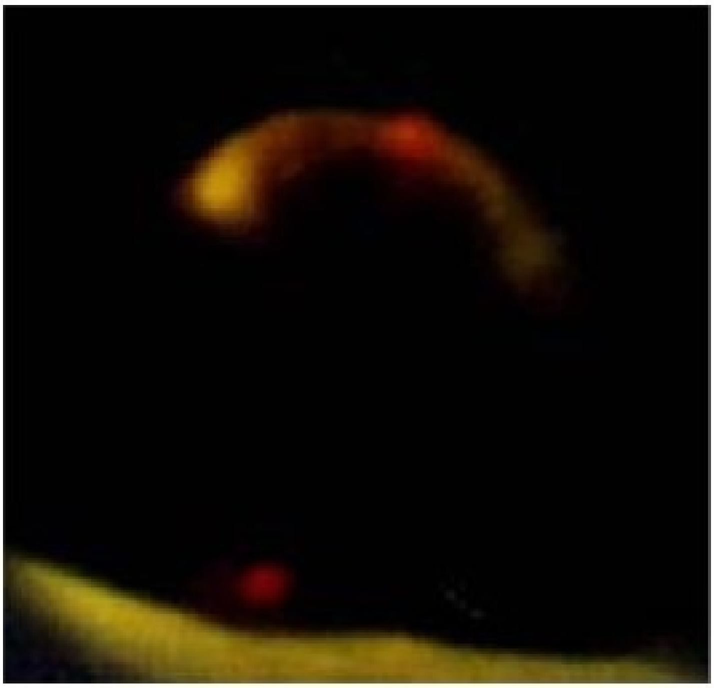

Figure 2. Images of the in vivo LIF emission coming from the HpD carrying atherosclerotic aorta and a primary tumor on the femoral area of an animal model.

Some of the groups use dual wavelength excitation for enhancing the contrast between the photosensitizer's emission and the autofluorescence. The search for chromophores other than HpD and Photofrin II is continuing, and other porphyrins (Uroporphyrin I) or Aluminum Phthalocyanine TS and Acridine Red have been extensively tested. Finally, time gating of the CCD camera's intensifier could also improve the signal to noise ratio in fluorescence images of tumors. By delayed observation of LIF it is possible to identify the contribution of the photosensitizer fluorescence to the overall emission signal. A prerequisite of this technique is the use of fast-pulsed lasers and a relatively expensive detection system.

Numerous groups continue the research on single point measurements of LIF, using simpler instrumentation and looking at the LIF distribution as a function of wavelength. Their prediction rates reached impressive levels in some cases (94%) but the same groups cautioned on the measurement of a relatively high number of false positives.

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**Biographical Sketches**

**Professor Costas Fotakis** is Professor of Physics at the University of Crete and Director of the Institute of Electronic Structure and Laser OF THE foundation for Research and Technology. His main research interests lie in the area of laser photophysics and laser materials processing and diagnostic applications. He is the author of more than 100 scientific publications. He has been invited speaker in numerous seminars and conferences and has received several academic awards. Also he has been project leader in major EU funded research projects and member of the organizational committees of several international conferences.

**Dr. Theodore G. Papazoglou** is a Research Scientist at the Laser and Applications Division of FORTH-IESL since 1991 and visiting Associate Professor at the Dept. of Physics, Univ. of Crete where he teaches Optoelectronics, Laser Applications and Medical Physics related courses. He received his Doctorate from the department of Biomedical Engineering of the University of Southern California in 1990. His thesis was on the use of Laser Induced Fluorescence Spectroscopy (LIFS) in the detection of atherosclerotic tissue during laser-assisted angioplasty. He served as research scientist at the Laser Research Center of the Cedars Sinai Medical Center, Los Angeles, CA. He has published 25 papers in refereed journals and over 30 articles in edited book volumes. He is the scientist in charge in various collaborations of FORTH-IESL (NATO-SfP, BIOMED II, INCO-COPERNICUS etc.). His current interests lie in the study of the optical properties of tissue using advanced opto-electronic devices such as ultra fast laser systems and fast detectors. He is involved in the design and development of optical systems for early cancer and arteriosclerosis detection using laser and photonic technology. His work has resulted in two patents. He is familiar with various data processing and system analysis techniques used in biomedical engineering applications. Furthermore he is involved in the design of various non-invasive detection medical workstations where linear and non-linear signal processing is involved.

**Dr. Vassilis Zafiropulos** is a Senior Research Scientist at FORTH-IESL since 1989. In parallel, he has been Visiting Ass. Professor at the University of Crete. He has coordinated nationally funded Programs and been responsible on behalf of FORTH-IESL for many EU Programs. He received his Doctorate from the department of Chemistry of the University of Iowa in 1988. His thesis was on Laser Spectroscopy and Photodissociation Dynamics of small molecules. He has also worked on research areas of diverse interest, ranging from Laser Induced Phenomena in gases to studies on Laser-Matter Interactions and development of Laser based analytical and diagnostic techniques. He is the scientist in charge of various European Research projects as BRITE/EURAM, EUREKA, SMT, CRAFT, COST etc. The last 10 years he is involved in research on the use of Lasers in Artworks Conservation and has been the vice-chairman of the first international workshop on 'Lasers in Artwork Conservation (LACONA I) ' and president of the relevant G7 COST Action. He is author and co-author of more than 60 articles.