

## LECTURE

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## Laser spectroscopy on organic molecules

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**Abstract** Various laser spectrometric methods have been developed until now. Especially, laser fluorometry is most sensitive and is frequently combined with a separation technique such as capillary electrophoresis. For non-fluorescent compounds, photothermal spectrometry may be used instead. A diode laser is potentially useful for practical trace analysis, because of its low cost and long-term trouble-free operation. On the other hand, monochromaticity of the laser is essential in high-resolution spectrometry, e.g. in low temperature spectrometry providing a very sharp spectral feature. Closely-related compounds such as isomers can easily be differentiated, and information for assignment is obtained from the spectrum. Multiphoton ionization mass spectrometry is useful for soft ionization, providing additional information concerned with molecular weight and chemical structure. A short laser pulse with a sufficient energy is suitable for rapid heating of the solid surface. A matrix-assisted laser desorption/ionization technique is recently employed for introduction of a large biological molecule into a vacuum for mass analysis. In the future, laser spectrometry will be developed by a combination with state-of-the-art laser technology. In the 21st century, new laser spectrometry will be developed, which may be based on revolutionary ideas or unexpected discoveries. Such studies will open new frontiers in analytical laser spectrometry.

## Introduction

The first laser was invented in 1960. Since then, new lasers were developed especially in the succeeding 5 years. Most of the continuous wave (CW) lasers such

as the HeNe laser (1961), the Ar ion laser (1964), or pulsed lasers such as solid-state lasers (1960–1962), the semiconductor laser (1962), and the N<sub>2</sub> laser were described. Many basic laser-related technologies were also found in this period, e.g. Q-switching (1961), second harmonic generation (1961), stimulated Raman scattering (1962), modelocking (1964), and parametric oscillation (1965). Other laser technologies, which should be noted here, are the invention of a dye laser (1966) and a rare-gas halide (excimer) laser (1975).

A laser has various advantages as a light source. For example, the laser has a good beam focusing capability, and it can therefore be applied to a small detector requiring a high power for sensitive detection. In addition, a short pulse width of the laser results in selectivity, e.g. in time-resolved spectrometry. On the other hand, good monochromaticity is essential in high-resolution spectrometry, which delivers information for assignment. Therefore, many spectrometrists started using lasers from the 1970's. Most people expected that a laser might be used practically as a light source from the 1980's. Several companies also tried to commercialize analytical instruments containing a laser as a light source. However, these challenges were unsuccessful. This was due to the cost of the laser and to difficulties associated with its maintenance and operation. However, this situation has completely changed now. Less-expensive and reliable lasers, such as the diode laser, the diode-laser-pumped solid-state laser, and the line-tunable HeNe laser, are now commercially available. Even for a high-power laser such as the Nd: YAG laser or an excimer laser, a long-term trouble-free operation (e.g. 10<sup>9</sup> shots) has become possible. These easy-to-use lasers are now employed for use in practical spectrometry.

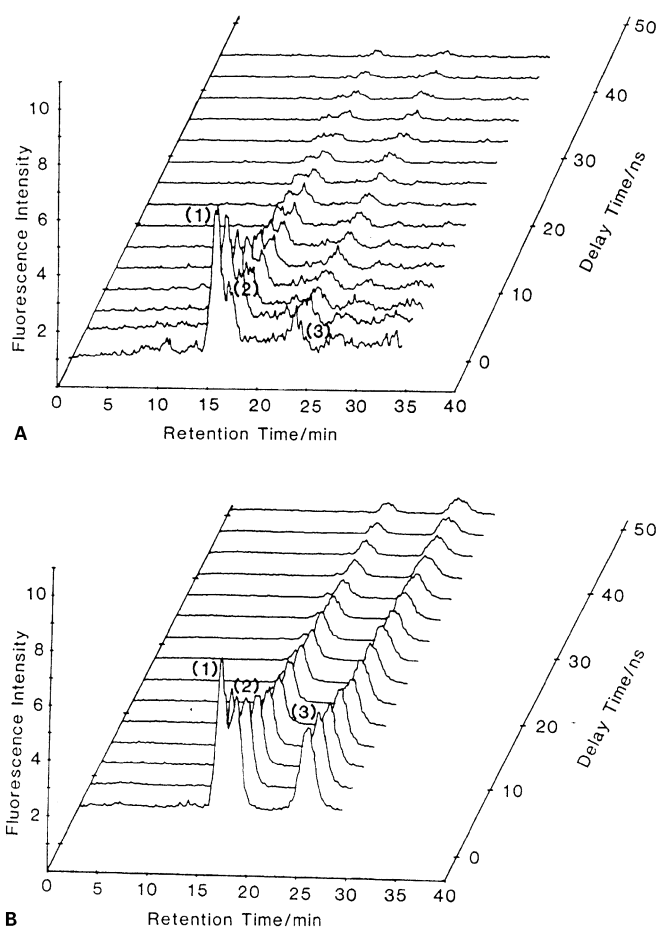
Therefore the development of laser spectrometry from the 1970's will be reviewed and the state-of-the-art of laser spectrometry will be summarized in connection with a preview of possible future developments, although not all the areas of laser spectrometry could be covered.

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## Landmark papers from the 1970's

A nitrogen laser has a simple structure. It produces short ultraviolet pulses and is suitable as a pump source for a dye laser and can be constructed even by a chemical engineer. The tunable range of the dye laser extends from the near-ultraviolet to the near-infrared, and it can be further extended to the far-ultraviolet by frequency doubling. Thus various kinds of spectroscopy and spectrometry were investigated using the nitrogen-laser-pumping dye laser from the 1970's. In this time, Winefordner et al. reported a series of papers about the detection of trace atoms in a flame. Almost all the elements, which were measured by atomic absorption or fluorescence spectrometry, were measured by laser fluorometry. For some elements, this method provides a very high sensitivity, allowing trace elemental analysis. Unfortunately, an atomic transition is easily saturated under moderate laser power, preventing a more sensitive analysis. In addition, pulse-to-pulse instability of the laser energy, which is unavoidable in a pulsed laser, made it difficult to subtract the background signal accurately. Thus this method raises several problems and cannot be used for real trace analysis. However, this work was probably the first demonstration presenting the advantage of the laser in spectrometric analysis and encouraged spectrometrists to use it.

In 1976, Zare et al. demonstrated the detection of a fluorescent organic molecule at sub-part-per-trillion (sub-ppt) levels [2]: they used a nitrogen-laser-pumped dye laser for sample excitation and interference and color filters for fluorescence isolation. Later, a CW HeCd laser emitting in the ultraviolet region was employed, due to a stable output power and its easy operation and maintenance. The detection limit could be improved 2–3 orders of magnitude by using a laser as an exciting source. Optical saturation is negligible for a condensed phase sample in most cases in contrast to atomic fluorescence spectrometry. In addition, the laser beam is monochromatic, and the stray light can be rejected completely by the use of simple optical filters. It increases the optical conductance in fluorescence isolation and improves the detection sensitivity. Such ultrahigh sensitivity was also obtained using a pulsed-gated photon counting system when a monochromator was used for fluorescence isolation [3]. The detection limit was determined by impurity fluorescence and Raman scattering of the solvent. So, additional selectivity was needed for a more sensitive detection, especially in practical trace analysis. A straightforward extension of this study might be the application to time-resolved fluorometry. A highly-repetitive nitrogen laser with subnanosecond pulse widths was constructed and used as a pump source for a dye laser [4], which produced nearly-transform-limited 200 ps pulses [5]. Time-resolved fluorometry



**Fig. 1A, B** Three-dimensional chromatogram of the sample extracted from airborne particulates (A) and standard sample (B)

is essentially useful for the suppression of impurity fluorescence, especially when the fluorescence lifetime of the sample is sufficiently long as in the case of polycyclic aromatic hydrocarbons. Moreover, it provides additional information, i.e. a fluorescence lifetime, for assignment. However, pulse-to-pulse instability of the laser energy produces a poor detection limit in comparison with CW-laser fluorometry, due to a poor background subtraction capability.

Laser fluorometry was combined with liquid chromatography and was found to be 2–3 orders of magnitude more sensitive than conventional fluorometry using an incoherent light source [6]. Time-resolved laser fluorometry provided additional selectivity [7]. As shown in Fig. 1, the sample can be identified from the fluorescence lifetime as well as from the retention time in chromatography. In another work, hydrophobicity of proteins was evaluated from the fluorescence lifetime of the dye attached on the surface of protein [8]. However, the detection sensitivity was poorer than that of CW laser fluorometry as described before. Unfortunately, the price of the CW laser, e.g. a HeCd laser, is almost identical to the price of a liquid

chromatographic system. Moreover, the laser line emitting in the ultraviolet region, which is important for a more universal detection of samples, is limited especially for a CW laser. On the contrary, a compact and less-expensive nitrogen laser was developed for practical spectrometry, but it suffered from the difficulties associated with maintenance and operation as described and also from large radio-frequency interference. Thus laser fluorometry combined with liquid chromatography has not yet been used practically.

Absorption-based spectrometry may be used for nonfluorescent samples. Since the sensitivity in conventional absorption spectrometry is independent of the intensity of the light source, it is difficult to improve the sensitivity by using a high-power laser. Thus photo-thermal phenomena succeeding to light absorption were measured to overcome this problem. An acoustic signal induced by the pressure change occurring from the energy conversion from light to heat was measured in photoacoustic spectrometry [9]. This approach was especially useful for the detection of trace gases, which was partly due to the high sensitivity of a microphone in the air. This technique was also applied to condensed phase samples, in which a piezoelectric transducer was used for better impedance matching [10, 11]. When the laser beam is focused into the sample, the local temperature increases. Thus the refractive index of this part decreases, which acts as a diverging lens to expand the transmitting beam. This approach was called thermal lens spectrometry [12]. The relative sensitivity of conventional absorption spectrometry was reported for liquid and gas samples under CW and pulsed laser excitations, indicating that a pulsed laser is useful for trace analysis of gases and a CW laser for liquids [13, 14]. Similarly, beam deflection resulting from the refractive index gradient was measured in thermal deflection spectrometry [15], and the beam diffraction resulting from an interference pattern thermally-induced by a crossed beam, was recorded in thermal grating spectrometry [16]. The electric current between two electrodes, which is induced under a strong radiation field, was recorded in multiphoton ionization spectrometry. This technique was developed for the trace analysis of gas phase samples but is also applied to condensed phase samples [17, 18]. Theoretically, the sensitivities of these spectrometric methods increase with increasing the laser power and are several orders of magnitude better than those of conventional absorption spectrometry. However, the detection limits were only 1–2 orders of magnitude better, since the background signal also increased with increasing laser power. These methods can also be combined to liquid chromatography. Unfortunately, these spectrometric methods are seldom used in practical trace analysis, since similar problems as in laser fluorometry arises and, in addition, a larger output power is generally needed.

## State-of-the-art laser spectrometry

The resolution in chromatography can be improved by using a narrow column, in which a small detector must be used to avoid a decrease in resolution. As a laser beam can easily be focused into a sub-nanoliter volume, laser fluorometry shows high sensitivity. Recently, capillary electrophoresis and micellar electrokinetic chromatography have been developed, which require a narrower, e.g. 10–50  $\mu\text{m}$ , column. Thus laser fluorometry is advantageous especially in trace analysis. An air-cooled Ar ion laser (blue, green) or a small HeNe laser (green, orange, red) has a sufficient output power, since optical saturation occurs above 20 mW. An avalanche photodiode with a high quantum yield is sometimes used instead of a photomultiplier for fluorescence detection. Even a single molecule in a liquid flow is detected by laser fluorometry; the fluorescence signal from a single molecule can be isolated as a burst photon signal against a background noise [19]. The detection limit is ten yoctomole or six molecules [20]. Glutathione derivatized with monobromobimane ( $\text{C}_{10}\text{H}_{11}\text{N}_2\text{O}_2\text{Br}$ , fluorescent probe for thiols purchased by Sigma Chemical, St. Louis, MO, USA) and inorganic ions such as  $\text{Li}^+$ ,  $\text{Na}^+$ ,  $\text{K}^+$  in a single erythrocyte have been detected directly and indirectly at low attomole and sub-femtomole levels, respectively [21].

The diode laser is more practical as a light source in spectrometry [22, 23] and is already applied to fluorometric detection in capillary electrophoresis and micellar electrokinetic chromatography [24, 25]. Figure 2 shows the trace analysis of amino acids labeled with a far-red dye. The detection limit is in the sub-attomole level. Now, various laser modules are commercially available, but the emitting wavelengths are located in the far-red or near-infrared region. Unfortunately, only several types of molecules exhibit a fluorescence in these spectral regions. Therefore, it is necessary to synthesize a new labeling reagent or to develop an analytical procedure allowing fluorometric

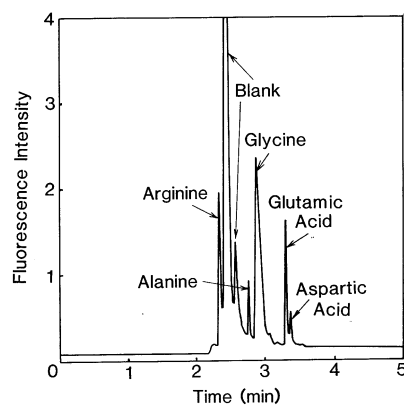


Fig. 2 Electropherogram for amino acids labeled with pyronin succinimidyl ester (50 fmol each)

determination of a nonfluorescent sample. A few biological molecules with SH groups or protein are measured by using far-red labeling dyes and also many aromatic hydrocarbons by indirect fluorometry.

Recently, narrow channels are produced on a glass plate by micromachining for application to capillary electrophoresis [26]. This approach is valid for the ultrafast separation of a sample. In the future, a light source, e.g. a diode laser, and a detector, e.g. an avalanche photodiode, will be integrated on the same chip. Such an analytical instrument might be employed even as a microsensor in chemical analysis.

For nonfluorescent samples, capillary vibration induced by light absorption and succeeding heat generation is detected either by using a piezoelectric transducer placed as a pivot to hold a capillary or by introducing the second laser beam to measure beam deflection by an acoustic wave [27].

For best use of laser spectrometry, the unique properties of the laser must be used advantageously. For example, monochromaticity of the laser is preferential in recording a high-resolution spectrum. Unfortunately, the spectrum of the sample measured at room temperature is generally broad. In order to obtain a sharp spectral resolution, it is necessary to decrease the temperature down to several degrees of Kelvin. Low temperature spectrometry such as Shpol'skii, matrix isolation, or supersonic jet spectrometry is developed and is verified to be useful for the differentiation of chemical species closely-related each other [28]. Especially, supersonic jet spectrometry is combined with multiphoton ionization/mass spectrometry giving information concerned with the molecular weight and the chemical structure. For example, as shown in Fig. 3, it is possible to measure the multiphoton ionization spectrum of chemical species giving a mass peak at  $M_w = 212$  for spectral identification. Furthermore, *p*-cresol is clearly shown to be present in the thermally

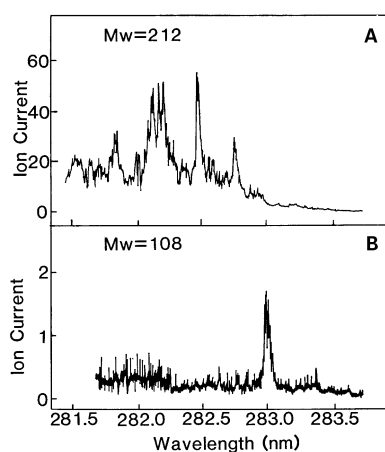
decomposed product, indicating that isomeric analysis is possible by this method. Recently, a femtosecond pulsed laser was used for multiphoton ionization and found to be useful for a softer and more efficient ionization in comparison with nanosecond multiphoton ionization currently used. It is noted that supersonic jet spectrometry can be combined with a separation technique such as gas, liquid, supercritical-fluid, or thin-layer chromatography [29]. Such a hyphenated technique gives physicochemical information, in addition to the spectrometric one.

Mass spectrometry is a sensitive analytical means and gives valuable information for structural analysis. However, it was difficult to apply it to a large biological molecule such as a protein. A laser can produce a short pulse with sufficient energy, and then it is suitable to use it in ablation and subsequent ionization of the molecule adsorbed on the solid surface. An organic compound is frequently mixed with samples for reduction or fragmentation. A large biological molecule has been introduced into a vacuum for mass analysis by employing this matrix-assisted laser desorption/ionization spectrometry. Even underivatized single-stranded DNA oligomers are measured [30]. Aromatic hydrocarbons floating on the surface of water are desorbed and multiphoton ionized in the air and are detected by measuring the electric current between the cathode immersed in water and the anode placed in air [31]. This approach allows sensitive surface analysis under atmospheric conditions.

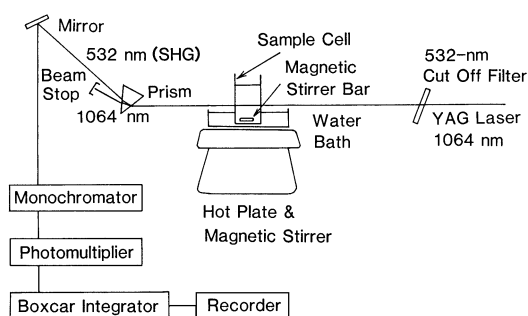
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## Preview

Analytical laser spectroscopy in the 20th century (next 5 years) may be previewed simply by extending present works and combining them with the state-of-the-art laser technology. For example, a widely-tunable and all-solid-state optical parametric oscillator will be available, which will make laser spectrometry applicable to practical analysis. It is also possible to combine present works with new optical engineering. For example, fiber optic technology is increasing its importance for data communication. Recently, various types of optical fiber sensors are developed; a compound, which acts as a transducer converting a chemical information to an optical one, is attached at the distal end of the optical fiber. Potassium concentrations can be continuously monitored by an optical fiber sensor based on diode laser fluorometry using a far-red dye [32]. Such an approach is useful even under a large radiofrequency interference. So, it will be useful in remote sensing and enables it to be combined with a local area network for human health care. New laser spectrometry will be developed to solve old problems remained. As shown in Fig. 4, the precipitation reaction of potassium dihydrogen phosphate (KDP) can be visualized in the presence of a large quantity of the inert

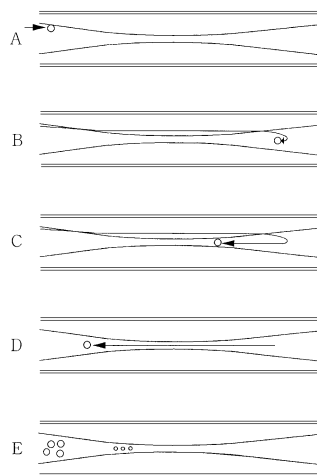


**Fig. 3A, B** Multiphoton ionization spectra for thermally decomposed products from polycarbonate measured by monitoring the signal at specified  $M_w$  values indicated in figures



**Fig. 4** Experimental apparatus for generation and detection of second harmonic emission from precipitates

**Fig. 5A–E** Schematic motion of particles. The laser beam is introduced from the right-hand side and the liquid from the left-hand side: **A** particle introduction; **B** focus of particle into beam center by gradient force; **C** acceleration of particle; **D** deceleration of particle; **E** particles drifting at equilibrium position



precipitate such as NaCl by monitoring the second harmonic emission [33]. Social needs will also stimulate laser spectrometry, e.g. a rapid and reliable spectrometric method for detection of toxic compounds artificially synthesized.

It is challenging to preview laser spectrometry in the 21 century. But, it is essentially difficult to predict a completely new approach, since such an approach might be based on revolutionary ideas or unexpected discoveries. Thus, what can be done is only to introduce the laser spectrometric work, which has been initiated by a new concept and an accidental finding.

Recently, a new technique has been invented for separation of small particles [34]. The separation scheme is shown in Fig. 5. A laser beam is focused into the solution, which contains particles counterflowing coaxially in a capillary. The particle is focused into the center line of the laser beam by radiation pressure. The particle is turned around, accelerated, passed through a beam waist, decelerated by a liquid flow, and drifts – at which point the radiation pressure is identical to the force induced by the liquid flow – resulting in a separation of particles as a function of size. This technique is expected to be useful for the separation of biological cells such as yeast, microorganisms, and

bacteria. Another possible application is the detection of proteins by an immunological reaction combining two particles by sandwich assay. In theory, single specified proteins can be detected by this method.

When we tried to convert a laser frequency by a stimulated Raman effect, we accidentally found that multifrequency laser emission were generated. This phenomenon was based on simultaneous generation of many rotational and vibrational lines by four-wave Raman mixing. We are now trying to generate an ultimately-short optical pulse by modelocking these emission lines, hopefully down to 1 fs or less. It is expected that a hard X-ray laser can be generated by tightly focusing such an ultrashort pulse onto a metal surface. Such a coherent ultrashort X-ray pulse might be useful in many fields, e.g. in crystallographic analysis and in X-ray microscopy. These approaches allow structural analysis of atoms, molecules, and even animals in the very short time scale, in which all the atoms cannot move. Such studies will open new frontiers in analytical laser spectroscopy.

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