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My Life with LIF: A Personal Account of Developing Laser-Induced Fluorescence

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Abstract

Laser-induced fluorescence (LIF) is a spectroscopic technique that involves the excitation of a molecular target by a beam of laser radiation followed by the detection of the subsequent emission of radiation from the target. LIF detection has several advantages over absorption spectroscopy. First, LIF has excellent detection sensitivity because a signal is observed against a dark background. Second, the emitted radiation can be collected at various angles with respect to the incoming laser beam, making it possible to obtain two- and three-dimensional images because the fluorescence is emitted in all directions. Third, by dispersing the fluorescence, it is also possible to learn about the transitions from the state excited to the various lower levels of the target species. Finally, because of the delay between the excitation and detection events, it is also possible to learn about what processes the excited target undergoes in the intervening time.

When I was first invited to prepare a prefatory review for the *Annual Review of Analytical Chemistry*, I agreed at once. But I confess I had mixed emotions: elation combined with dread. I am very honored by the invitation. However, at the same time, I realized that these invitations often signal that others think that a person's career is essentially at an end. At an age when many of my friends have retired, are thinking about retiring, or have died, I nevertheless vigorously reject the notion that my career is drawing to a close. Scientific research is too much fun for me to walk away from it now, as long as my health permits it. So I am not composing my obituary ahead of schedule here. Instead, I offer you something rather different: reminiscences about laser-induced fluorescence (LIF) and my role in making this technique so useful. If you want to learn about my dysfunctional family and how I was a weird and nerdish child, please read my autobiography, available in paperback: *Richard N. Zare: Molecule e vita* (1). If you are looking for biographical information about me, I urge you to consult my Web site (<http://www.stanford.edu/group/Zarelab>).

My story about LIF begins with the birth of the laser. The concept of the laser goes back to Albert Einstein in the early twentieth century, but the 1958 groundbreaking paper, "Infrared and Optical Masers," by Charles H. Townes and Arthur L. Schawlow at Bell Telephone Laboratories (2), inspired the laser's development. This paper proposed an extension into the infrared and visible regions for what had already been achieved in the microwave region of the electromagnetic spectrum, work for which Townes and [Aleksandr M.] Prokhorov would receive the Nobel Prize in Physics in 1964. Gordon Gould, who was a graduate student of Townes at Columbia University, also came up with important concepts and coined the term laser, an acronym for light amplification by stimulated emission of radiation.

On May 16, 1960, Theodore Harold "Ted" Maiman demonstrated the first laser at the Hughes Research Laboratories in Malibu, California. This feat was achieved with a synthetic ruby-crystal rod measuring 1 cm by 1.5 cm. It produced a pulsed beam of deep-red light that was brighter than a million suns. Unlike the light from a flashlight, which rapidly spreads in shape, the beam of the laser stays tightly bunched together. A laser beam can travel for miles before its spread becomes comparable to that of a flashlight beam going across a room. This property in which all the light waves oscillate in phase is known as coherence.

Maiman held a press conference on July 7, 1960, in New York City to introduce his coherent light source to the world. But what good could the inventor of the laser imagine for this device? Maiman stated, "a laser is a solution seeking a problem" (3). This sentiment was repeatedly uttered by many experts in the physics community who pondered its possible uses.

In 1960, I was a junior (third-year undergraduate) at Harvard University, pursuing a double major in chemistry and physics. I was spending the summer in my hometown, Cleveland, Ohio, working for Clevite Corporation, measuring the intensity of scattered X-rays from a single crystal of cadmium selenide to learn about the structure of this semiconductor material. This work would lead to my first scientific publication (4). When Maiman's press release appeared, we avidly discussed around the lunch table what scientific advances might come from the laser. We were sure that it would profoundly change the world, but at the time, we had not imagined how ubiquitous the laser would become. These days, the laser has a multitude of applications, from bar-code readers in supermarkets to devices that help reattach detached retinas.

Maiman's ruby laser produced millisecond pulses of light and could not be fired at a rapid rate. The next big advance came on December 12, 1960, when Ali Javan, William R. Bennett, and Donald R. Herriott (5) demonstrated at Bell Telephone Laboratories the first continuously working (cw) laser. It contained helium and neon gases and was called the He-Ne laser. Unlike Maiman's laser, which was pumped by a flashtube, the He-Ne laser was pumped by an electrical discharge inside the gas mixture. The electrical energy was turned into coherent radiation.

At the time, lasers were quite exotic, and only a few existed. I thought of the idea of using a laser to excite molecules and cause them to fluoresce when I was a beginning faculty member of the Department of Chemistry and the Department of Physics and Astrophysics of the University of Colorado (both without tenure) as well as a member of what was then known as the Joint Institute for Laboratory Astrophysics, later renamed simply JILA, in Boulder, Colorado. One of my Harvard professors told me that this idea would never work: On the basis of what was found in Gerhard Herzberg's encyclopedic treatises on molecular spectroscopy, many molecules fall apart when electronically excited. I was crestfallen by this offhand comment, but I felt that the pessimism was too severe and was determined to try it anyway.

First, though, I had to build my own He-Ne laser. With the help of the JILA staff, this task was easily accomplished in about three months. Next, I needed to find a molecule that I could put into the gas phase to absorb the coherent red light from the He-Ne laser. The first LIF experiments were successfully carried out on the potassium dimer molecule, K_2 , which is made by heating potassium metal in an evacuated glass cylinder fitted with proper windows to let the laser beam in and out. Hot potassium discolors normal glass, so a special glass cell had to be constructed. The first full report (6) of this experiment was published in 1968. I fell in love with the fluorescence streak, which, when dispersed in a spectrograph, showed transitions from the electronically excited vibrational-rotational level pumped by the He-Ne laser to the different vibrational-rotational levels of the ground electronic state of K_2 .

In this setup, we observed high vibrational-rotational levels of the molecule that had never been seen before. The experiment extended our knowledge of the forces that pulled together two potassium atoms as a function of separation distance. I had picked this molecule because I was familiar with it from my graduate studies in Professor Dudley Herschbach's research group, first at the University of California at Berkeley, then at Harvard University. The dimer was actually a thorn in the side of many of my fellow graduate students, as it was an unwelcome fellow traveler in atomic beams of potassium in their crossed-beam reactive scattering studies. The fluorescence process for exciting the potassium dimer molecule (K_2) in the red is very similar to that for the iodine (I_2) molecule in the green (**Figure 1**).

When I look back on my career, I see that the idea of using lasers to excite molecular fluorescence was a most natural extension of some of the studies I had done as a graduate student under the guidance of Professor Herschbach. My PhD thesis was titled "Molecular Fluorescence and Photodissociation." I had become deeply acquainted with fluorescence, starting with a seminar at Berkeley's chemistry department given by a graduate student Robert A. Berg, who was studying under the direction of Professor Leo Brewer and developing a way to measure the radiative lifetime of the I_2 molecule excited by the mercury green line using a phase-shift fluorometer (7). **Figure 2** shows the coincidence of the atomic mercury line and the I_2 absorption spectrum taken at high resolution.

During Berg's seminar, Herschbach asked, "What causes the wildly varying intensity pattern of the resolved fluorescence?" Berg had no answer, and Brewer also sat silently in the audience. Herschbach stated that any good graduate student should be able to calculate this intensity pattern in two weeks. The next day I found that I had been given this exercise to do.

As the great American scientist Edward Condon (9) had pointed out, the intensity pattern is an example of matter-wave diffraction, which he named internal diffraction. Assuming that the dipole transition moment was independent of internuclear distance, the intensity pattern was proportional to the square of the overlap of the ground-state and excited-state vibrational wave functions. Herschbach had imagined that all that was necessary to complete this exercise was to use the analytical wave functions for excited-state and ground-state potential energy curves when expressed as Morse oscillators. If only this problem had been so simple! As I bitterly discovered, it was not.

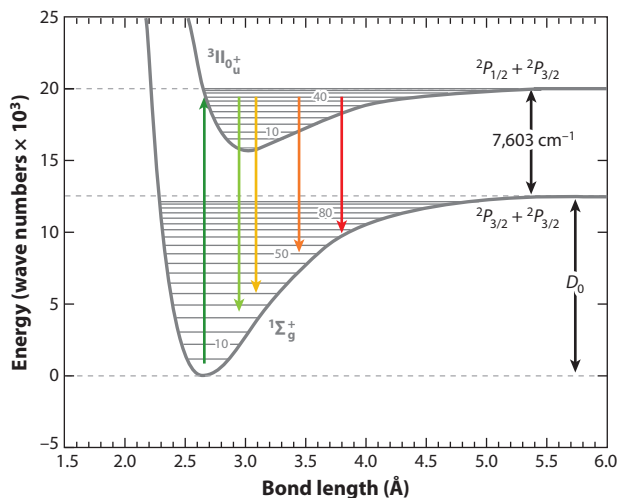


Figure 1

I_2 B–X fluorescence process. A particular vibrational-rotational level in the $\text{B } ^3\Pi_0^+ \text{u}$ state is excited in absorption, and the subsequent emission from this level is to various other vibrational-rotational levels of the $\text{X } ^1\Sigma_g^+$ state, governed by the dipole selection rules, to give the fluorescence spectrum.

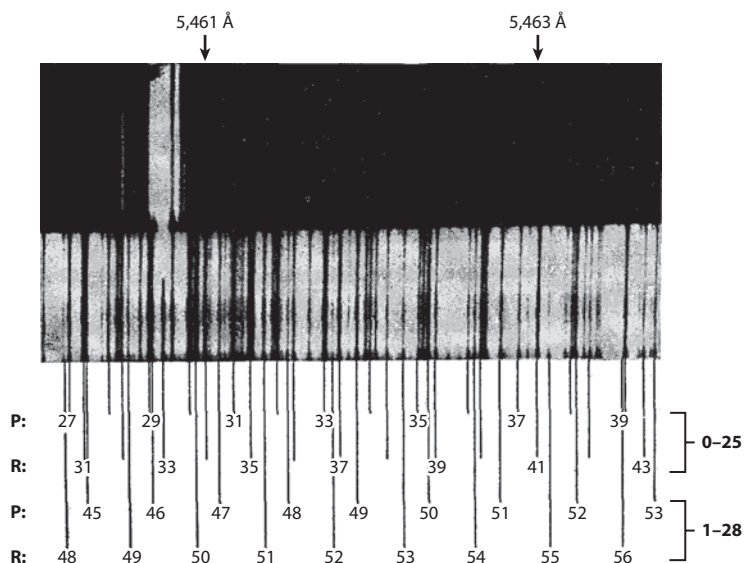


Figure 2

The green mercury 546.075-nm emission line (showing hyperfine structure) in the upper panel of the diagram is compared with a high-resolution portion of the I_2 B–X absorption system in the lower panel, showing how the frequency of this atomic line coincides with the frequency of the transition of the $v'' = 0, J'' = 32$ level of the X state to the $v' = 25, J' = 33$ level of the B state. This figure is adapted from Reference 8 with permission. Prior to that work, the vibrational numbering of the upper level had been incorrectly assigned to $v' = 26$.

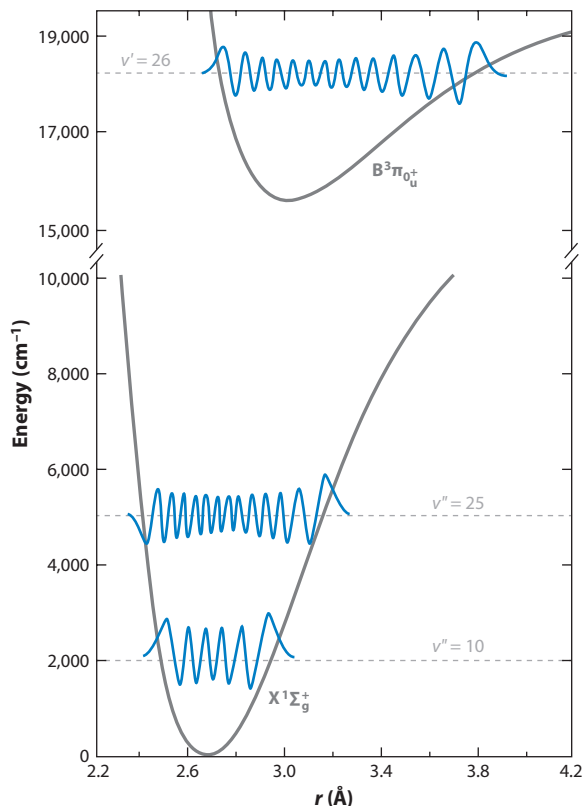


Figure 3

Rydberg-Klein-Rees potential energy curves and vibrational wave functions for the I_2 B and X states. Adapted from Reference 10 with permission.

The vibrational overlaps between the various ground-state vibrational levels, with the $v' = 26$ level of the excited state, involved many cancellations (**Figure 3**). The two vibrational wave functions constructively and destructively interfere, going in and out of phase with one another. As is true for so many problems involving diffraction, the results depend on the exact shapes of the upper-state and lower-state potentials, which in turn determine the forms of the vibrational wave functions.

First, I labored to construct from theory, using the published spectroscopic constants for the X and B states, realistic potentials by means of the Rydberg-Klein-Rees method. Next, I numerically solved the Schrödinger equation in this potential for the vibrational wave functions, computed the square of the overlap, and compared the numbers my computer program punched out against the measured intensity data, which, at that time, had been recorded photographically. I could not make a good fit between theory and experiment and suggested that the spectroscopic constants must be slightly in error (10). In the meantime, my advisor had been called back to Harvard, where he had been a Harvard junior fellow, as a full professor. Jeffrey Steinfeld, a graduate student of Professor William Klemperer, later showed in 1965 that the real problem was that the vibrational numbering of the excited state had been misassigned and needed to be reduced from 26 to 25. Once that was done, the comparison between the theoretically calculated and experimentally measured intensity patterns was rather satisfying (**Figure 4**) (8). This supposed two-week exercise stretched

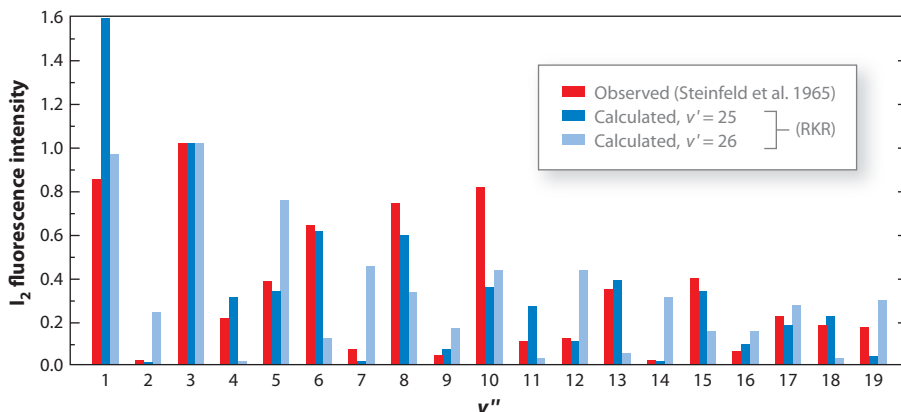


Figure 4

Comparison between experimental and calculated I_2 fluorescence intensity distributions excited by the mercury green line for assignments $v' = 25$, and $v' = 26$, normalized to the (25,3) transition. Abbreviation: RKR, Rydberg-Klein-Rees. Adapted with permission from Reference 8.

into three years of graduate research for me, but today it is commonly used as an experiment in undergraduate physical chemistry laboratories.

My first experiments with LIF were with the He-Ne laser. But the advent of the argon ion laser soon after the introduction of the He-Ne laser made many more sharp lines of a single color available as monochromatic cw excitation sources. The field really took off with the invention of the tunable dye laser by Mary Spaeth at the Hughes Aircraft Company in 1966, which allowed experimentalists to dial up whatever color was desired. Credit for the invention of the dye laser also needs to go to Peter P. Sorokin at IBM, Yorktown Heights, New York and Fritz P. Schäfer, Max-Planck-Institut für biophysikalische Chemie, Göttingen, Germany.

LIF has so many advantages. It gives a bright signal against a dark background. As all students of chemical analysis know, the size of the signal is not as important as the ratio of the signal to the noise. LIF excels on this point. This feature would later become the basis for ultrasensitive chemical analysis, enabling detection limits to be pushed to that of a single molecule. LIF permits preparation of a well-defined excited state whose properties—radiative and collisional—can be studied in great detail. Molecules can be probed by LIF in extremely hostile environments, such as flames, arcs, and sparks. LIF can also be used in other amazing ways, such as sorting cells one at a time, prospecting for petroleum leaks in the ocean floor, and distinguishing between cancerous and noncancerous tissue in a patient. But I am getting ahead of the story here.

When I moved to Columbia University in 1970, I had the idea that LIF could be used to separate the isotopes of elements. All atoms of an element have the same number of protons in the nucleus, which defines the name of the element. But atoms of any particular element can differ by weight because they contain different numbers of neutrons. These variants in neutron numbers are known as isotopes. For example, the element uranium consists of the more common isotope U-238, which has 92 protons and 146 neutrons with a natural abundance of approximately 99.28%. But it also has the rarer isotope U-235 of 92 protons and 143 neutrons (basically, three fewer neutrons) with a natural abundance of only approximately 0.72%. Much attention is attached to this lighter isotope of uranium because it sustains a nuclear fission chain reaction and is the material for nuclear reactors and nuclear bombs.

Because of the differing weights of the isotopes (and the different structures of their nuclei, such as nuclear spin states), their absorption spectra are distinctly different. A laser beam of the correct wavelength (color) can excite atoms or molecules containing one specific isotope and not the other. I thought that this idea was an important one and even tried to patent it. I asked my colleagues at the university's chemistry department for help. At that time, Columbia University did not have an intellectual property and technology transfer office, so I was advised to try the Research Corporation, whose offices were located in downtown Manhattan, a simple subway ride away. The Research Corporation was famous for reaping the rewards of the patent on the Cottrell precipitator, an electrostatic device for removing dust from gases. I went there, armed with convincing data that we could separate isotopes of elements by laser excitation. To my great disappointment, I was told that patenting the idea was foolhardy. I was informed that the laser isotope separation technique would be valuable only for the isotopes of uranium for which the U.S. government would not pay anything. Nothing was done.

Nevertheless, we did write several papers describing one particular technique for laser isotope separation (11–13). This technique also led me to write a nontechnical article on this topic for the popular science magazine *Scientific American* (14). I was pleased that this work led me to become a consultant to both Los Alamos National Laboratory and Lawrence Livermore National Laboratory. Both of these government research laboratories were soon locked in an intense, and often bitter, competition to determine which one would come up with a viable laser-based separation procedure for the isotopes of uranium. This situation became a real challenge to me, for I had to make sure that each group trusted me and that I did not divulge the progress of one group to the other.

In the process of writing the article for *Scientific American*, I had the pleasure of working with the great photographer Fritz Goro. **Figure 5** depicts an illustration we made together, in which the beam from an argon ion laser passes through an evacuated glass cell containing I_2 vapor. The fluorescence is clearly visible, as shown by the yellow streak inside the glass cell. We placed a diffraction grating beneath the cell. The grating allowed us to catch the reflection of the yellow streak and disperse it into the colors of the different I_2 molecule transitions, as the molecule reradiated its excitation as it fell from the specific vibrational-rotational (v' , J') level of the excited state to the various different vibrational-rotational (v'' , J'') levels of the ground state. The intensity of each transition is governed by the Franck-Condon principle involving the square of the vibrational wave function overlap integral and the $\Delta J = J' - J'' = \pm 1$ dipole selection rules.

There is so much more that can be learned from a careful study of **Figure 5**. Close examination shows that the yellow streak is wider than the green laser beam. The explanation is that the pumped I_2 molecules remain in their excited state for such a long time that the random motion of the molecules causes the image of the fluorescence to spread in space. Careful measurements of this spread leads to an estimate of the radiative lifetime of the excited I_2 molecule on the order of a microsecond, that is, one-millionth of a second, in good agreement with the more precise measurement made earlier by Brewer et al. (7).

If some gas is admitted to the evacuated cell, the spread shrinks because of collisions between I_2 molecules and the gas atoms or molecules. The collisions stop the excited I_2 molecules from flying in straight lines. Depending on the admitted gas, the intensity of the lines decreases because of the fluorescence quenching caused by energy transfer between the collision partners. New lines appear on the grating because some collisions populate new (v' , J') levels of the excited I_2 molecule. So much information can be obtained about the reactive and inelastic collisions that excited molecules undergo from these studies. The interested reader can see a video of me illustrating LIF of I_2 excited with a green laser pointer (see Supplemental Video 1; follow the Supplemental Materials link at <http://annualreviews.org>).

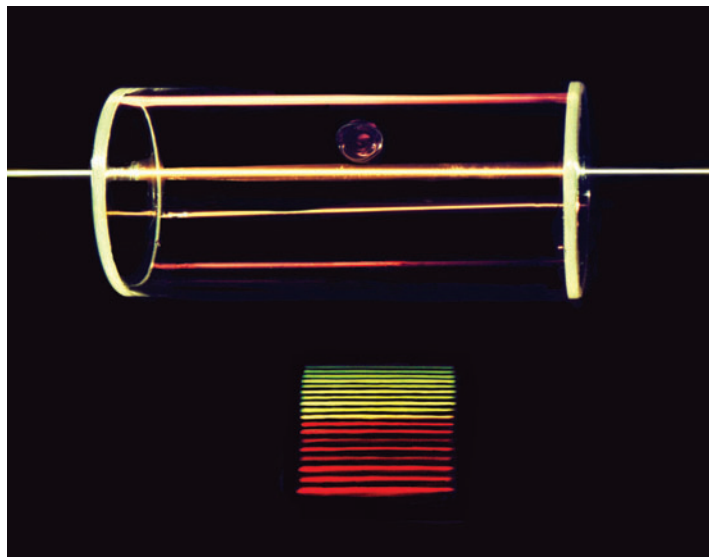


Figure 5

Photograph of laser-induced fluorescence of iodine (I_2) vapor taken by Fritz Goro and the author in the author's laboratory at Columbia University, 1977. A green laser beam enters an evacuated glass cell containing I_2 vapor, causing a yellow streak of fluorescence to appear. Below the glass cell, the light from the yellow fluorescence streak strikes a reflective diffraction grating, where it is dispersed into its different colors, each corresponding to a particular molecular transition from the pumped vibrational-rotational state of the excited I_2 molecule to one of the vibrational-rotational levels of the I_2 molecule in its ground electronic state. The appearance of the yellow color in the undispersed fluorescence is the sum of the different colors of the various molecular transitions, weighted by the intensity of each transition.

I need to emphasize the sensitivity of LIF and its ability to detect molecules under extreme conditions. Most stable molecules have an even number of electrons, paired together to make bonds between the atoms of the molecule. In chemical reactions, transient intermediates that have an odd number of electrons are often formed. These intermediates are known as radicals. Although their existence is often fleeting, radicals play a critical role in diverse areas of chemistry, such as atmospheric chemistry, polymer chemistry, and even human biochemistry. LIF has been able to capture the presence of these elusive entities before they disappear. According to a review (15) published in 2002, the following species, most of which are radicals, have been observed in flames through the use of LIF: H, C, O, C_2 , CH, OH, CO, C_3 , HCO, CH_2 , C_2H , C_2O , CH_2O , HCCO, CH_3O , $C_2H_2O_2$, C_3H_5 (allyl), C_7H_7 (benzyl), N, CN, NH, NO, NS, NH_2 , HCN, HNO, NCO, NCN, NO_2 , NH_3 , CCl, CF, CHF, CF_2 , CF_2O , PO, S_2 , SH, SO, SiO, and SO_2 . By now, the list has grown longer.

It was an easy step to turn LIF over to the detection of nascent reaction products under single-collision conditions. This was achieved in 1972 (16). **Figure 6** illustrates the experimental setup.

This apparatus allowed us to determine the internal energy distribution of reaction products, information that was missing when a mass spectrometer was used as a reaction product detector (17). **Figure 7** shows an early example. Moreover, using the polarization of the light, we also learned about the alignment of the reaction products.

One illuminating example of the power of LIF was in examining the internal state distribution of Na_2 formed in a supersonic expansion. My laboratory was the first to report that the supersonic expansion caused extensive cooling of the molecules, which greatly simplified the spectral analysis

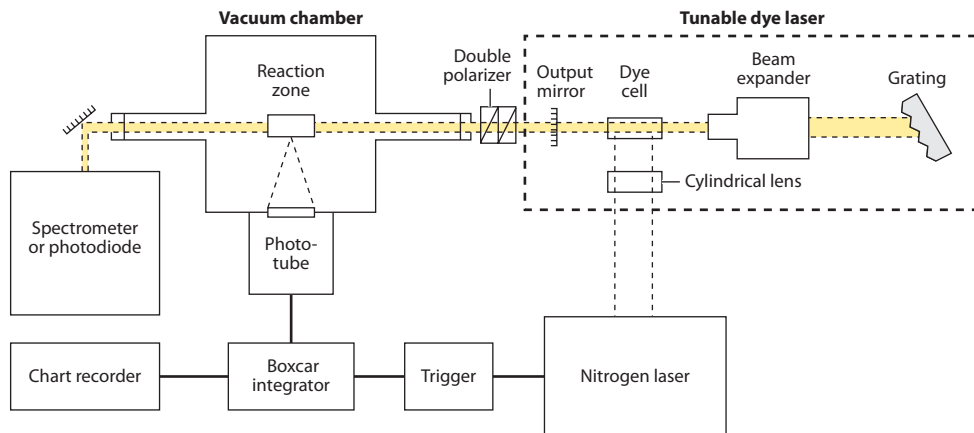


Figure 6

Schematic diagram for the laser-induced fluorescence detection of reaction products. Adapted with permission from Reference 17.

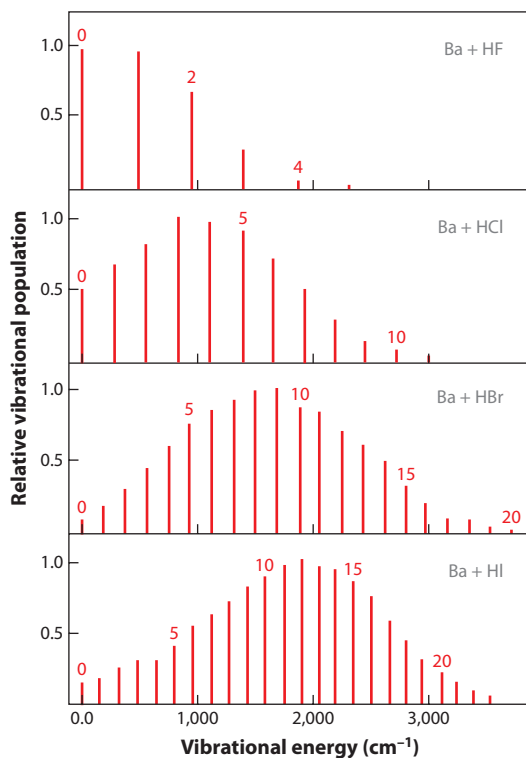


Figure 7

Relative population distribution of BaX vibrational levels in the reaction Ba+HX. Adapted with permission from Reference 18.

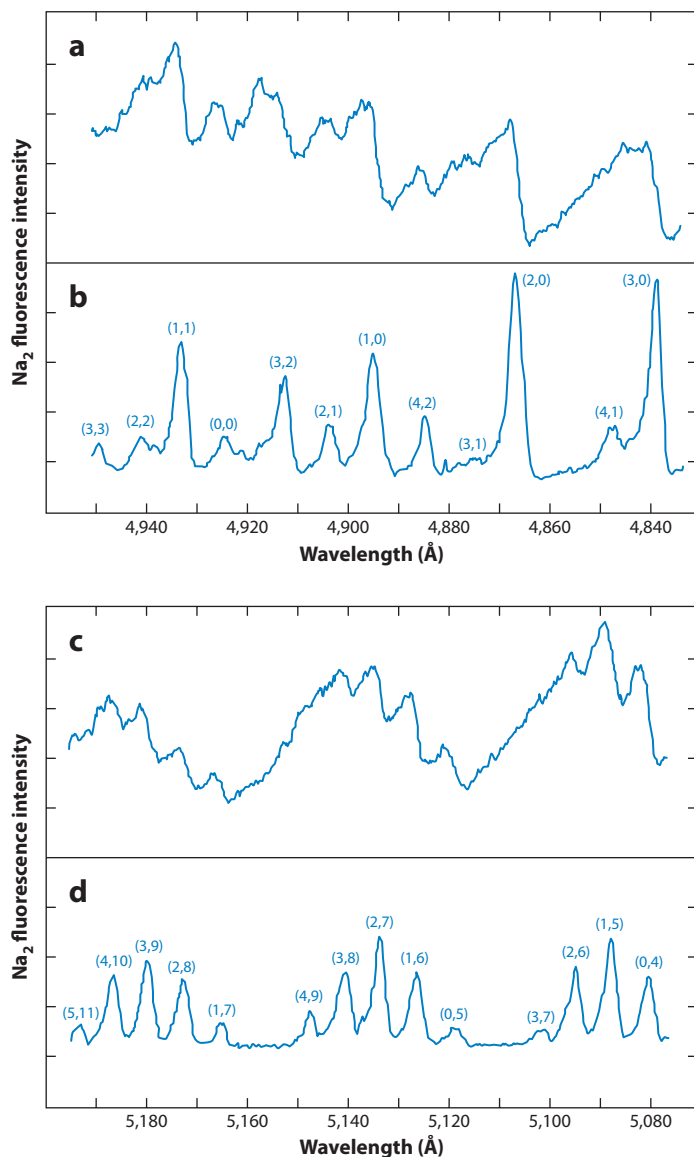


Figure 8

The Na₂ B ¹Π_u-X ¹Σ_g⁺ fluorescence spectrum excited by white light (*a,c*) in a cell at 590 K and (*b,d*) in a nozzle beam with an oven chamber temperature of 920 K and a nozzle chamber temperature of 970 K. The vibrational bands are identified by (v',v''). Adapted with permission from Reference 19.

of the molecules (19). **Figure 8** shows an example: Subsequent work by Sinha et al. (20) would show that the Na₂ molecules are not randomly distributed in the flow but align with the stream direction, like logs floating in a river.

In 1977, we found that converting molecules to molecular ions by a state-specific resonance-enhanced multiphoton ionization (REMPI) process was even more sensitive (21). It has become the predominant method for understanding fundamental elementary reactions, such as the simplest bimolecular reaction, $\text{H} + \text{D}_2 \rightarrow \text{HD} + \text{D}$. I studied this reaction system for many years in

great detail after moving to Stanford University in 1977. Other elaborations involved multilaser experiments: One infrared laser prepared reagents in a specific vibrational-rotational level, another laser generated fast atoms in laser photolysis as one of the reagents, and a final laser system detected the reaction products in a state-specific manner through either LIF or REMPI (22).

A key turning point occurred in 1976, when I attended a national meeting of the American Chemical Society (ACS). A U.S. Department of Agriculture chemist, Dr. Larry Seitz, searching for a particular session, walked into the wrong room at the huge ACS meeting. In that room, I was giving a presentation on the benefits and promises of LIF. During the question-and-answer session, Seitz asked me whether I could detect aflatoxins. I did not know what aflatoxins were, but ignorance has never stopped me from making up answers. I told him that if aflatoxins fluoresced and entered the gas phase, I could easily detect them. Seitz seemed quite excited by this answer and explained that these fungal metabolites are potent carcinogens for which the food supply has to be screened, especially moldy nuts and grains that have been stored for a long time.

Subsequent correspondence with Dr. Seitz revealed that some aflatoxins decompose when they are heated, so it really is difficult to do the gas-phase experiment I first had in mind. I began to think about the possibility of doing LIF on compounds in liquids. I tried to interest new prospective graduate students in the Columbia chemistry department in this project as a thesis topic—including Jacqueline Barton, the present chair of the Caltech department of chemistry—but no one was willing. Dr. Gerald Diebold, who is now a professor at Brown University, was a postdoctoral research associate in my group at that time. He was willing to boldly go where others had not gone before so together, we did the first LIF experiment on aflatoxins. We used liquid chromatography to separate different aflatoxin molecules in a mixture and then applied LIF as a detector (23). **Figure 9** illustrates the experimental setup.

Once I moved in that direction, a whole new world of applications appeared. It was a classic case of “once you have a hammer, everything begins to look like a nail.” One notable example is when I started developing LIF as a detection method for capillary electrophoresis (CE). I had visited Professor James W. Jorgenson at the University of North Carolina, where he showed me the remarkable separation power of this new technique. In CE, a strong electric field is applied across the length of a glass capillary filled with a liquid. The electric field causes ions to move at different speeds along the capillary. The current generated by the moving ions produces much heat, but the small cross section of the capillary, only thousandths of a centimeter across, dissipates the heat by conduction through the capillary walls with great effectiveness. This process allows different bands of ionic species that travel along the length of the capillary to be resolved with almost no broadening, giving pure and cleanly separated aliquots of the components in a mixture.

Jorgenson had been using absorption spectroscopy as a CE detector, but the short path length of the capillary greatly limited the sensitivity. To me, the solution to this problem was obvious. The ability to focus laser light into tiny volumes and its extraordinary sensitivity made LIF an ideal detection method. The first demonstration of its power was achieved in 1985 (24). This work led to commercialization by Beckman-Coulter, Inc., in their PACETM system (25). For many years, this commercial success supplied my research group with substantial financial support, until the patent expired.

CE separations with LIF detection led to one of the greatest achievements of the end of the twentieth century: the sequencing of the human genome, in which the millions of bases in DNA were read by automated CE-LIF machines. I feel very proud to have played a part in this advance, which has revolutionized our understanding of biology and offers so many hopeful possibilities for future medical treatments.

The exquisite sensitivity of LIF also had another consequence. In the 1990s, with my postdoctoral research associate Shuming Nie, currently a professor at Emory University, and my

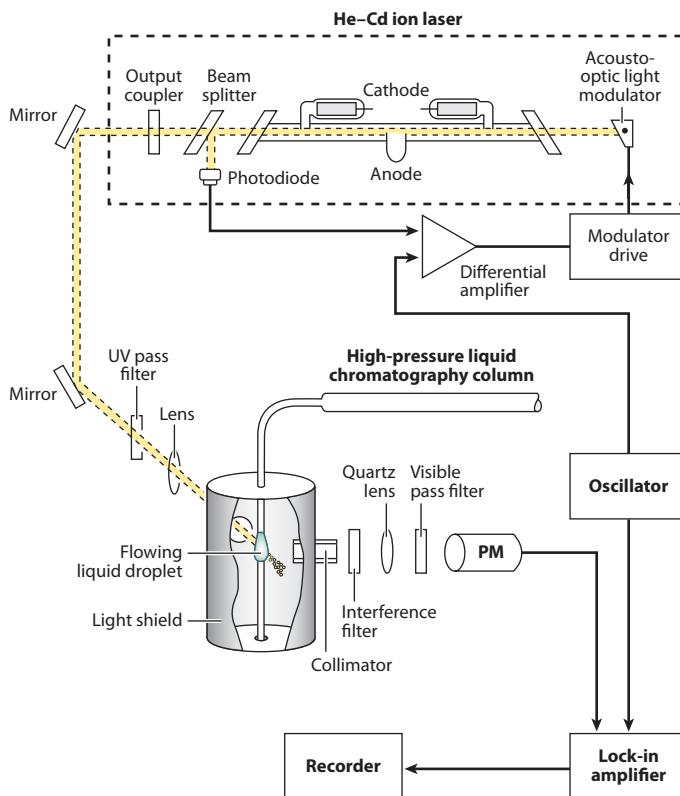


Figure 9

Laser-induced fluorescence detection of analytes separated by high-performance liquid chromatography. Abbreviations: PM, photomultiplier; UV, ultraviolet. Adapted with permission from Reference 23.

graduate student Daniel Chiu, now a professor in the chemistry department at the University of Washington, we were able to detect single molecules in room-temperature liquids by using LIF (26). This had been previously done in cryogenic matrices, first by my Stanford colleague W.E. Moerner. Since then, the field of single-molecule spectroscopy has exploded with all types of biological applications. In particular, it is now possible to count the copy numbers of proteins in a single cell (27) and to probe the whole genome of a single cell (28), both of which have been accomplished using a microfluidic platform.

Figure 10 illustrates the first demonstration of this technique for single molecules of fluorescein. At the time, this was big news, but today you can set up this experiment as part of an undergraduate laboratory. I have certainly done that at Stanford, where I designed a course for undergraduates to take as part of a new biological chemistry track for majoring in chemistry.

Various applications of LIF are still unfolding. It was once thought that the spatial resolution of LIF was limited by the wavelength of the excitation source. A point of emitted light is always accompanied by a diffraction pattern whose size is proportional to the excitation wavelength. This diffraction pattern limits the ability to locate the point's position in space. For this reason, the diffraction limit constrains how close together two (or more) emitting molecules can be located relative to each other. Today, new ways are being developed for breaking this diffraction limit with great success. Many are based on the idea that high spatial resolution can be achieved by acquiring LIF data in which only one of the emitting molecules is excited at a given time or in

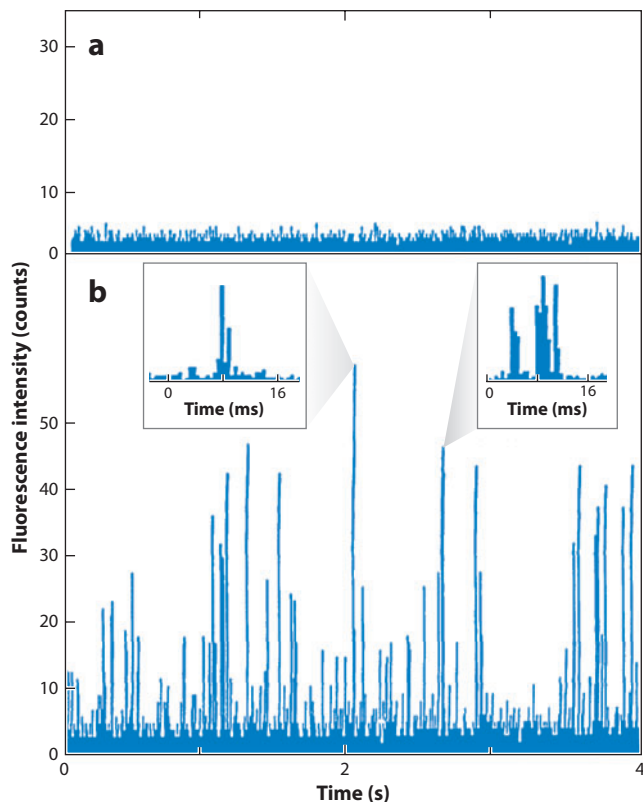


Figure 10

Laser-induced fluorescence detection of single molecules of fluorescein in aqueous solution: (a) blank consisting of distilled water; and (b) 3×10^{-10} M fluorescein. The excitation is a cw (continuously working) laser beam at 480 nm with an intensity of 0.1 mW. The inserts show expanded views of the designated peaks. Adapted with permission from Reference 26.

which the angular form of the emission is modified by applying another excitation step at the same time. By these methods, the spatial resolution of visible light microscopy has been increased to approximately 10 to 20 nm, allowing some biological processes to be described at the molecular scale (29).

Today, LIF has become globally popular. New developments include the ability for LIF to resolve features on the nanometer length scale, thereby breaking the diffraction limit set by the wavelength of the excitation radiation. The various applications of LIF give me much pleasure because I have shared its success with many people. Now that I am a bit older, I have met young students who explain to me what LIF is, how it works, and why it facilitates what they are investigating. They are blissfully unaware that I am the originator of this technique. This situation is wryly amusing but sobering. It allows me to assess the worth of what I have done and how we all take for granted the struggles of others who came before us.

DISCLOSURE STATEMENT

The author is not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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