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International Journal of Mass Spectrometry 200 (2000) 71–77

International Journal of Mass Spectrometry

# Matrix-assisted laser desorption/ionisation, an experience

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Received 1 June 2000; accepted 4 August 2000

**Abstract**

MALDI, matrix-assisted laser desorption/ionisation has been a success, no doubt. Together with ESI, electrospray ionization it has revolutionized the analysis of macromolecules from identification to function, the former a key element, e.g. in proteomics, the latter partially still a dream, a not so unrealistic one, though. Thousands of MALDI instruments can be found in research as well as in industrial laboratories all around the globe and even more scientists and developers find it useful in the pursuit of their diverse projects. Once discussed in little attended sessions outside the prime time it is now one of the centers of attention and discussion in literally all conferences on mass spectrometry and increasingly so also on bioanalytics. Yes, MALDI has come of age! (Int J Mass Spectrom 200 (2000) 71–77) © 2000 Elsevier Science B.V.

*Keywords:* MALDI; LAMMA; Mass spectrometry; Time-of-flight; TOF; Laser desorption

## 1. Introduction

How did this all come about? Do we know, do we understand? We are not sure. Perhaps as the inventors we are also too close to it, too overwhelmed by the success and too biased to give a fair account of the history of matrix-assisted laser desorption/ionisation (MALDI). Therefore, this is not a history of MALDI, it is not even intended to be one. It is rather an account of some experiences and thoughts, afterthoughts mostly, in fact, which we got out of the MALDI development. Are they representative? Can they be generalized? Some of them possibly, hopefully, we are inclined to believe, but primarily they are personal experiences, not easily reproduced. They are arranged partially randomly under a few pairs of seemingly antagonistic in reality, however, mostly synergistic terms.

## 2. Demand and options

Yes, there was a demand for mass spectrometry in the life sciences around 1970 when our first attempts started. It was clear that a better understanding of the structure and function of biological systems on the cellular and subcellular, hopefully even on the molecular level, required new, more sensitive and more informative analytical techniques. Was it really that obvious? Well, it quickly became obvious when in 1971 one of the authors (F.H.), then working at a German National Laboratory on Radiation and Environmental Research in Munich, looking for good applications for the ruby lasers he had built, almost by chance met Raimund Kaufmann, a physiologist at the

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*PII* S1387-3806(00)00300-6

University of Freiburg, some 300 miles away, who wanted to measure the distribution of $Ca^{++}$ ions in heart muscle cells. Calcium antagonists for the treatment of coronary diseases were the big theme then until beta blockers took over most of the field. We looked into laser induced emission spectroscopy as one of the option first, but soon settled for mass spectrometry because of its inherently higher sensitivity. A few papers had appeared in the literature by then on laser mass spectrometry [1–3] which certainly influenced our decision. LAMMA, the laser microprobe mass analyzer was developed, then manufactured commercially and, in the end, used mostly in the semiconductor industry. The $Ca^{++}$ distribution, incidentally, was never measured by LAMMA. Did we know then that laser mass spectrometry would solve the problem and, if yes, why and how? Of course not. It was a dream at best, dreamt at the side of a public pool after we had scanned the scarce literature and it was definitely not shared by our superiors. Did we believe in it? Years later in 1982, when LAMMA had already become reality, but MALDI was still some years away, Peter Roepstorff at an IFOS symposium asked me (F.H.) whether I believed that one would ever be able to record mass spectra of proteins. The answer was that I had not given up the dream but found it hard to believe in it. Only a year or so later the Uppsala and Odense groups published their first plasma desorption spectra of proteins, based on the ground breaking work by Ron Macfarlane and coworkers [4]! 1988, at the International Mass Spectrometry Conference in Bordeaux we presented our first MALDI spectrum of a protein with a mass >100 kDa (Fig. 1) [5], which actually looked more like a series of broad humps rather than a real mass spectrum. On the way to the conference dinner Marvin Vestal asked, whether this was more than just a record, whether it might ever be of commercial value. We were as uncertain as he was. Still, it took less than a year until his company, Vestec, decided to develop the first commercial MALDI instrument, after Ron Beavis and Brian Chait had demonstrated the simplicity of putting together a time-of-flight (TOF) spectrometer and had confirmed our results. Doubts have stayed with us all along much as have the hopes that

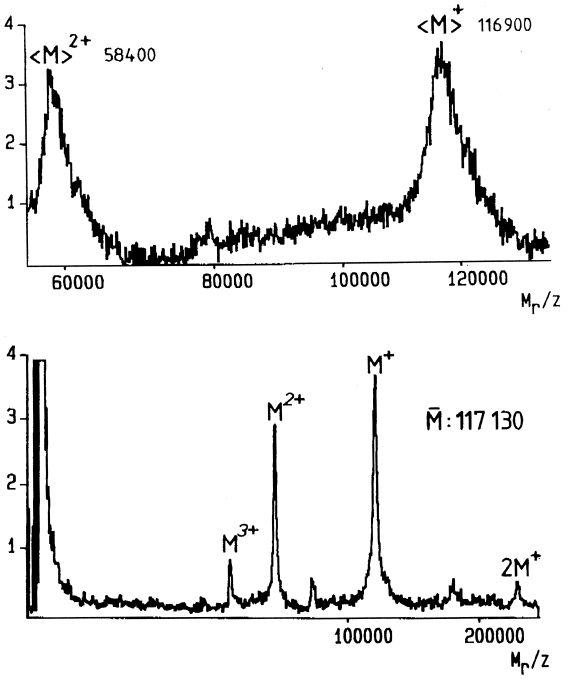


Fig. 1. Spectra of $\beta$-D-galactosidase. Matrix: nicotinic acid; wavelength: 266 nm. (Top) Early spectrum, presented at [31]. Sum of 100 shots. (Bottom) Spectrum recorded only a few weeks later, demonstrating the fast progress. 1 pmol prepared amount, sum of 30 shots. Adapted from [32].

a dream would eventually come true. In the end, many factors contributed to the success. Should one of them be singled out besides, of course, the serendipity, it should be interdisciplinarity. Now as much as then it was the combination of experiences from very different fields of science which was seminal. Interdisciplinarity, i.e. cooperation across the often so rigid borders between the disciplines, talked about much and implemented with reservations still, was one of the most important ingredients in the development of LAMMA and, indeed later of MALDI.

## 3. Potential and prejudice

Was there a way to assess the practical potential of the concept then? Well, no research and development without funds and no funds without reviews. This is what one of the reviewers thought of it: “. . . this

project is at the edge of physical feasibility. The intended sensitivities have, so far, never been reached and will, most probably, not be reached by the suggested project either . . . " Was she/he wrong with this analysis, was it prejudice against the new and unproven? No, she/he was quite right with the analysis. It were our ideas as to how it would work which were wrong and which we had been careless enough to elaborate in the proposal. By now we know quite well that, would we really have ignited a dense plasma of the organic sample with high power lasers, as suggested in the proposal, very few ions if any, would have made it through a mass spectrometer and have reached the detector, in MALDI even more so than in LAMMA. How many useful developments originate from at least partially incorrect or incomplete concepts? But then, our real goal was to solve a problem, not to prove a principle. The very first experiments immediately told us to steer clear of real plasma generation. The reviews would actually most probably have been the end of the story, would it not have been for an unusually curious and courageous program officer at the VW Foundation, who decided to go for it anyway. He must have felt, though, that a word of caution would be appropriate in the letter of allowance: " . . . in accepting the funds, you will also have to accept the high risk for a successful completion of the project . . . "

The every day reality of project reviewing and proposal granting soon caught up with us, though. One case is particularly memorable, because it is pretty close to the musical chairs, we used to play as children. Two times the German National Research Council (DFG) had turned down our request for an Excimer pumped tunable dye laser, each time granting only the funds for a one year graduate student salary " . . . to conduct more preliminary experiments." When this happened a third time we were ready to finally turn down the grant. What other preliminary experiments could we do on the wavelength dependence of laser desorption without a laser which would allow us to scan the wavelength range of interest? However, since we did not seem to be able to get the laser, we at least wanted to know the reviewer's argument against it (no "pink sheets" at the DFG!). The information which was finally conveyed was as short as it was simple " . . . it is known from the literature that the wavelength is not an important parameter in laser desorption . . . " At least the reviewer was fair enough to list the source of the information [6]. It took us less than one hour to resolve the controversy and the DFG less than two weeks to grant the laser, after we had informed them. The reference the reviewer had cited was a review article by David Hercules et al. which indeed contained a short statement "It is generally conceded that the wavelength of the laser is not an important parameter" but it also continued to say "although this conclusion has been established from studies on a limited number of systems." Hercules et al. actually had taken this statement from yet another review [7] which indeed at that time was the most comprehensive account of the state of the art. The statement in that review is based on a number of original publications, all of which had used largely different sets of experimental parameters, a fact the authors explicitly mention as a caution. In none of these projects had, in fact, more than one single wavelength been used and the most recent of them was six years old at the time of the proposal review and was, surprise over surprises, our own description of the very first LAMMA results [8]. It did not take us long to prove the importance of the laser wavelength in MALDI, after we had the tunable laser installed and running.

## 4. Technology, new and revived

Technologies are as important a part of a development as are ideas. Lasers were barely a decade old when the work started and little did we and others know then about their potential for analytical applications, the interaction with organic specimen in particular. Nonlinear optics was an emerging technology and the first commercial *Q* switches and frequency doublers came just in time to allow us to switch from the visible to the UV, key to LAMMA as well as MALDI. The first usable nitrogen lasers appeared at about the same time, only to disappear from the market again until many years later. TOF

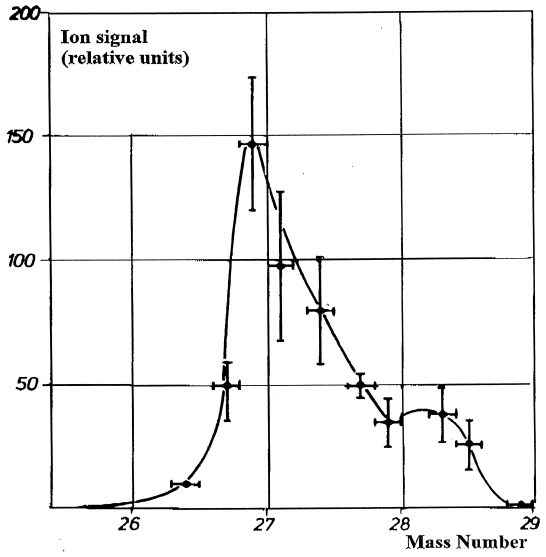


Fig. 2. Early quadrupole LAMMA spectrum of an aluminum coated cover slide. Each point is the average of several hundred laser shots.

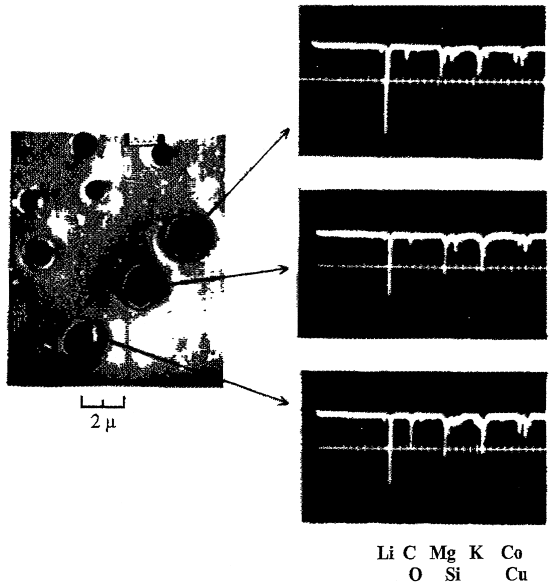


Fig. 3. Spatially resolved LAMMA spectra of a 0.1 $\mu$m thick section of epoxy doped with 3 ppm of Li, Mg, and Co ions in crown ethers. The spectra are screen shot from a fast Tektronix oscilloscope.

Mass spectrometers had been developed in the fifties [9], but soon forgotten because of their poor performance except for a short revival for laser applications [2,3] and later by Macfarlane and Torgerson [4] for plasma desorption and by us for the laser microprobe LAMMA [10]. Those were the days of quadrupoles and such monsters as four-sector instruments (what a prejudice!). We started out with a quadrupole, only to find out that it took us about half a day to collect a spectrum covering a range of only four masses and barely resolving two adjacent ones (Fig. 2). Our first TOF had a vertical tube, a concept which seems to get popular again nowadays and spectra had to be photographed by very short lived Polaroid 410 films from the 4 by 6 cm screen of the then fastest oscilloscope for transient signals (Fig. 3). Those were still the analog times, the digital age was just dawning. What a relieve was the first transient digitizer with an only nominally 8 bit resolution and 1024 channels, hooked up later to one of the first Apple computers. The first MALDI mass spectra of larger proteins were actually recorded with a time resolution of 500 ns/channel in order to obtain the necessary time and mass window. Actually, TOF development had been continued in the Soviet Union largely unnoticed by the West, but Mamyrin's ion reflector [11] was eventually implemented in one of the first commercial LAMMA instruments. The other revolutionary development, key to the success of MALDI, was actually the rediscovery of delayed ion extraction simultaneously by several groups many years later [12–15] and first implemented commercially by Vestal/Vestec then already called Perseptive Biosystems [16]. Who would have believed that a TOF instrument could have a mass resolution of 20 000 or better just a few years before? Would MALDI have been nearly as successful as it is without all these quite diverse technologies, coming along just in time? Hardly!

## 5. Plan or chance

Around 1980 we began to expand the LAMMA applications to look at small organic compounds, triggered by the observation of the chemical noise in the spectra, which apparently represented fragments of the organic matrix in our samples. The first results were moderately successful at best and a manuscript

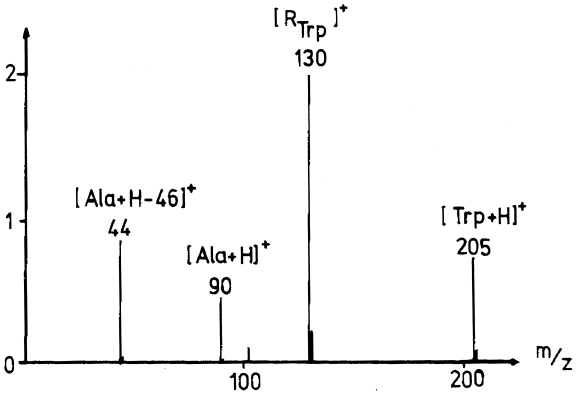


Fig. 4. Laser desorption spectrum of a mixture of the aliphatic amino acid alanine and the aromatic amino acid tryptophane recorded at the threshold fluence of tryptophane. The codesorption of alanine was the first observation of a matrix effect. Wavelength: 266 nm. Adapted from [33].

describing the results of a systematic collection of spectra of all amino acids was rejected by one of the leading analytical journals. After Michael Karas had joined the group, he one day ran a mixture of Tryptophane and Alanine and saw signals of both, even though the laser fluence had been adjusted to a value, at which only Tryptophane should have gotten desorbed and ionized. We would, most probably, have missed the message, would we not have known from the earlier systematic investigations that Alanine had a more than 10 times higher desorption threshold fluence than Tryptophane at the wavelengths of 266 nm: Alanine came riding along piggyback with the Tryptophane (Fig. 4) [17]. The idea of matrix assisted desorption was born almost instantly. Of course, the idea of a matrix, being important for the desorption of labile organic compounds, was a quite popular concept at that time. The role of glycerol in fast atom bombardment was discussed extensively in the mass spectrometry community [18,19], as was that of nitrocellulose in plasma desorption [20]. Cooks had also suggested beneficial matrix action in laser desorption [21,22]. It was the concept of a controllable deposition of the energy per unit volume into the sample which made all the difference and distinguished the function of the matrix in MALDI from that in the other desorption techniques, in which this control was essentially not possible. The role of resonant versus nonlinear light absorption in the interaction of laser radiation with organic samples, collected mostly in work on medical laser application by us and others, helped in designing and interpreting the key experiments for the MALDI development. Considerable further work by several groups was, however, necessary before the now popular matrices such as 2.5-dihydroxybenzoic acid (DHB) [23], the cinnamic acid derivatives [24,25] and 3-HPA [26], to name just a few, were discovered. It is also almost forgotten by now that for many years infrared lasers and UV lasers were competing strongly for the largest ions and best spectra. The 1978 paper by Posthumus et al. [27] on the $CO_2$-laser desorption of organic molecules up to about 1000 Da was long considered the gold standard in the field. So, our experiments on UV-laser desorption in those days were planned as well as we could plan them, given the limited knowledge and understanding, but chance came in to help at the time we were ready to recognize its significance.

## 6. Theories or models

If there was a demand and goal at the outset but we had the wrong theory as to how it was to be achieved, do we have a complete and quantitative theory of the mechanisms underlying laser desorption/ionization now, almost thirty years later and after contributions from so many research groups all around the globe? We have learned a lot, but the answer is nonetheless essentially “no.” Some of the basic conditions which rule the desorption part are understood and molecular modeling is beginning to reveal some important aspects of the processes even though realistic volumes of $\mu m^3$ in size and time scales of tens of nanoseconds are still beyond the capacity of even very large computers [28]. Ionization is even more of a challenge still, even though type and relative abundance of the ions can be extracted directly from the spectra. Systematic experiments have mostly contradicted early models based on photoionization or proton transfer from excited states of matrix molecules. Charge disproportionation in clusters and separation

by fracturing have been suggested and could, in principle, explain ion formation in UV as well as IR MALDI, but neither has been checked by systematic experiments yet. Was and is it all just trial and error then? The answer is no again. We and others have had at least qualitative models all along, some of them more intuitive, like the "volcano" model [29], preferred in our team by Michael Karas, some at least partially quantitative more the preference of Franz Hillenkamp [30], both important in their own right. Such models have guided the research of our and many other groups. Even though they remain at any time imperfect, they do predict trends of results upon variation of selected parameters and play an indispensable role in the progress in MALDI as in many other fields of science. It is, most probably, fair to say, that the great success of MALDI, offering solutions for a wide variety of analytical problems has somewhat deceived us and others to pursue preferentially applications and leaving the investigation of the mechanisms mostly for later. We suggest that it is time to go back to some more basic research. There is all reason to believe that a better, a more detailed understanding of the MALDI processes will open new applications and optimize existing ones. MALDI mass spectrometry of DNA, e.g. is still lagging far behind that of proteins with a range of potential application comparably large.

## 7. Cooperation or competition

Interdisciplinarity has been described above as one of the key aspects in the MALDI development. Cooperation, has been the equally important sister of it. We have been privileged to have had an intense exchange of ideas and results with many colleagues and groups over the years, some of them have even led to real friendship. We have always felt that in the end both sides have gained more from these exchanges than either side had given and MALDI has been the real winner. Joint projects, e.g. with the MIT group in Boston and Peter Roepstoff's group at Odense University, more recently also with companies such as PE Biosystems and Bruker Daltonik have contributed significantly to the MALDI development. This is not to say that competition is not also an important driving force in modern research and development. I (F.H.) well remember one evening with Brian Chait on the board walk at the 1989 ASMS conference in Miami. We began to speculate about the next steps in the MALDI development. We quickly agreed that it would, in all likelihood, be advantageous to go from the then exclusively used 266 nm wavelengths of the quadrupled Nd:YAG laser to one longer than 300 nm, preferentially that of the $N_2$ laser at 337 nm. We admitted that we were both not sure whether that would work and whether we would find suitable matrices for that wavelength. We consciously decided to not tell each other, which matrices we were considering as candidates and both left Miami knowing that we better work hard on this problem, would we want to be the first to do it. In the end two very different solutions, the cinnamic acid derivatives, pursued in New York and, after a little detour via 2-aminobenzoic acid, DHB in Münster were found, both still the most favored matrices for different types of applications. It is the right mix between cooperation and competition that is needed, but it is good to keep in mind that human attitude results in what might be called the second law of science: left to itself, in a closed system of scientists competition always increases, whereas cooperation requires an open system and some expense of energy. We would like to encourage more open systems and more (emotional) energies put into collaborations in the true sense of the word.

## 8. Labor or fun

Was it hard labor then, over these years? It certainly was. But, much more so, it was a lot of fun. During the years when we, i.e., the two authors, both worked together on MALDI, we almost never agreed right away with each other on concepts, experiments, results and their interpretation. What for us was a constant struggle for the best next step to be taken, the best understanding of what had been measured, conducted often in loud and prolonged debates, has made

many visitors wonder, how we could efficiently work together at all. For us, however, it was a fun game of constant challenge with wins and losses pretty evenly distributed. Mostly, though, we ended up with a result which somehow reflected both inputs. We both miss the almost daily challenge and fun now, that we are not working in the same place any more. The real second law does not allow us to go back and do it all over again, even if we would want to, but the memories remain of a great time with MALDI.

## Acknowledgements

Many have contributed to our work, family, friends, colleagues, and even reviewers and funding agencies. Above all, however, our thanks go to the students and postdoctoral fellows, who have passed through our labs during these years. Without their genuine curiosity, their enthusiasm and hard work, most of what is now MALDI mass spectrometry would not have happened, at least not in Münster and Frankfurt.

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