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REVIEW

Proteome imaging: A closer look at life's organization

Ron M. A. Heeren

FOM-Institute for Atomic and Molecular Physics, Amsterdam, The Netherlands

Imaging the proteome is a term that is used in many different contexts. The term implies that the entire cohort of proteins and their modifications are visualized. This unfortunately is not the case. In this mini-review, a concise overview is provided on different imaging technologies that are currently used to investigate the structure, function and dynamics of proteins and their organization. These techniques have been selected for review based on the unique insights they provide in subsets of the proteome. These techniques have been illustrated with practical examples of their merits. Mass spectrometry-based imaging technologies are playing a key role in proteome research and have been reviewed in more detail. They hold the promise of detailed molecular insight in the spatial organization of living system.

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1 Introduction

1.1 General remarks

The study of protein structure, function and their interactions with their environment constitutes a multibillion euro research effort throughout the biological, biomedical, biochemical and biophysical sciences. Many studies are directed at proteins studied in isolation, yet it is the complex dynamic interplay between different entities in biological systems that is key to life. As insights move on, integral systems approaches are becoming more prevalent. Proteomics, centered on MS technology contributes an augmenting amount of data, information and knowledge to this quest for insight into life's organization [1]. Imaging technologies are becoming more and more important in proteomics research as they are capable of representing very complex results in a form that is easily interpretable by the human brain. Many different types of imaging techniques are being used to chart the proteome of different species, and a selected set of proteome imaging technologies will be touched upon in this mini-review. Although each of these methodologies employs images in very different manners, they all serve the same purpose: Study the identity, distribution, dynamics and interaction of proteins in biological systems. No technique to date exists that provides a full-proteome image, *i.e.* every methodology discussed in this report images a sub-set of the entire proteome. In that sense, one can argue that the definition of proteome imaging really depends on the type of interrogation to which the proteome is submitted. It is clear that imaging technologies play an important role for many different applications. In proteome research, three types of image usage can be distinguished as depicted in Fig. 1. These are: (1) the use of optical images for direct inspection; (2) the use of images to represent large spectral data sets; and (3) the use of molecule-specific images. Many different types of subdivisions are possible in imaging technology, yet this division will be shown to be able to describe most of the current developments in proteome imaging. This mini-review will focus on molecule-specific imaging technology. However, in everyday proteome imaging the first two types of images are the bread-and-butter of most researchers. A brief discussion is therefore merited.

Correspondence: Professor R.M.A. Heeren, FOM-Institute for Atomic and Molecular Physics, Kruislaan 407, 1098 SJ Amsterdam, The Netherlands
E-mail: heeren@amolf.nl
Fax: +31-20-6684106

Abbreviations: FTIR, Fourier transform infrared; **LAMMA**, laser microprobe mass analysis; **SIMS**, secondary ion mass spectrometry

1.2 General optical image technologies

Optical image technologies play an important role especially in disease proteomics [2]. It is nowadays often combined with affinity-based capturing or labeling techniques. The

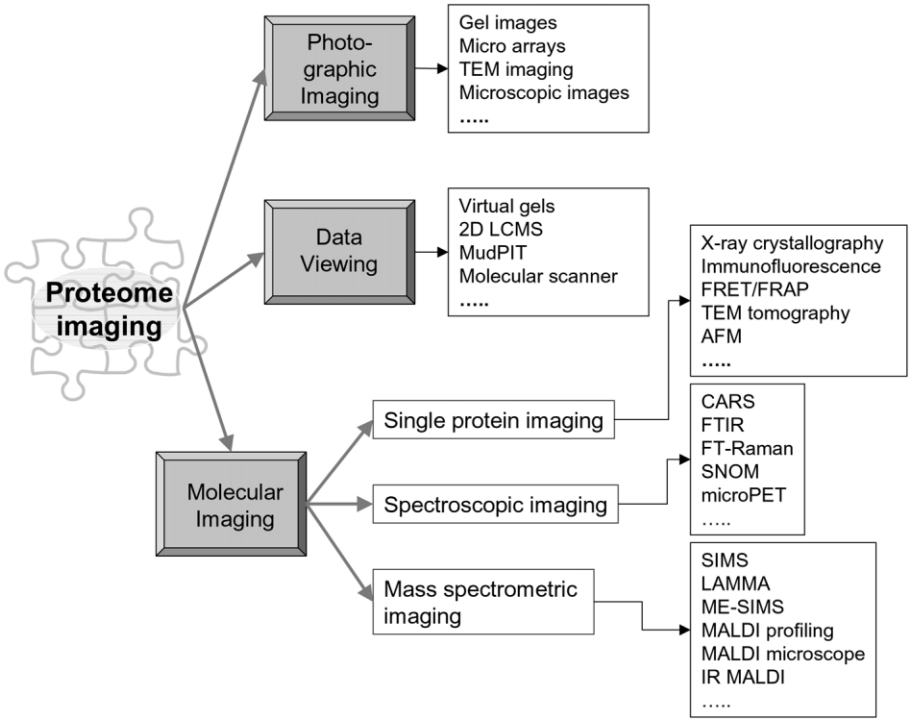

Figure 1. An overview of different imaging techniques that provide information in relation to structure, function or organization of a biological microenvironment.

purpose of this strategy is to find deviations from "normal" protein expression in a quest for biomarkers for specific diseases. Studies of this type focus on obtaining a better fundamental understanding of the biochemistry of a disease as well as the development of novel diagnostic strategies. Unfortunately, most diseases are the results of the derailment of heterogeneous regulatory processes, that occur on both genome and proteome level. As a result, it is not a single biomarker that needs to be elucidated, rather a more global analysis of the molecular landscape is required [3]. As far as gene and protein expression levels are concerned a differential analysis strategy is often employed. Gene microarray technology is the technique of choice for the evaluation of gene expression and single nucleotide polymorphism (SNP) mapping. DNA microarray technology provides a detailed image of the simultaneous expression of entire genomes. The use of this technology augments with the elucidation of more and more genomes. New insights into gene function, disease pathophysiology, disease classification, and drug development are obtained by automated comparative image evaluation of photographic array images directly showing gene expression up- and down regulation [4, 5]. For protein expression analysis the first step is often the isolation and enrichment of a complex protein mixture from a biological system. The mixture is analyzed using 2-DE that separates the mixture by p*I* and molecular weight. The gel is stained and a scanned image of the gel is obtained. Two images from a control and a test sample or samples stimulated under two different environmental conditions are obtained and compared for differences in protein expression or protein modification [6, 7]. An example is provided in Fig. 2 where a comparative proteome analysis of *Saccharomyces cerevisiae* is performed under glucose or ethanol limiting conditions [8]. The differences found are subsequently analyzed with a plethora of analysis techniques for protein identification. Non-enrichment-based strategies such as protein microarray technology or direct MS analysis offer alternative protein expression analysis approaches that also rely on imaging technologies in a very similar manner. [9] The use of photographic images is not restricted to the visualization of complex mixtures of proteins. Rapid imaging techniques are also commonly employed to study the motion of protein filaments in live cells directly using phase contrast microscopy as demonstrated in Fig. 3. These images are being employed by biophysicists to measure the mechanical forces and dynamics of complex protein systems that are exerted by the biopolymers [10]. Advanced Cryo-EM imaging (such as 3-D molecular tomography) technologies are employed to study the structure of large biomolecular complexes such as the 20S structure of the GroEL chaperonin complex [11, 12].

1.3 Visualization of large datasets

In proteome studies, many different techniques lead to very large datasets. High-throughput MS approaches contribute largely to the generation of voluminous datasets. There are many examples to illustrate this, but we will restrict ourselves to the most common approach, the so-called bottom-up approach, using PMF. Two main implementations of this technology exist, one where peptides are generated from

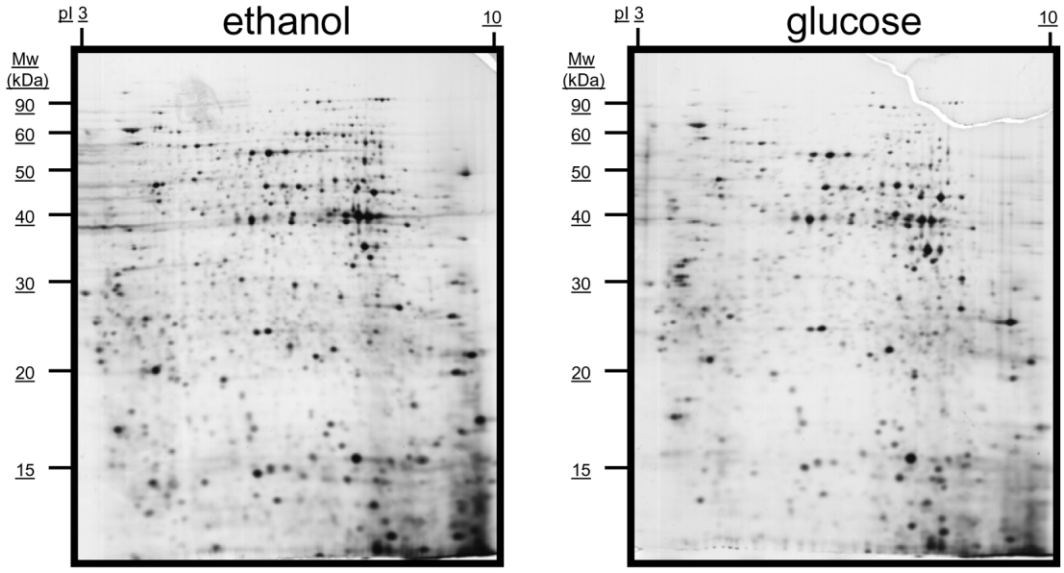

Figure 2. Scanned images of silver stained 2-D SDS-Page gels demonstrating relative changes in protein expression in ethanol-limited versus glucose-limited cultured (published with kind permission of A. J. R. Heck, taken from [8]).

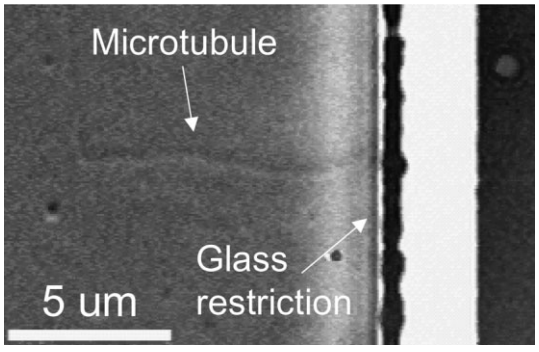

Figure 3. Phase contrast microscopy snapshot image of a growing and buckling microtubule. The tubule is dynamically polymerizing from α- and β-tubulin molecules under physiological conditions (image courtesy of M. Dogterom).

proteins that have been pre-separated using 1-D or 2-DE followed by in-gel digestion [13] and the multidimensional protein identification technology (MuDPIT) approach [14] where a complete protein mixture is enzymatically digested into an even more complex peptide mixture. The first method relies heavily on the photographic image of a stained gel in order to cut out selected (or all) protein spots prior to digestion. MALDI-TOF MS is the preferred analysis technique to obtain a protein ID using the resulting peptide mixtures from each gel spot. MuDPIT uses LC separation coupled to ESI MS/MS [15] for peptide identification. Hunt *et al.* have pioneered the use of LC-MS for peptide identification [16]. The sheer volume of mass spectra generated by both of these approaches has long been a bottleneck in the data analysis pathway. Presenting the analytical data and results is challenging by itself, but fortunately, the use of image technology provides some relief. The application of virtual 2-D gels representation has been applied to many different studies. Smith *et. al.* used a virtual gel presentation to compare the protein expression levels with a reproduced Swiss-Prot 2-D-PAGE photographic image of an *Escherichia coli* lysate [17]. They found a good correlation between the protein distributions, but more importantly demonstrated the added value of accurate molecular weight information. Although the information density in their CIEF-FTICRMS study was much greater, the use of image representation permits an easy comparison between the two approaches. Orgozalek-Loo *et al.* have employed a similar visualization strategy to reconstruct a virtual 2-D gel, using data collected from each excised gel band that was analyzed using a protocol combining gel excision, in-gel digestion, reduction, alkylation and trypsin digestion followed by MALDI-MS [18]. To deal with this wealth of data various bioinformatics initiatives have been started aimed at finding novel ways to process and extract information from data and combine different information streams. The molecular scanner approach, pioneered by the Hochstrasser group [19] employs a similar visualization strategy. Recently, the same group has substantially simplified the procedure by the application of a blotting technique [20] that employs a trypsin immobilized membrane to digest the migrated proteins on their way from the 1-D gel to a capture membrane. The latter constitutes a PMF image of the original 1-D gel that can be read-out directly with MALDI MS. An example is provided in Fig. 4. This technology is now in the author's lab beginning to be employed for the direct analysis of tissues combined with a direct molecular imaging technique [21].

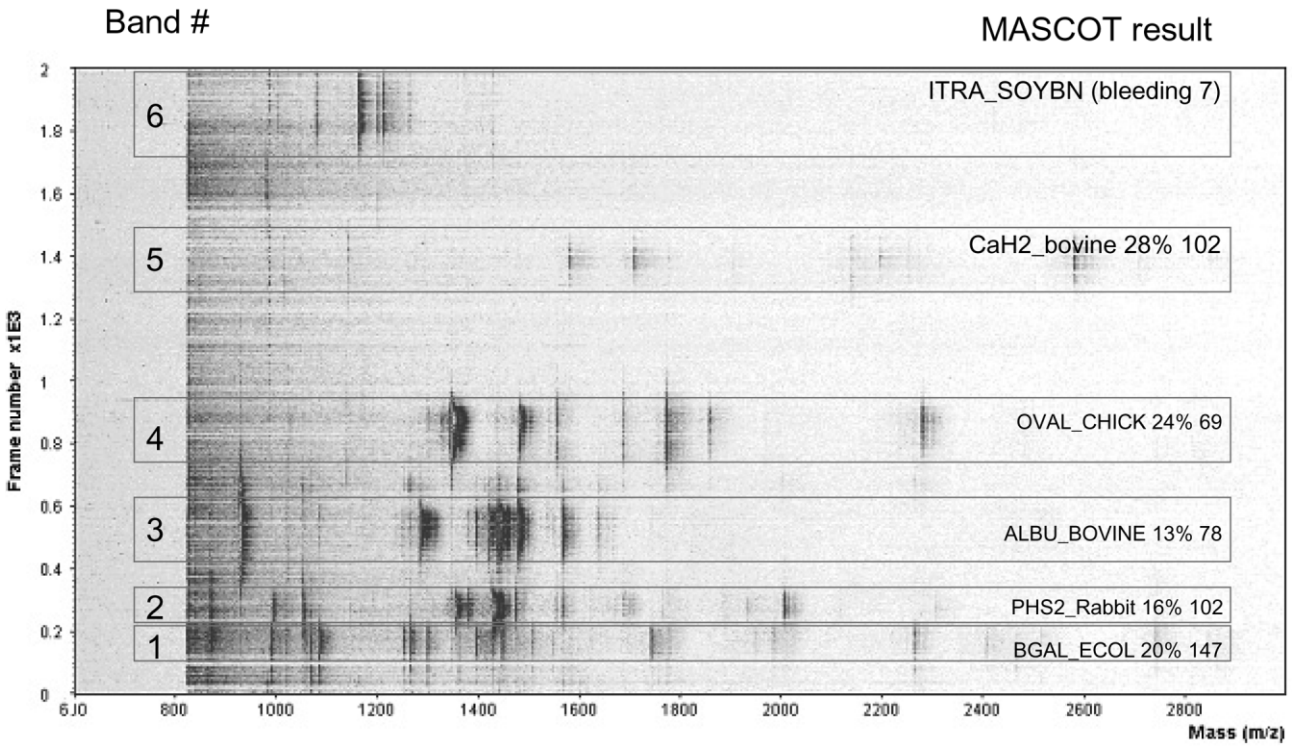

Figure 4. Molecular scanner virtual gel representation of a protein calibration mixture that has been migrated on a 1-D SDS-PAGE gel and subsequently blotted through a membrane in which trypsin had been immobilized. The membrane was read out with microscope mode MALDI imaging in collaboration between FOM-AMOLF and the BPRG group in Geneva. Membranes were kindly provided by A. Vaezzadeh of the Geneva group. This 2-D image was created with the AWE3D software package developed by M. Konijnenburg from the AMOLF informatics department.

2 Molecular imaging technology

2.1 General observations

Molecular imaging techniques in the life sciences aim at the investigation of the relation between spatial organization and function of molecules in biological systems. A distinction in molecular imaging classes can be made based on the type of image information generated combined with the proteome subset that is targeted. The following three will be discussed: Single protein imaging, spectroscopic imaging and MS imaging.

2.2 Single protein imaging

The first class of molecular imaging targets the smallest proteome subset, single proteins or protein structures. Using multiple fluorescent labels it is now possible to annotate the spatio-temporal distribution of selected biomolecules in parallel in a single experiment [22]. Impressive advances have been made in this field, that now allow *in vivo* visualization of cellular biology and the use of clinically important staining techniques [23, 24]. Such microscopy studies have already been used to substantiate the intuition that a protein's function is correlated with its localization in the cell [25, 26] and to demonstrate that several diseases are associated with altered molecular distributions [27–29]. Using targeted fluorescent molecular probes or through the insertion of GFP or equivalent sequences in the organism's genome a particular protein or set of proteins can be labeled for high resolution imaging with confocal microscopy [30]. A major advantage of this approach is that it can be applied *in vivo* and can provide detailed insight in the dynamics of molecular organization and protein synthesis pathways. The drawback of the approach is that it is imperative to know in advance which subset will be studied and little information on the alteration of structure and functionality is provided.

2.3 Spectroscopic imaging

Spectroscopic imaging is the second class of molecular imaging technology. It targets the largest proteome subset, all proteinaceous material. Micro-spectroscopic imaging tools are used to study the distribution and interactions of biomolecules within cells/tissue [22, 31–33]. Generally, vibrational spectroscopic imaging in the life sciences is employed to image the distribution of functional groups on biological surfaces. Fourier transform infrared (FTIR) imag-

ing, for example, has matured into a technique that provides insight in the spatial organization of various classes of molecules in complex matrices.

At this point in reviewing molecular imaging technology, it is important to make a clear distinction between microprobe mode imaging and microscope mode imaging techniques. The first mode deals with the acquisition of spectra from a small area by selective illumination or selective detection. The sample is subsequently moved to the next detection area and after the entire surface of interest has been scanned, a retrospective image can be constructed from the acquired data set. This measurement mode is also often referred to as "mapping". In the microscope mode, an entire (panoramic) image passing through the microscope or spectrometer is acquired at different wavelengths, optical path length differences or molecular masses. No local distortions occur that might influence the spectrum taken from the next, adjacent spot, no image aberrations or scattering resulting from the use of diaphragms occur and the speed of spectral image acquisition is usually one or two orders of magnitude higher compared to the microprobe mode.

FTIR imaging is one key example of microscope mode spectral imaging. The field of view, spatial resolution, speed of analysis and information density of FTIR microscopic imaging have all improved tremendously by the relatively recent availability and implementation of infrared cameras or focal plane array detectors. As a molecular imaging technique, it allows the visualization of the microscopical distribution of functional groups on or in a complex surface in a fast and non-destructive way. Pioneering work of Levin and Lewis [33] has led to the commercial availability of FTIR chemical imaging systems and their application in biomedicine [34]. With the advent of new, dedicated faster detectors and electronics, it now is possible to study the dynamics of biomolecular chemistry at surfaces with micrometer size spatial detail. These advanced molecular imaging systems are still mostly in the hands of academic research groups, but are starting to find their way into to industrial research laboratories and hospital laboratories. As an example, the identification of different functional groups in arteriosclerotic tissue sections is demonstrated using Fourier transform infrared imaging microscopy in Fig. 5. Functional group images were obtained without prior staining of the tissue samples and allowed the molecular distinction between the plaque material and the healthy arterial wall. Diffraction limited absorption images show the presence of the proteinaceous material in the arterial wall with approximately 6-μm spatial resolution in a 400×400-μm field of view. Imaging the Amide-band at $1650\,cm^{-1}$ clearly shows the distribution of protein rich material in arteriosclerotic plaques deposited on the arterial wall. The absence of staining chemicals in this non-destructive analytical technique allows further analysis of the tissue section surface with other molecular imaging techniques. The drawback of this spectroscopic imaging technique is the requirement of flat or thin samples to prevent total IR absorption. Other spectroscopic imaging techniques such as coherent anti-Stokes Raman scattering (CARS) [35] or micro-magnetic resonance imaging (MRI) allow the generation of 3-D images of particular classes of molecules such as lipoproteins or lipids in

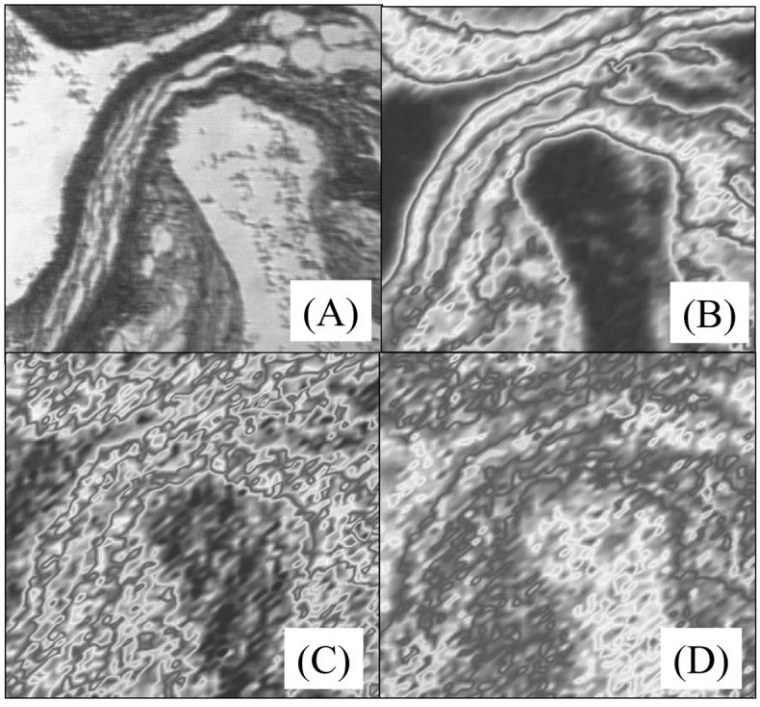

Figure 5. FTIR microscope mode images of 10-μm thin sections of mouse arterial tissue. The optical image of conventionally stained sections is shown in panel (A). Field of view is 400 by 400 μm; panels B–D show the amide distributions at $1650\,cm^{-1}$ (B), Amine image at $3300\,cm^{-1}$ (C) and a CH functional groups image at $2920\,cm^{-1}$ (D). Images taken at AMOLF in collaboration with N. de Bont and P. Demaecker, Radboud University Hospital, Nijmegen, The Netherlands.

living systems. In this category, micro-computed tomography (μCT) allows the nondestructive, noninvasive *in vivo* imaging of a number of disease processes, proliferation of metastases and response to therapeutic agents. Generally, all the molecular imaging techniques lack the capability of molecular speciation, but rather present a more general picture.

2.4 Mass spectrometric imaging

The last molecular imaging technology discussed in this review provides a small to medium size subset of the entire proteome. It, however, has the added advantage that it provides detailed structural information on the biomolecules found at surfaces using the enabling technology in proteome research, MS. In contrast to the non-destructive spectroscopic techniques described earlier, MS imaging relies on the removal of attomolar amounts of molecules from the surface under study. For this purpose routine methods for desorption and ionization can be used that are commonly employed in proteomics investigations. Spatial resolution, molecular selectivity, depth of analysis, sensitivity, destructiveness and the required sample modification are key issues in molecular imaging of biological surfaces. No technique exists (yet) that does not compromise on at least one of these issues. Throughout the last five decades, there have been many different incarnations of imaging MS. Many different research groups have early on speculated about the possibilities of visualizing macromolecular distributions directly from organic surfaces. It was not until the conception of MALDI as a desorption and ionization technique for intact macromolecules [36, 37] that it became conceivable to image proteins from surface directly with MS. Before the application of matrices to surfaces (see Fig. 6), two different types of mass spectrometric imaging were common: SIMS and LAMMA. The major part of the early MS imaging developments came out of the physics labs and found a multitude of applications in semiconductor research and industry. This community focused on the use of energetic atomic ion beams to generate secondary ions from surfaces that were subsequently mass analyzed. The technique was and is referred to as secondary ion mass spectrometry (SIMS). SIMS has demonstrated to be an excellent technique to image the distribution of elements and small organic molecules with spatial resolution around 100 nm [38, 39]. It is currently undergoing a revival with the advent of novel primary ion beam types using molecular clusters (Gold or Bismuth cluster sources) [40] or larger molecules (C60) [41]. This approach extends the useful mass range into the realm of lipids [42] and cholesterol [43]. Yet, the real promise of high-resolution imaging of larger biomolecules (peptides and proteins) has yet to be shown with SIMS imaging without surface modifications. After the successful introduction of organic matrices in MALDI, it became clear that a similar beneficial effect was found when a MALDI preparation was examined with SIMS [44]. Protein ions up to 12 kDa were found using a standard MALDI sample preparation protocol, indicating that similar gas-phase ion molecule reactions must play a role during matrix enhanced SIMS (ME-SIMS). Remarkably, it has been found that irrespective of the use of a UV laser or a 15-keV indium ion beam similar spectra are obtained from tissue sections and cells [45]. Recently, this methodology has been applied for the successful analysis of low abundance lipids [46] in neuropeptides from tissue [47], elucidating surface topography and peptide localization, respectively.

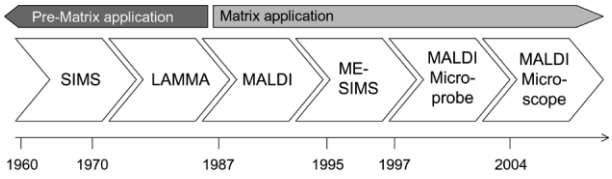

Figure 6. Historical development of MS imaging.

The second early MS imaging method used a microfocused laser beam shortly after the introduction of lasers in MS. Its acronym became LAMMA for LAser Microprobe Mass Analysis [48, 49]. It employed a microfocused UV laser beam for laser desorption and ionization from complex organic and inorganic surfaces. This was the conception of direct molecular imaging of biological tissue with MS. When a few years later the first MALDI results were reported demonstrating the direct desorption and ionization of intact proteins it was obvious that the LAMMA approach would soon be applied to surfaces that were covered with matrices [50]. In the next section the current status of MS-based proteome imaging technology will be reviewed and the microprobe and microscope approach for MS-based biomolecular imaging compared.

3 Recent developments in MS-based proteome imaging

Molecular imaging with MS, which provides both the chemical information of a mass spectrometer and the spatial organization of each component on a surface, is revolutionizing the field of biological surface analysis. MS imaging in particular brings chemical specificity to this field. In modern molecular imaging techniques, such as immunocytochemistry or immunoelectron microscopy as described earlier, molecule-specific labeling is required. Imaging MS can spatially map surface components without pre-selection (or labeling) using an intrinsic molecular property; the molecular mass. In proteome imaging it is increasingly important to be able to visualize protein expression directly on cellular surfaces or tissue sections. This provides a similar approach as in histopathology but with the enormous advantage of molecular information. This form of proteome imaging also benefits from the sensitivity these MS desorption and ionization techniques bring to the field. It is the combination of high spatial resolution with high sensitivity for high mass

molecules that makes molecular imaging MS attractive. Both MALDI-[51, 52] and SIMS [53, 54], [55] based approaches are being developed to provide more information by enlarging the proteome subset that can be imaged. These two techniques offer different starting positions for the ultimate goal of high mass high resolution imaging, namely high mass + low spatial resolution (MALDI) and low mass + high spatial resolution (SIMS). While MALDI is commonly used to generate ions of up to 100 000 Da, typical pixel sizes of the images are $\geq$ 25 μm [56]. On the other hand, SIMS routinely delivers images with submicron ($<$ 1 μm) spatial resolution, [53] however, the sensitivity rapidly decreases with increasing mass such that these high-resolution images are typically obtained from ions having an *m/z* under 500. In addition to the primary ion beam developments described previously, different sample preparation protocols are being implemented to increase the mass range that can be imaged with a SIMS approach. The application of a very thin layer of common matrix material (such as 2,5-dihydroxibenzoic acid, sinapinnic acid *etc.*) directly to tissue for ME-SIMS purposes is one of them [47]. Figure 7 shows an example of direct tissue imaging with ME-SIMS.

The application of a matrix for high spatial resolution imaging does have a number of limitations. First of all the final resolution will be governed by the size of the matrix crystals. Secondly, that application of the matrix has to be carefully tuned to minimize diffusion of molecules of interest on the surface, but still be in wet contact with the surface long enough to extract relevant molecules from the tissue. Many different sample preparation protocols have been developed but it is still is a craft rather than a science. The criteria of a good matrix deposition depend strongly on the required resolution and sensitivity. Generally lower spatial resolution (*i.e.* larger crystals $\gg$ 10 μm) provides more intense signals than higher spatial resolution (*i.e.* smaller crystals $\ll$10 μm). This also applies to MALDI-based molecular imaging techniques. One approach that seems to enhance molecular ion yields seems to be the deposition of thin layers of metals (gold or silver in particular) on top of a surface of interest. So far this technology has been used to enhance the yield of lipids and smaller biomolecules from a variety of surfaces [57], [43, 58, 59]. The exact mechanism of action of this thin metal coating is not fully understood. One of the hypotheses is that reduced surface charging enhances signal persistence and hence sensitivity.

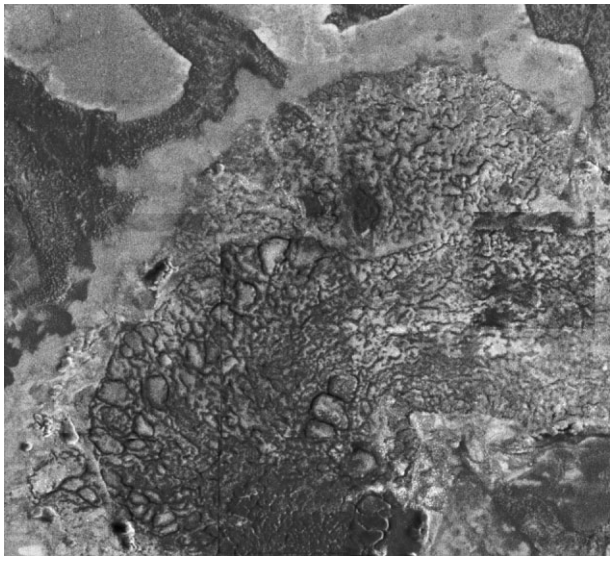

Figure 7. ME-SIMS total ion image of *Lymnaea stagnalis* nervous tissue (mosaic kindly prepared by A. F. M. Altelaar).

One area of MS imaging that is applied more frequently is MALDI MS imaging in the microprobe mode. Activities in this field have boomed as a result of the pioneering work of the Caprioli group [60] [61]. At the end of 1997, they employed position-correlated MS to record the spatial distributions of proteins in rat pituitary tissue. Line-scan images of a section of pituitary, revealed localization of several peptides such as vasopressin, α-MSH and beta-endorphin, in the anterior, intermediate, and posterior pituitary. More recently, this technique has been used to investigate cancer (Fig. 8) and Alzheimer's disease at the molecular level [62, 63]. The image of human glioblastoma for instance showed that by means of imaging MS a molecular based distinction could be made between the distal and most active area of tumor proliferation, an ischemic area and a necrotic area of the tumor. These examples of direct high molecular weight imaging have been the onset on a number of new developments. Protocols for subcellular MALDI-MS have been described for comparative peptide profiling on single isolated neurons [64,65]. From these and other studies it is clear that sample preparation protocols are still in need of further development. A strong approach is the combination of complementary proteome imaging techniques. Chaurand *et al.* [66] combined laser capture micro dissection (LCM) followed by conventional proteome work-up with MALDI MS profiling directly on mouse epididymis tissue. In this experiment the conventional proteome image (Virtual gel/PMF) can be considered an atlas that serves to find the interesting proteome subset for MALDI MS imaging purposes. In this particular case, a 400-protein subset was monitored in the course of the experiment.

Molecular imaging with MS is also finding its way into pharmaceutical research. Recent examples include the investigation of the permeation of a pharmaceutical compound into the skin after application through MS imaging a cross-sectional imprint of treated tissue [67]. In addition, anti-tumor drugs have been investigated with imaging MS to asses if these compounds end up at the targeted location in the tissue. It was demonstrated that the MALDI-MS response was proportional to the amount of drug in the tissue [68]. New developments in protein array technology use MALDI-MS imaging as a read-out for pore chip protein arrays [69] and combine the MS results with a fluorescent read-out scheme for immediate validation. Not only MALDI-MS imaging is used in pharmaceutical studies. Using dy-

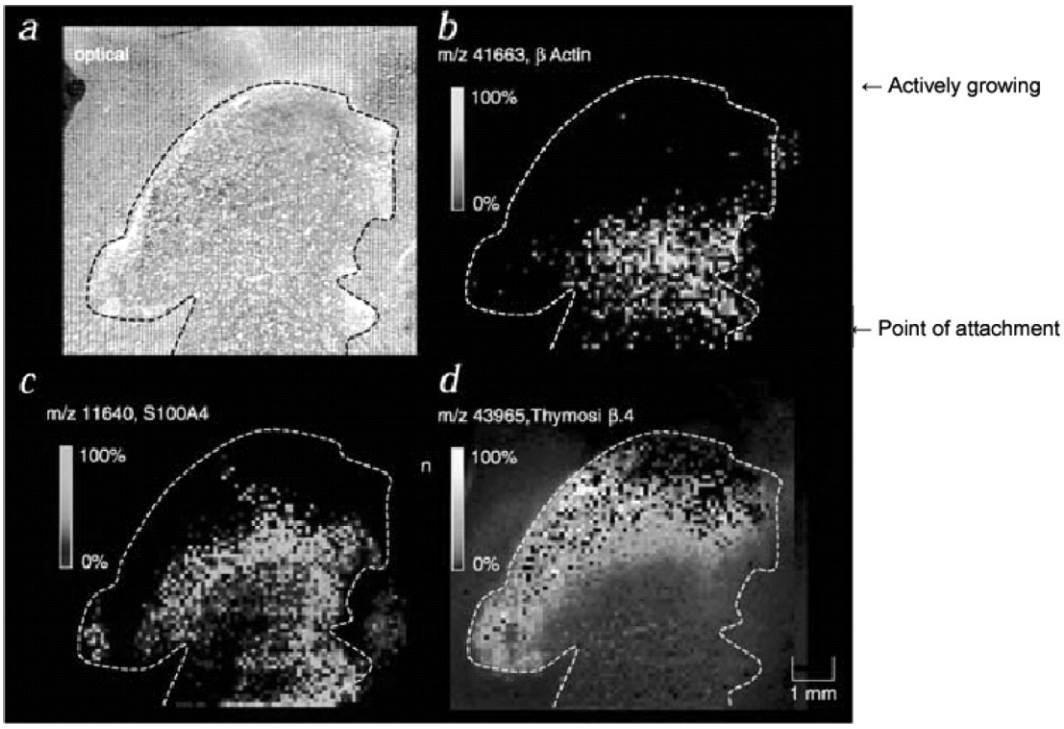

Figure 8. MALDI microprobe tissue image of actively growing tumor tissue (published with kind permission of M. Stoeckli taken from [63]).

namic SIMS with nanometer size probe beams it has been demonstrated to reveal boron neutron capture therapeutic agents in single cells with 50-nm resolution based on Boron subcellular distributions [70]. TOF-SIMS is often employed for the study of novel drug delivery systems [71]. A decade ago, SIMS and X-ray photoelectron spectroscopy (XPS) imaging were used to investigate peptide distributions in biodegradable polymeric slow-release systems [72]. The improvement in preparation of tissue samples by freeze fixation and substitution further extend the limit of molecular imaging in pharmaceutical research. Direct imaging of trace elements and molecules marked with a tracer make it possible to determine their targets by comparison with images of subcellular structures [73].

The achievable spatial resolution and sensitivity (sample preparation) are still the limiting factors for the widespread application of macromolecular imaging MS. The speed at which images with a field of view in the order of several centimeters are being generated is still low in both MALDI and SIMS imaging. The images can take up to 24 h to be acquired with approximately 50-μm MALDI probe size. It is conceivable that surface alterations can readily occur on a matrix-covered surface in the course of an experiment. One approach that partially alleviates this problem is the use of rapid microscope mode MALDI imaging. It has been demonstrated to generate 200-μm field of view peptide ion images with micrometer resolution in single laser shots [74]. Currently, microscope mode MALDI imaging allows the generation of selected peptide ion images or total ion images with a repetition rate of 10 Hz. An example of rapid imaging is shown in Fig. 9 where a few frames from total ion movie of a 200-μm wide line-scan taken at 10 Hz are presented. In comparison with microprobe mode, MALDI at the same spatial resolution the MALDI microscope generates images approximately four orders of magnitude faster [45]. The microscope approach has an additional advantage. As the spatial resolution is limited only by the quality of the ion optics and the spatial resolution of the detector, and not by the size of a probe beam, it allows for the use of ionization techniques that normally provide little or no spatial resolution. Infrared MALDI is such a technique of which the spatial resolution is generally diffraction limited to tens of micrometers. The microscope approach has recently demonstrated the generation of peptide ions images with the same resolution (4 μm) as microscope mode UV MALDI. For the first time, MS images were obtained a factor of 3 below the Abbé diffraction limit (the minimum focus size that can be generated with an optical set-up) at regular laser fluences [75]. The IR techniques potentially can be applied to cells and tissue without matrix application. The hydroxyl groups ofcytosolic water could be used to resonantly couple the desorption laser's energy into the surface and generate biomolecular ions [76]. Yet to date, it seems that the amount of energy needed for significant biomolecular signal is too high to be of

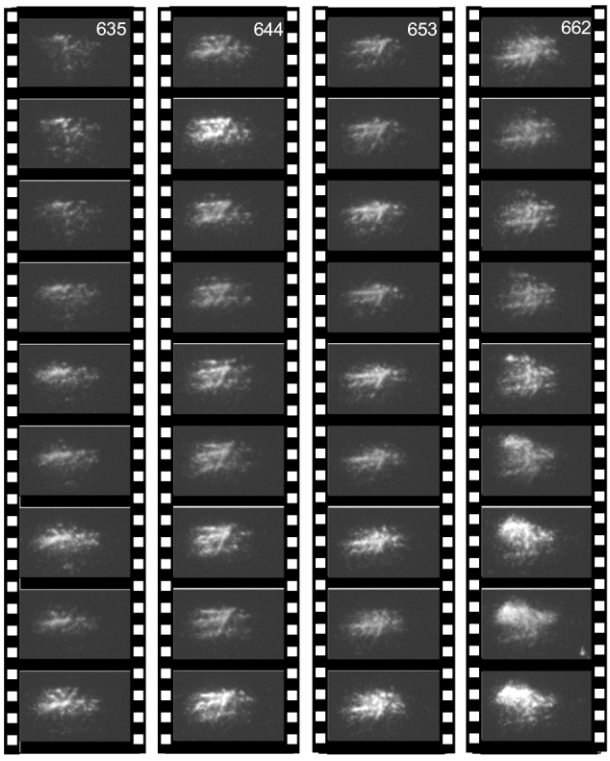

Figure 9. Mass microscopic total ion movie of a matrix covered fibrous membrane.

practical value. New sample preparation and substrates are under investigation [77], [78] that could bring the practical application of IR MALDI imaging closer.

4 Concluding remarks

A proteome can be imaged at many different levels. Several imaging methods and approaches have been reviewed that all contribute to the understanding of life's complexity. Here a subjectively selected set of imaging techniques have been discussed and many others (such as X-ray crystallography) have been omitted. This review is therefore far from complete, but it does provide the reader with different views to the proteome. Some vistas show a distribution of a single protein while others show a complete protein complex or an even larger subset of the proteome. Not a single approach exists that fully captures the complexity of a proteome, and every single imaging technique described in this mini-review somehow compromises molecular specificity, resolution, speed, sensitivity or another key parameter of interest. Imaging MS has been shown to provide the broadest view to the proteome, but still suffers sample preparation issues, ion suppression effects and its applicability is far from general. It is a field that is, however, rapidly developing, judging by the number of publications in between 1988 and 2004 depicted in Fig. 10.

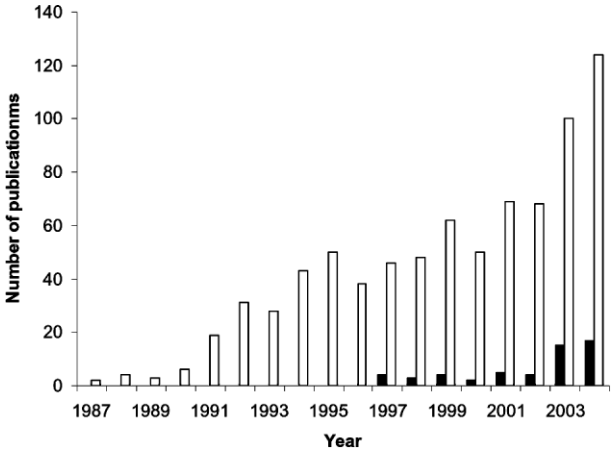

Figure 10. Publication history of imaging MS and its MALDI imaging subset. Bibliographical information was extracted from the ISI Web-of-science database.

As seen in this figure, the developments in MSc imaging were threatening to stagnate around 1997, but the conception of MALDI MS imaging has clearly turned it into a booming field. This, in my opinion, is just a prelude of what is still to come in proteome imaging.

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