Talanta 102 (2012) 16–25

Contents lists available at SciVerse ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

History and present status of imaging analysis

Freddy Adams [a,*], Carlo Barbante [b,c]

[a] Micro-Trace Analysis Centre (MiTAC), University of Antwerp, Wilrijk, Belgium
[b] Institute for the Dynamics of Environmental Processes – CNR, Venice, Italy
[c] Department of Environmental Sciences, Informatics and Statistics, Ca' Foscari University, Venice, Italy

ARTICLE INFO

Available online 29 June 2012

Keywords:
Imaging analysis
Tomography
Depth profiling
Super-resolution microscopy
Spatial resolution
Near-field optics
Coherence
Synchrotron imaging

ABSTRACT

In this tutorial review we give a concise and general overview of the development of imaging analytical techniques from its early stages in the late 1950s up to the present. Analytical techniques that are available for the characterization of the atomic and molecular composition as well as the structure at the bulk level often fail for the analysis of heterogeneous materials. Over the last 50 years a number of specialized analytical techniques were developed – or adapted from existing techniques – that, with time, matured into powerful tools for visualizing structural and compositional heterogeneity in nanotechnology and for the study of natural objects. These techniques evolved first at the microscopic and then the mesoscopic level (the range 100–1,000 nm), and later onto the nanoscopic scale between a few nm and 100 nm, where quantum effects start affecting the properties of materials.

1. Introduction

Many complex functional materials usually have limitations in atomic, molecular or structural ordering. At present, heterogeneities are important up to the nanosize level. For nanomaterials there is often an intimate relationship between the composition and structure on one side and the properties and function on the other side. The properties of nanosized materials significantly depend on their three-dimensional morphology (size, shape, surface topography) and on the heterogeneity of their composition. These parameters must be precisely correlated with their properties, which are often unexpectedly unusual, for the design and fabrication of new materials for potentially new applications [1]. The complexity of nanomaterials often leads to inherent instability and lack of robustness.

Heterogeneity on the nanolevel also plays an important role in the natural world. For instance, in geological and environmental materials heterogeneity can result from numerous different processes. In the case of materials such as minerals, soils or sediments, heterogeneity is the result of diverse bio-geochemical processes that operate over extremely long time periods. In the case of biological samples, heterogeneity is the result of development processes responsible for the differentiation of tissues, cells and or sub-cellular compartments. The overall result of all these processes is increased system complexity.

The heterogeneity of materials is a relative concept; many materials can easily be considered as homogeneous in structure or composition above a certain macroscopic size level and then become heterogeneous when the size of observation is reduced. Over the past decades scientific and technological interest has gradually shifted from the macroscopic world to miniaturized objects and over the past decade the nanoscale (<100 nm) size level has been reached. With the growing importance of increasingly smaller natural and synthetic materials that need to be characterized it became increasingly necessary to be able to analyze local differences in composition and structure.

Irrespective of the mechanism driving heterogeneity, the overall result is a system complexity that requires the systematic measurement of heterogeneity. The orderly collection of such heterogeneities in composition using imaging or chemical imaging tools is often a necessity. This requires the acquisition of large datasets in order to properly understand the intrinsic order (or disorder) of materials.

In a recent report the National Academy of Sciences of the USA said that the importance of imaging in chemistry is motivated as follows [2]:

…sustained efforts are needed to facilitate understanding and manipulation of complex chemical structures and processes. Chemical imaging offers a means by which this can be accomplished by allowing the acquisition of direct, observable information about the nature of these chemistries. By linking technological advances in chemical imaging with a science-based approach to using these new capabilities, it is likely that fundamental breakthroughs in our understanding of basic chemical processes in biology, the environment, and human creations will be achieved.

* Corresponding author.
E-mail address: freddy.adams@ua.ac.be (F. Adams).

0039-9140/$ - see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.talanta.2012.06.057

"All pictorial form begins with the point that sets itself in motion…" was stated by Paul Klee in his notebook [3]. Most imaging methods are based on zero-dimensional (point analysis, 0-D) observations that can be systematically moved over a line (1-D) and then to a surface to produce two-dimensional (2-D) images. While it is sometimes possible to derive 3-D information from such images through stereological considerations, it is often desirable to obtain more direct 3-D information on the structure and composition of a particular object. Such 3-D images can be obtained by collecting 2-D images in successive slices obtained through sequential sectioning by e.g. the removal of material from the surface with ion beam etching (sputtering, depth profiling). A more or less accurate full 3-D representation can thus be obtained. The alternative consists of the measurement inside of a given sample by using matter-penetrating radiation such as X-rays, particles or electron beams. Tomographic or confocal techniques then allow the direct 3-D measurement and analysis of microscopic or nanoscopic objects. More exceptional is the situation when a 2-D image is directly obtained in one operation by direct imaging microscopy.

The Nobel Prize of chemistry or physics has been awarded several times for the development of new imaging techniques: Richard Zsigmondy in 1903 for the development of optical ultramicroscopy and the study of colloids, Frits Zernike in 1953 for phase-contrast microscopy, Dennis Gabor in 1971 for his discovery of holography, Ernst Ruska in 1986 for the development of electron microscopy and, jointly the same year to Gerd Binnig and Heinrich Rohrer for scanning tunnelling microscopy. In addition, there were other Nobel Prizes that led to important tools that are now used in nano-analysis. Kai Siegbahn obtained the Nobel Prize for physics in 1981 for his work on electron spectroscopy for chemical analysis/X-ray photoelectron spectrometry (ESCA/XPS), which is now a prominent surface analysis technique. He shared the Prize with Nicolaas Bloembergen and Arthur Schalow for their research on laser spectroscopy. Both XPS, which is important for surface characterization and laser spectroscopy play a key role in imaging analysis.

2. Imaging and analytical chemistry

Chemical or structural composition comes in an addition to direct observational tools such as the optical or electron microscopy. The majority of analytical techniques for spatially confined and imaging analysis are based on spectroscopic techniques covering various areas of the electromagnetic spectrum or mass spectrometry and derive from methodologies that are summarized under the denominator of "beam analysis". In addition to these techniques, electron microscopy developed potential for chemical analysis especially through the detection of emitted X-rays, in principle for surface analysis but—through the combination with sputtering tools—can also be used for depth profiling and 3-D analysis.

At present, there are many highly sophisticated analytical tools available for the characterization of inorganic and organic materials with a high structural complexity and heterogeneity. A number of these techniques have the potential to perform 2-D and 3-D imaging on the sub-microscopic, or even on the nano-size level. In chemical analysis at the nano-level a number of techniques can be used that complement information available through physical techniques such as atomic force microscopy (AFM) and scanning tunneling microscopy (STM).

Among the many alternative techniques, a number of prominent imaging analytical techniques can be defined:

- Secondary ion mass spectrometry (SIMS) using focused primary ion beams or with broad beams relying on ion optics to spatially resolve the secondary ions;
- Laser microprobe mass spectrometry (LMMS) and specifically laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) for elemental analysis and matrix assisted laser desorption ionization (MALDI) for organic analysis;
- Electron spectroscopies (XPS, scanning Auger electron spectroscopy (AES) and scanning Auger microscopy (SAM);
- Transmission/scanning electron microscopy (TEM, SEM), electron probe microanalysis (EPMA);
- Ion beam analysis (IBA) with techniques such as micro-proton induced X-ray emission and Rutherford backscattering spectroscopy (μ-PIXE, RBS);
- Microscopic X-ray fluorescence analysis (μ-XRF) and related inner-shell ionization techniques.

All these methodologies belong to the category of beam (probe) methods of analysis in which a sample is excited by an energetic beam of particles or radiation. Their spatial resolving power depends on mastering ways to obtain increasingly smaller impinging beams. Nevertheless various additional factors influence the spatial potential, the most important being the degradation of beam dimensions while the beam is travelling inside the sample, or the absorption or scattering of outgoing radiation or particles.

In 3-D imaging we should make a distinction between the lateral dimensions defined by the beam hitting the sample and the third dimension (the depth inside the sample). The surface composition of solid materials usually differs considerably from that of the bulk. This is a deliberate change in many technological materials (e.g. in coatings and catalysts) or is produced unintentionally during e.g. weathering processes for natural materials or surface patina generation in archeological objects.

It is only quite recently that surface analysis was developed into instrumental tools mainly through the development of powerful probe techniques. When the surface is operationally defined as the layer in which the material composition and properties differ from those of the bulk material, its thickness can differ enormously from atomic layers, as in catalysts, to several micrometers, as in coatings, or several mm as in metallic art artefacts.

2.1. Historic development

Up to the early 1970s analytical techniques lacked the potential to analyze differences in composition and structure well below the macroscopic level. Interest was focused rather on the determination of the average composition through the application of strict sampling protocols. It was therefore necessary to obtain a representative sample for analysis and it was fundamental to perform repetitive analyses as an estimate for the reliability of the processes (see Fig. 1, top).

The first technique for local analysis on a size reduced scale was the EPMA which resulted from the combination of an electron microscope with crystal diffraction (wavelength dispersive)

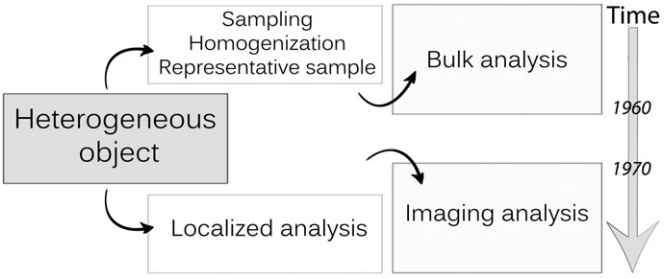

Fig. 1. Classical analytical chemistry and the "birth" of analytical instrumentation and analytical imaging in the 1960s.

XRF analysis. This instrument appeared in the late 1950s as a commercial instrument and was gradually refined in the 1960s. The instrumentation served originally for the analysis of the grain structure and phase recognition of geological materials and compositional differences in metal production.

It is only in the early 1970s that the interest shifted to the direct study of the heterogeneity of materials with the appearance of localized and imaging analytical techniques (Fig. 1, bottom) it was a paradigm shift that was brought about as much by the appearance of microelectronics and computing possibilities as enabling technologies for analytical instrumentation [4].

The ion microprobe is similar to the electron microprobe and was first introduced by Liebl and Herzog in the early 1960s [5]. The lateral resolution is dependent on the primary ion beam that is raster scanned over the sample. Around the same time Castaign and Slodzian built the first sophisticated ion microscopes [6]. The development of the technique as an imaging tool resulted in the development of a range of instruments based on ion microscopy instead of ion probe measurements. In these instruments (Cameca 3F to 5F, NanoSIMS50), ion optics are used to preserve the spatial distribution of the secondary ions sputtered from the surface of a sample. Resolution is then entirely dependent on the quality of the secondary ion optics and not on the impinging beam. The basic advantage compared to the ion probe techniques is that all ions of the analyzed area are detected simultaneously. Secondary ion mass spectrometry also differentiated between dynamic instruments that continuously sputter away surface layers and static secondary ion microscopes that remain virtually on the uppermost surface layer [7].

The laser was applied in analytical chemistry as early as the 1960s. Lasers offer a highly versatile energy source for atomization, excitation, and ionization. Any samples with known light absorptivity can be analyzed by laser ablation and ionization to provide elemental information. Important factors in laser sampling are the crater depth and its size. The widely accepted technique of laser sampling has many advantages, e.g. there is little or even no sample size requirement and sample preparation, low risk of reagent or solution waste, the introduction of contamination is avoided, and high spatial (lateral and in-depth) resolution. The power density deposited on the spot area can easily be controlled by adjustment of laser parameters (mainly pulse duration, wavelength and energy) in comparison with other ionization sources. Significant benefits and capabilities are brought into practical chemical analysis with laser sampling. In combination with a proper detection system, it is an attractive technique for the elemental and isotopic analysis of samples through various spectroscopic methods [8].

Over the entire twentieth century there was a steady development of mass spectrometric techniques which differentiated into a large number of separate approaches according to the tools used for ionization and the techniques for ion separation and handling [9]. Mass spectrometry thus evolved from a crude tool for isotopic measurements and separation in the early 20th century to a powerful methodology for sensitive inorganic, organic and structural biological analysis. As an imaging instrument for inorganic analysis it contributed to ion bombardment (SIMS, 1960s), glow discharge mass spectrometry (1980s) and laser ablation ICPMS (1990s).

SIMS ion imaging was adapted from a wide beam technique for macroscopic analysis (with *ca* 300 μm beam size) in the 1960s to a confined beam technique with 10 μm lateral resolution in the early 1970s, a lateral resolution of 1–2 μm in the 1980s, <0.1 μm in the 1990s, 50 nm in 2003 and now an imaging resolution of *ca* 20 nm is possible. SIMS has experienced increased application for high spatial resolution imaging with the development of cluster and polyatomic primary ion sources (Au and Bi clusters, C_{60} and, more recently, massive argon clusters). Such projectiles provide simultaneously improved secondary ion yield and decreased fragmentation of surface species.

The unique properties of synchrotron radiation (SR) generated by third generation sources coupled with state-of-the-art X-ray optical elements/detectors, offer new possibilities in various X-ray microscopy and microprobe methods both in terms of achievable spatial resolution and sensitivity. In addition to characteristics of SR light, such as high intensity, high degree of polarization and energy tunability, a valuable feature is the capability to provide highly spatially/temporally coherent X-ray illumination. Due to their high penetrating power in materials, X-rays also allow bulk regions of thick samples to be studied in their natural environment (air, water etc.). They also offer possibilities for direct 3-D imaging through tomographic or confocal techniques. Finally, when we exclude possible radiation damage, measurements are also non-destructive and samples remain available for other purposes.

2.2. Imaging and analysis

The featured actors in the development of microscopic and nanoscopic imaging as described above, originated with developments in analytical chemistry on one side and with the much earlier appearance of conventional imaging tools (optical and electron microscope) on the other side. Their combination and interaction for the characterization of materials over the last 50 years led to possibilities that are now culminating in the development of sensitive high-resolution analytical imaging tools with unprecedented spatial resolution down to the level of a few nm.

With the appearance of spatially discriminating methods of analysis, observation and analysis were effectively combined as illustrated in Fig. 2. The Fig. presents a general overview of development aspects that led to imaging analytical chemistry as it exists today. The acquisition of a visual (optical image) is only the first step toward comprehensive data analysis and is shown on the lower level of the Fig. At present, microscopic techniques accomplish this first observational step in conjunction with electronic detectors, image processors, and various display devices as integrated extensions of a powerful imaging system.

An alternative approach to the optical and electron microscopic visualization methods are techniques based on confined scanning probe techniques including STM, AFM, scanning near-field optical microscopy (SNOM), confocal laser scanning microscopy (CLSM), fluorescence and nano-particle labeling. These techniques followed the introduction of the STM in the early 1980s by Binnig and Rohrer, a technology that was the first to enable the imaging of surface features down to the atomic scale [10–12]. They are valuable tools for understanding the complex architecture of nanoscale structures and are important in studying microscopic and sub-microsocopic objects below the limit imposed by the wavelength of light. Imaging

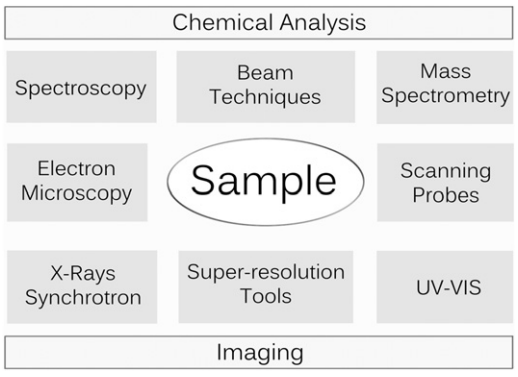

Fig. 2. Imaging analysis: the relation between chemical analysis and imaging.

STM and AFM have now become standard techniques in materials science laboratories. The power of STM and related techniques go way beyond imaging, they have unique support capabilities for manipulation of materials at the atomic scale and for local spectroscopy such as the combination of the AFM with near-field light microscopy and Raman spectroscopy. It is also important that it became possible to image beyond the limitations resulting from diffraction in super-resolution techniques (see further).

2.3. Beam analytical techniques

When we need information on local variations of the composition of a material a general experimental approach is based on scattering: we shoot radiation or particles in a well-prepared state on the target and detect radiation or particles coming out of the target as a result of various forms of interactions. In the case of surface analysis the most basic questions to be answered with this approach are sometimes quite simple such as, e.g., whether the surface is clean or which elements are on the surface. More complex are questions such as which chemical compounds are present, what is the exact geometric structure of the surface or what is the surface coverage and the homogeneity of its distribution. Under more general conditions where lateral dimensions are also concerned (for 3-D analysis) questions are related to the elemental or molecular composition, the heterogeneities in composition and whether the heterogeneity is random or structured. Most techniques achieve these goals by making use of intense particle or X-ray beams and provide essential information down to the atomic scale with a sensitivity and specificity that is impossible to reach by conventional techniques.

Fig. 3 gives a summary of some beam techniques that are now available with high sensitivity and imaging resolution. Beam methods of analysis can be classified according to the excitation mode or according to the relaxation mechanism. Particles that are used for excitation of a sample surface are electrons, ions, atoms and photons. The same particles can also be used in the de-excitation process for getting information about the composition of the sample. A most important issue is the sensitivity of a technique. Another factor of importance is the localized analytical potential. The present analytical characteristics of a few of the many possibilities for localized analysis are summarized in Table 1, listing lateral and depth resolution, the major features of interest, and their capability at present as updated from Adams et al. [13].

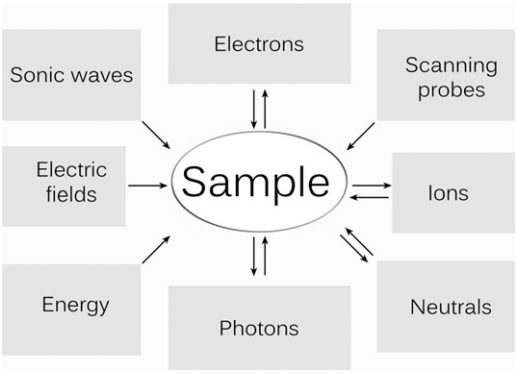

Fig. 3. Input energy channels exciting a sample and output phenomena that can be measured in spectrometric techniques.

Some of the intense particle and X-ray beams and nuclear methods are only available through the use of large and complex infrastructures in specialized research centers or using complex laboratory equipment. Special emphasis can be given to synchrotron radiation because it provides exciting new possibilities for imaging and elemental and structural analysis.

2.4. Imaging resolution

In microscopy, resolution is not an unambiguously defined quantity; it depends on operational parameters and on definitions. In imaging analysis it is defined in two quite distinct ways:

(i) For the definition of the size of a two- or three-dimensional element (a 2-D surface element or pixel or a 3-D volume element or voxel) for imaging of the composition and/or structure in larger heterogeneous objects (image resolution), and
(ii) for making the ultimate distinction between two separate objects or the position in space of one particular object e.g., an atom in an aggregate of other atoms or, as in astronomy, for determining the position of a distant star (positional or point resolution).

Overall, the issue is that the center of the image can be determined with much higher precision than its width.

2.5. Lateral image resolution

The lateral imaging resolution as defined for single point measurements (spot analysis) and through systematically repeating measurements over a sample surface, 1-D and 2-D imaging, is the most relevant quality parameter of maps and line scans. In such 1-D and 2-D imaging the geometric quality factor for imaging is defined by the pixel dimensions of the image. In beam analysis, the resolution cannot be better than the achievable beam size used for imaging but, in addition can be degraded by the interaction characteristics of the incoming and the outgoing radiation and by diffraction related effects as we will see in the next section.

Well defined procedures are necessary for the determination of lateral image resolution. In image analysis, spatial resolution refers to the smallest diameter (lateral dimension within a thin specimen) from which spatial information can be obtained.

Table 1
Present spatial resolution characteristics of selected techniques (adapted from reference [13]).

Technique	Lateral resolution (nm)	Depth resolution (nm)	Analyte	Sample characteristics
SIMS, dynamic and static	50–200	1–10	inorganic/organic/ speciation/isotopic (limited)	quantitative information limited
AES	20	*ca* 1	inorganic/speciation	conductive samples
TEM	$<$1.50–100 pm	$<$1 position determination	inorganic/organic (limited)	specimen preparation; beam damage
Synchrotron X-rays	100–500	none, (tomography *ca* 100)	inorganic (XRF) structural (XRD) speciation (XAS)	damage limited, can be used in air
Three-dimensional atom probe 3-DAP	atomic	atomic	elemental	conductors, semiconductors UHV, cryogenic temperature

The equivalent quantity is the minimum useful pixel size, below which there is no substantial gain in information content when a heterogeneous object is visualized and characterized with a beam analysis technique (photons, electrons and particles).

The resolution can be measured by the visualization of a discrete object such as a thin wire of a diameter considerably smaller than the beam size. When the beam is scanned over such a sample a Gaussian-shaped response is observed whose width is dependent on the beam size and on various other factors depending on the imaging tool and the interaction processes between the beam and the sample. The resolution in this direction is then usually defined as the width of the response function between 14 and 86% of the curve, which corresponds to two standard deviations from the mean of probabilities (2 s statistical criterion). More commonly the resolution is obtained with a discrete sharp object defined as a knife-edge providing the same information from the sigmoid shaped curve representing the cumulative distribution of the probability distribution. The other dimension is then defined in the same way and the 2-D pixel element represents 95% of the total response.

Although this is apparently a straightforward measurement there are numerous possibilities for errors, amongst which are statistical effects, specimen erosion, distortion due to implantation and other kinds of specimen damage. It is important to take these errors into account to avoid a misinterpretation of the result of the measurement and an incorrect estimate of the beam size. Note that the Fig. of merit of resolution depends on the standard deviation of the probability distribution.

2.6. Depth resolution

Similar procedures based on the analysis of objects with well-known layer thicknesses obtained e.g. by ion implantation or sputtering, are used for the depth resolution when depth profiling tools are employed. With the decreasing thickness of extremely thin layers, it became necessary to do film characterization with a depth resolution of a few inter-atomic distances. For a single interface between two compositionally different layers A and B, the most common definition of the depth resolution as recommended by IUPAC and ASTM E-42 is Δz, which corresponds to the interval of the depth coordinate z at an interface over which there is (again) a 84% to 16% change in intensity [14].

The depth resolution function obtained by sputtering is often asymmetric and governed by three fundamental parameters: atomic mixing length, roughness and information depth. The application of the mixing-roughness-information depth (MRI) model to reconstruct in-depth distributions with a depth resolution of the order of a few monolayers has been demonstrated for SIMS and AES depth profiles. The use of low-energy molecular ions has pushed the mixing length down to 0.4–0.6 nm The ultimate depth resolution is predicted to be in the range 0.7–1.0 nm and values of 1.4–1.6 nm have been reported [15,16].

Depth resolution on the basis of sputtering is now able to reach 0.5–1 nm, an improvement by a factor of 10 since 1975 [17]. XPS and AES provide quantitative chemical characterization of nano-scale thin films with increased depth information by angle resolved XPS or sputter techniques. State of the art spectrometers perform elemental mappings of surface structures with a lateral resolution of less than 3 μm [18]. AES became recently a standard technique in semiconductor manufacturing for defect analysis of sub-micron particles; a modern field emitter AES reach a lateral resolution of 10 nm.

In the past, instrumental analytical chemistry kept pace concerning its lateral and depth resolution with the development of e.g. semiconductor devices whose lateral resolution increased from ca. 300 μm in the 1970s to less than 32 nm—in line with the microelectronics roadmap—which was predicted in 1990 to reach the 35 nm features and 10^8 transistors per cm^2 in 2012 [19]. In a recent paper we went into detail on this evolution and compared the performance with that of semiconductor miniaturization [18].

2.7. Positional or two-point resolution

Optical microscopy is used with a multitude of optical contrast methods in biology and materials science but suffers from the fundamental limitation to the resolution that can be obtained, i.e. the diameter of the smallest object that can be resolved. This limitation applies to any tool used for analysis, using electromagnetic radiation or particles such as electrons or ions. Objects whose diameter is less than a particular wavelength defy observation.

In 1873, Ernst Abbe first postulated that diffraction fundamentally limited the resolution that the microscope could achieve to *ca* half the wavelength of the light used. In a more rigorous treatment than that made by Abbe, Lord Rayleigh defined the minimal resolvable as the distance between two equal brightness point sources imaged by a diffraction-limited imaging system [20]. The Abbe–Rayleigh criterion is the basic motivation in employing ever-more-energetic (lower wavelength) radiation or particles with which to observe samples.

Two-point resolution is a criterion for the resolving capability of an imaging system and is defined as the system's ability to resolve two point sources of equal brightness e.g. two stars in distant space or two adjacent atoms that emit fluorescence radiation. Due to the finite size of the system's optical components, such point sources are imaged as a diffraction pattern of the system's effective aperture instead of as a point. This diffraction pattern then represents the

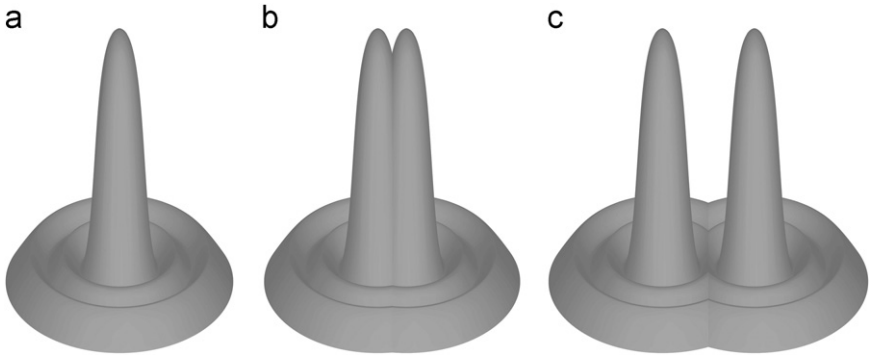

Fig. 4. Diffraction patterns and the limit of optical resolution for 3 different spacings between two point objects (e.g. two Au atoms). In the right image the two objects become resolved and are seen as two discrete objects

system's "point spread function" (PSF) or the "Airy disk" (for George Biddell Airy who was the first to write an explanation for the phenomenon of the series of concentric bright rings around a point object such as a star in 1835) [21]. The principle is schematically illustrated and explained in Fig. 4. In practice, the point resolution is often measured with two Au atoms located at different distances from each other to define the distance where they can be recognized as two distinct objects.

3. Spatially confined spectroscopy

Despite many advances, microscopes did not challenge the Abbe–Rayleigh limitation since its description in the late 19th century for more than a century. The diffraction barrier triggered the invention of X-ray microscopy (with energy 1–50 keV and wavelength 1–50 nm) and electron microscopy with electron wavelengths in the TEM ranging from 0.4 to 4 pm. For instance, when an electron is accelerated in a 100 keV microscope, its wavelength is ca. 4 pm, a distance far shorter than that separating the atoms in a solid or molecule. In addition, the electron microscope is able to provide structural information, whereas light microscopes, with the best resolution provide only the coordinates of their position [22].

Several approaches demonstrate how super-resolution microscopy (localization microscopy) can be optimized to obtain a spatial resolution considerably better than the limit imposed by the wavelength of light, down to a few nm for visible light. Such approaches towards super-resolution have been first introduced by Edward Synge who published the first paper challenging the diffraction limit in 1928 [23]. It took until 1984 before the proposition was realized with the technique of scanning near-field optical microscopy (SNOM), developed independently by Pohl et al. [24] (they called it the optical stethoscope) and Lewis et al. [25]. The technique uses a near-field optical probe, having a sub-wavelength-sized opening, within a distance comparable to the aperture's radius, r, to a sample. Subsequent progress in near-field microscopy has led to the development of "nano-optics", concerned with the manipulation of light in sub-wavelength dimensions and nano-local spectroscopy [26]. It has become a common tool for sub-100 nm spatial resolution optical imaging [25].

At present this multidisciplinary field has provided the development of exciting new nano-optical probes and the real promise of optical microscopy with true nanometer resolution [27], as we will see further in this article.

3.1. The electromagnetic spectrum for imaging

At present practically the entire electromagnetic spectrum (Fig. 5) can be used for imaging purposes. The region between infrared and high energy X-rays is prominent in a number of imaging applications, such as those of probe analytical techniques that give rise to a variety of de-excitation processes involving electrons, ions, neutrals or photons. Sub millimeter terahertz radiation i.e. the frequency range between the microwave and the infrared remained undeveloped until recently because suitable radiation sources and detectors were lacking [28]. The production and the detection of coherent terahertz radiation was technically challenging until the late 1990s but this situation is now changing rapidly with application areas appearing thanks to the recent developments in materials issuing from nanotechnology. This starts now to open up new opportunities for imaging, if not directly for analysis, then surely as a supporting tool for the observation of materials.

Multispectral imaging and hyper-spectral imaging deal with obtaining multiple images covering a spectral area. Up to now such techniques are used mostly in spectral reflectance for remote imaging in earth and planetary sciences and applications, but increasingly also for the study of art works such as paintings. Developments in sensors and radiation sources are expected to increase the range of applications in analytical imaging techniques.

3.2. X-ray coherent imaging

For X-ray imaging several modes of interaction of the radiation with matter can be used: X-ray absorption, elastic and inelastic scattering. The conventional approach for X-ray imaging was based on the use of X-ray absorption as the source of contrast, ignoring another source of contrast: phase information. It is only over the last decade that solutions to the use of coherent radiation for imaging started appearing. They were identified as a "phase odyssey" by Nugent et al. [29].

The basis of phase contrast coherent imaging is schematically represented in Figs. 6 and 7. X-rays passing through regions of differing composition pick up different relative phases, which corresponds to being refracted and produce a distorted wave front resulting in a phase contrast that can be observed.

The behavior of X-rays as they travel through a sample is described using a complex refraction index. For X-rays the refraction index deviates only slightly from unity and contains two main terms, an absorption (extinction) term and a phase-shift term incorporating refractive effects (the phase retardation index, elastic and inelastic scattering). At X-ray energies of 15–25 keV, the

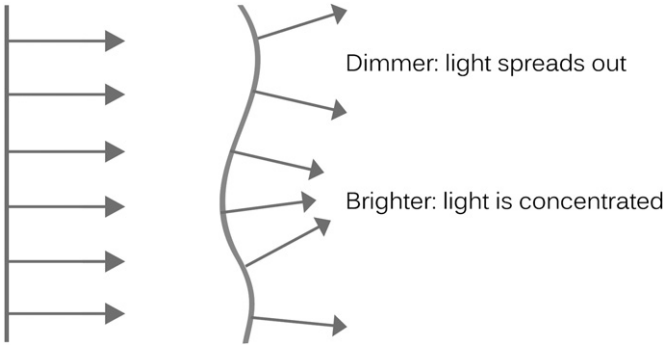

Fig. 6. When an originally flat coherent wave field passes through a material object the wavefront and the phase is changed and the phase gradient can be visualized or measured by the propagation of the intensity. As light travels in the direction perpendicular to the wavefront the light becomes concentrated (brighter) or dimmer.

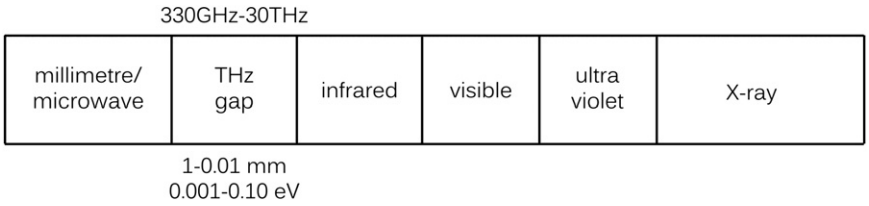

Fig. 5. The electromagnetic spectrum from the X-rays (right) to millimeter (microwave) radiation.

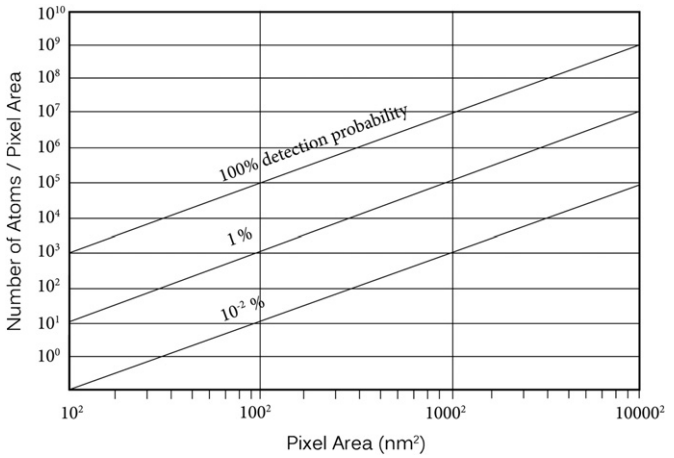

Fig. 7. Number of atoms per pixel versus the pixel area for imaging (adapted from H.F. Arlinghaus, personal communication).

phase-shift term is up to 1,000 times greater than the absorption term, of the order of 10^{-7}, compared to 10^{-10}. This makes it possible to observe phase contrast when absorption contrast remains undetectable. Phase differences are detected by the various phase-contrast techniques. Research has been going on for almost two decades to explore ways of exploiting phase information as a source of image contrast. The approaches used fall into three broad categories: interferometry, diffractometry, and in-line holography.

The evolution of SR sources has resulted in a dramatic increase of brilliance and a high flux of sufficiently coherent X-rays that can be used for coherent imaging [30], and triggered the development of a number of techniques based on coherence properties of light such as scattering of coherent X-ray radiation, X-ray photon correlation spectroscopy and phase contrast imaging.

3.3. Sensitivity

Present research explores the extreme limits of analytical chemistry: the characterization of attomole (10^{-15}) to zeptomole (10^{-18}) quantities of atoms or molecules [31]. Presently, the aim is to detect such amounts of analyte within nanometer size surfaces or volumes, while the ultimate goal is visualization and chemical imaging at the level of individual atoms and molecules. Fig. 7 illustrates the sharply increasing need for sensitivity with decreasing pixel size. Chemical imaging and the spatial and temporal characterization of the molecular composition, structure, with dynamic visualization of chemical events in space and time is essential to the future development of many fields of science [32].

Fig. 6 gives an indication on the number of atoms that are involved when addressing increasingly smaller size samples or portions of samples in heterogeneous objects, here indicated by the 2-D pixel that is addressed for analysis and imaging on the basis of given lateral pixel size. It is clear that – as a matter of detection probability – increasingly higher performance systems will be necessary to deal with the signals generated in the beam interaction as the image resolution increases.

3.4. Scanning near-field optical microscopy

Imaging techniques with a spatial resolution down to a fraction of a micrometer can be obtained using either by confocal microscopy or near-field optical techniques. Fluorescence imaging is the most frequently used provided that the sample fluoresces or can be labelled with a fluorescent dye. An attractive alternative is Raman (vibrational) spectroscopy.

SNOM was developed as the optical equivalent of other scanning probe microscopic techniques and is able to observe samples with a lateral resolution equal or better than 100 nm [32]. One of its features is to deliver a continuous or pulsed laser beam to a spot size of 30–100 nm. SNOM has been developed into a tool for highly localized laser-based analysis using Raman spectroscopy/imaging, fluorescence detection/imaging and laser ablation mass spectrometry. All these techniques allow in situ molecular and elemental analysis. SNOM-Raman usually relies on the use of surface-enhanced Raman scattering (SERS) and allows only the observation of surfaces with a special composition. The SERS enhancement varies across the sample (through electromagnetic field enhancement effects, so-called "hotspots") and depends critically on the substrate preparation, which limits its applicability for quantitative measurements. In SNOM-laser ablation mass spectrometry, the ablated material is ionized and then analyzed by a mass spectrometer [33]. SNOM tips are thus designed for high optical transmission and for sustaining pulsed laser radiation.

There are also possibilities to integrate scanning force microscopy with ion beams. The scanning probe produces images of surface structure. The scanning probe images surface structures non-invasively and aligns the ion beam to particular regions of interest [34]. In the same way AFM/STM tips are used to monitor the position of an X-ray beam of nanoscopic dimensions [35,36] and nanoscopic features too small to be resolved by optical microscopy. An AFM tip can also be used to position nanostructures in the X-ray beam focus and to manipulate the nanostructures.

Tip-enhanced Raman spectroscopy [37] (TERS) is a combination of Raman, SERS and AFM. A fine metal-coated AFM tip is used as an antenna to give strong enhancement of Raman scattered light from the sample. Since the AFM tip is on the nanometer scale, it is possible to obtain localised enhancement on the same scale. This allows molecular analysis with excellent spatial resolution, which is only limited by the tip's size and shape, thus allowing reliable surface-enhanced Raman spectroscopy measurements. While scanning the tip over the sample surface, topographic information is obtained simultaneously and can be directly correlated with the spectroscopic data. With TERS 128×128 pixel, 50 nm full spectral resolution images of binary lipid mixtures could be obtained [38]. The same technique provided the identity of defects and contaminants due to differences in spectroscopic signatures and chemical imaging with a resolution of < 12 nm [39].

3.5. Electron microscopy

Richard Feynman foresaw the potential of manipulating matter at the atomic scale in 1959 in his now often quoted visionary lecture "There's plenty of room at the bottom" [40]. He postulated there the need to improve the performance of instruments such as electron microscopes to observe individual atoms and described the exciting possibilities that would open up if scientists could learn how to control single atoms and molecules. Now, 50 years later these objectives have been achieved. At present aberration corrected electron microscopes achieve better than 50 pm resolution and magnification of up to about 10,000,000 times. This resolution is sufficient to "see" individual atoms i.e. to determine their 3-D position with high overall accuracy.

The information limit for high resolution TEM (HRTEM), when pushed to 50–100 pm (picometer) in aberration-corrected microscopes still remains roughly a factor 50 larger than the electron wavelength at 300 keV. Presently instruments are available with atomic resolution and a precision for the determination of atom positions of 0.05 nm, the possibility of measuring atom columns atom by atom and 3-D visualization and analysis by electron

tomography. The use of electron energy loss spectrometry (EELS) allows the identification of individual atoms in array positions and provides speciation information (e.g. graphite and diamond differentiating sp^2 or sp^3 bonds).

The potential of the electron microscope to resolve three-dimensional (3-D) structures on the atomic scale is continuing to be used in different fields, including materials science and biology. Major development is now occurring in transferring 3-D EM into a four dimensional technique introducing time as an extra dimension, thus opening up possibilities for dynamic measurements [41].

4. Imaging in biology

Because many biological processes take place on the length scale of a few nm, the ideal imaging resolution in bio-imaging is on this length scale. Fluorescence microscopy is a well-established imaging method, and one that has made many important contributions to the understanding of biological processes, aided by the availability of different kinds of fluorescent tags. Fluorescence microscopy is well suited for imaging of complex objects in vivo, three-dimensionally, and in their native environment.

4.1. Localization of molecules

Near-field techniques are used for nano-local studies of scattering, fluorescence, Raman scattering and nonlinear optical processes that provide information on the properties of single molecules and functional materials down to the level of 10 nm. When used for 3-D visualization such as for studying biological phenomena, confocal fluorescence microscopy cannot be used to investigate below the size level of 200 nm due to the Abbe–Rayleigh criterion, but several techniques are used to break the diffraction limit and have been applied to live-cell imaging [42–44]. Two-photon excitation microscopy is used as an alternative to confocal imaging and provides distinct advantages for three-dimensional imaging. The advantages are reduced phototoxicity, increased imaging depth, and the ability to initiate localized photochemistry.

Single molecule spectroscopy and imaging (SMS) for measuring the signal of individual fluorescent labels in living cells has recently grown exponentially. The main reason for SMS is that it provides information on a single cell instead of the population average of many cells, thus exposing normally hidden heterogeneities [45]. We cannot go into detail on the topic here and we refer readers to a recent collection of articles as a "virtual" issue of Analytical Chemistry under the heading "On novel optical probes for advanced analytical imaging" which provides an overview of the methodologies that fall in the realm of imaging [46]. It includes, among other topics reviews on: surface enhanced Raman scattering for imaging using nanoparticles for signal enhancement [47], the development of quantum dots for their use as probes in optical imaging [48], and near-field laser ablation mass spectrometry [49].

Different types of localization microscopy provide images from locally positioned single molecules, with a spacing error much smaller than the optical resolution. Using photo-switchable fluorescent molecules it is identified as photo-activated localization microscopy (PALM) [50], or stochastic optical reconstruction microscopy (STORM) [51], but many other acronyms are used to describe similar or variant methodologies.

4.2. Molecular imaging, imaging mass spectrometry

With its growing interest in biological and medical research, mass spectrometric imaging is presently in full development as an instrumental technology. There is increasing emphasis on speed of analysis, robust data processing and normalization strategies [52], means for increasing spatial and mass spectrometric resolution [53], atmospheric pressure measurements [54] and the extension of the mass range to higher masses. We refer readers to recent reviews on mass spectrometric imaging [55,56]. Molecular imaging using mass spectrometry has expanded with the advent of new ionization techniques over the years and was driven by uses for the analysis at the single cell level for cells and tissues. The most commonly used methods are time-of-flight SIMS (TOF-SIMS) [57], MALDI [58] and desorption electrospray ionization (DESI) [59]. Chemical imaging of small sections in biological systems is possible with high sensitivity and spatial resolution. A wide range of analytes, including drugs and their metabolites, hormones, lipids, peptides and proteins can be imaged and quantified directly from tissue sections [60].

The spatial resolution of imaging mass spectrometry techniques in, e.g., SIMS and MALDI is typically limited by the inability to detect the low number of ions sputtered from the sample using the smallest diameter beam achieved with an instrument. In practice, resolution is further degraded by other factors affecting the incoming and outgoing signal. One example of further degradation of resolution in MALDI is related to molecular delocalization on the biological target surface when the matrix solution is deposited. A non-homogeneous crystallization, or changes in concentration of the matrix in the tissue section, can be responsible for loss of reproducibility of the spectra and a decrease in lateral resolution.

The NanoSIMS 50 instrument is able to detect and quantify ions under 300 Da with a very high spatial resolution and high mass resolution at high transmission; it is able to yield an isotopic image of the surface with a lateral resolution of around 50 nm [61]. With this instrument, the proportion of ions recovered (the useful yield) from the sample is close to or even greater than 3% of the CN ions produced under bombardment of C- and N-containing samples. Using a NanoSIMS 50, Cabin-Flaman et al. [62], were able to detect and image labeled DNA with isotopically modified nucleotides and then aligned on silicon surfaces using the NanoSIMS 50. The technique is called Combing–Imaging by SIMS (CIS). The isotopically labeled, single DNA fibers, uniformly aligned on this surface, were detected with a resolution of 50 nm (i.e., 150 base pairs).

Metals are present in a considerable fraction of the proteins (identified as the metallome) where they serve many important functions in biological processes. In conjunction with the analysis and study of biological samples the term of metallomics is now used to describe this field as a complement to genomics and proteomics [63].

Up to now hyphenated techniques such as liquid chromatography coupled to LA-ICP-MS has been used to detect metal containing compounds and then use electrospray tandem mass spectrometry and other organic mass spectral tools for their identification. Now attention is moving to direct imaging the spatial distribution with advanced mass spectrometric techniques such as static and dynamic SIMS and MALDI, with MS/MS e.g. time-of-flight/time-of-flight, TOF/TOF [64].

Another technique that is finding use for this type of work is synchrotron micro/nano-XRF imaging. It has a nearly full elemental chemical profiling ability for tissue samples. Both LA-ICP-MS and micro-XRF techniques were compared for the analysis of 200 nm thick dehydrated sections of biological material and were found to be comparable for this application [65]. Advantages of nano-XRF imaging reside with the extremely high spatial resolution (now down to 30 nm) that can be obtained and the possibilities for imaging speciation analysis through X-ray absorption measurements [66].

For molecular imaging, scanning near-field Raman optical microscopy also provides information on the sub-μm level.

5. Conclusions

Advances in image analysis are based on developments intended to make reliable tools that respond to scientific and technological needs. Their main concerns are based on advances in spatial resolution, detection sensitivity, reliability, accuracy and economy (speed).

The unabating progress in mass spectrometric methodology and the technical implementation of ionization, mass separation, and mass measurement has been reviewed by Yates [9]. All these advances contributed to the fast increasing imaging potential (spatial resolution, mass resolution, versatility etc.).

At present, practically the entire electromagnetic spectrum can be used for imaging purposes. The progress is largely based on developments in a variety of probe analytical techniques that have given rise to a variety of de-excitation processes involving electrons, ions, neutrals or photons that can be measured with a range of detectors or using increasingly more powerful mass spectrometric techniques. Through the development and refinement of a number of surface specific analytical techniques, highly sensitive and spatially limited analytical information for surface and thin films can also be obtained. Spatial resolution now extends far into the nano-domain.

One of the most rapidly expanding areas for analytical imaging is biology and biomedicine. Developments in contrast agents and biosensors have provided many new possibilities for bio-imaging. Nanoparticulate contrast systems such as quantum dots, gold nanoparticles, and dye doped silica nanoparticles provide increased photo stability, higher quantum yields and stability compared to the conventional contrast dyes. Modern biomedical imaging technologies have led to significant advances in diagnosis and therapy [46,67].

Continuing progress in third generation light sources has led to extremely brilliant X-ray beams in stable conditions that are able to provide nano-size tunable X-ray beams of a few tens of nm dimensions. X-ray imaging and tomography are becoming faster with the development of pixellated detectors [68].

The potential of SIMS for imaging has increased considerably through the commercial availability of ion sources for clusters (e.g. Ar, Au, Bi etc.) and polyatomic (e.g. C_{60}) ions. Applications of the technique now are expanding in genomics, proteomics and metabolomics.

Limitations in mass coverage and mass resolution are now overcome by the use of ultra-high resolving powers as made available through Fourier transform ion cyclotron resonance, demonstrating a mass resolving power in excess of 100,000 ($m/\Delta m$) [69]. Time-of-flight systems also improved resolution consistently over the last decade. Multiple reflection systems with $m/\Delta m$ in excess of 50,000 are now commercially available.

LA-ICP-MS is improving its spatial resolution through developments in laser ablation and by assisting the laser with several positioning tools [70]. New developments in mass spectrometric detection such as new multi-element simultaneous detection systems based on the Mattauch–Herzog magnetic analyzer with position sensitive detectors allow quicker imaging possibilities [71,72].

"As conceptual elements, the point, line, plane, and volume are not visible except to the mind's eye" is stated by Paul Klee in his notebook [3]. A specific pixel or voxel only represents spatial coordinates and serve to order information in an image; it derives its importance from the data it is associated with. The data content of a specific pixel or voxel may be quite complicated, e.g. sets of high resolution mass spectra combined with numerous fragmentation patterns, or complex X-ray spectra with, for each pixel, associated diffraction patterns and/or X-ray absorption information. Moreover, it is often necessary to combine data from images obtained with different imaging tools (for instance, MALDI, DESI and electron microscopy). Care is presently taken for optimising the interpretation of such extensive datasets generated in mass spectrometric imaging [52], X-ray microscopy and hyper-spectral imaging.

Acknowledgments

We gratefully acknowledge the precious contribution of Fabio Polo (University of Venice) for the editing of the figures and of our colleague Warren Cairns (IDPA-CNR), Renaat Gijbels (University of Antwerp) and Victor Norris (University of Rouen) for useful comments.

References

[1] Y. Song, Z. Zhang, H.E. Elsayed-Ali, H. Wang, L.L. Henry, Q. Wang, S. Zou, T. Zhang, Nanoscale 3 (2011) 31–44.
[2] Committee on Revealing Chemistry through Advanced Chemical Imaging National Research Council, Visualizing Chemistry: The Progress and Promise of Advanced Chemical Imaging, The National Academies Press, 2006.
[3] P. Klee, The Thinking Eye: The Notebooks of Paul Klee, , 1961, pp. 1–32 (English translation).
[4] F. Adams, Spectrochim. Acta B: At. Spectrosc. 63 (2008) 738–745.
[5] H.J. Liebl, R.F.K. Herzog, J. Appl. Phys. 34 (1963) 2893.
[6] R. Castaign, G. Slodzian, J. Microsc. 1 (1962) 395–410.
[7] A. Benninghoven, Surf. Interface Anal. 43 (2011) 2–11.
[8] Y. Lin, Q. Yu, W. Hang, B. Huang, Spectrochim. Acta B: At. Spectrosc. 65 (2010) 871–883.
[9] J.R. Yates III, Nat. Methods 8 (2011) 633–637.
[10] G. Binnig, C.F. Quate, C. Gerber, Phys. Rev. Lett. 56 (1986) 930–933.
[11] G. Binnig, H. Rohrer, Surf. Sci. 126 (1983) 236–244.
[12] L.L. Soethout, H.V. Kempen, G.F.A. Van De Walle, Electron. Electron. Phys. 79 (1990) 155–269.
[13] F. Adams, L. van Vaeck, L. Barrett, Spectrochim. Acta B: At. Spectrosc. 60 (2005) 13–26.
[14] A. Fick, in: ASTM International, 100 Barr Harbor Drive, PO Box C700, West Conshohocken, PA, 19428-2959 USA, 1996.
[15] R. Escobar Galindo, R. Gago, A. Lousa, J.M. Albella, TrAC-Trends Anal. Chem. 28 (2009) 494–505.
[16] S. Hofmann, Philos. Trans. R. Soc. London, Ser. A 362 (2004) 55–75.
[17] S. Hofmann, Surf. Interface Anal. 30 (2000) 228–236.
[18] R. Merz, NanoS-GUIDE 2007, Wiley-VCH Verlag GmbH, 2007.
[19] R. Compaño, L. Molenkamp, D.J. Paul, Technology Roadmap for Nanoelectronics, European Commission IST Programme, Future and Emerging Technologies, 1990, pp. 46–58 (Chapter 4).
[20] J.W.S. Rayleigh, Philos. Mag. XLII (1896) 167–195.
[21] G.B. Airy, Trans. Cambridge Philos. Soc. 5 (1835) 283–291.
[22] A.H. Zewail, Philos. Trans. R. Soc. A 368 (2010) 1191–1204.
[23] L. Novotny, The History of Near-field Optics, Elsevier, 2007.
[24] D.W. Pohl, W. Denk, M. Lanz, Appl. Phys. Lett. 44 (1984) 651–653.
[25] A. Lewis, M. Isaacson, A. Harootunian, A. Muray, Ultramicroscopy 13 (1984) 227–231.
[26] D. Richards, Philos. Trans. R. Soc. London, Ser. A 362 (2004) 699–700.
[27] V.T. Yadugiri, R. Malhotra, Curr. Sci. 99 (2010) 900–907.
[28] A.G. Davies, E.H. Lindfield, M. Pepper, Philos. Trans. R. Soc. London 362 (2004) 197.
[29] K.A. Nugent, D. Paganin, T.E. Gureyev, Phys. Today 54 (2001) 27–32.
[30] D.H. Bilderback, P. Elleaume, E. Weckert, J. Phys. B 38 (2005) S773–S797.
[31] F. Adams, Talanta 85 (2011) 1230–1232.
[32] R. Zenobi, Anal. Bioanal. Chem. 390 (2008) 215–221.
[33] J.S. Becker, A. Gorbunoff, M. Zoriy, A. Izmer, M. Kayser, J. Anal. At. Spectrom. 21 (2006) 19–25.
[34] A. Persaud, S.J. Park, J.A. Liddle, T. Schenkel, J. Bokor, I.W. Rangelow, Nano Lett. 5 (2005) 1087–1091.
[35] W. Ludwig, E.M. Lauridsen, S. Schmidt, H.F. Poulsen, J. Baruchel, J. Appl. Crystallogr. 40 (2007) 905–911.
[36] M.S. Rodrigues, O. Dhez, S. Le Denmat, J. Chevrier, R. Felici, F. Comin, J. Instrum. 3 (2008) P12004–12008.
[37] R.M. Stockle, Y.D. Suh, V. Deckert, R. Zenobi, Chem. Phys. Lett. 318 (2000) 131–136.
[38] L. Opilik, T. Bauer, T. Schmid, J. Stadler, R. Zenobi, Phys. Chem. Chem. Phys. 13 (2011) 9978–9981.
[39] J. Stadler, T. Schmid, R. Zenobi, ACS NANO 5 (2011) 8442–8448.

[40] R.P. Feynman, Eng. Sci. 23 (1960) 22–36.
[41] A.H. Zewail, Science 328 (2010) 187–193.
[42] L. Schermelleh, P.M. Carlton, S. Haase, L. Shao, L. Winoto, P. Kner, B. Burke, M.C. Cardoso, D.A. Agard, M.G.L. Gustafsson, H. Leonhardt, J.W. Sedat, Science 320 (2008) 1332–1336.
[43] W. Wang, G. Li, C. Chen, X.S. Xie, X. Zhuang, Science (2011) 1445–1449.
[44] K. Isobe, A. Suda, H. Hashimoto, F. Kannari, H. Kawano, H. Mizuno, A. Miyawaki, K. Midorikawa, Biomed. Opt. Express 1 (2010) 791–797.
[45] S.J. Lord, H.L.D. Lee, W.E. Moerner, Anal. Chem. 82 (2010) 2192–2203.
[46] D.A. Higgins, Anal. Chem. 83 (2011) 8048–8049.
[47] S. Lee, S. Kim, J. Choo, S.Y. Shin, Y.H. Lee, H.Y. Choi, S.H. Ha, K.H. Kang, C.H. Oh, Anal. Chem. 79 (2007) 916–922.
[48] J. Liu, S.K. Lau, V.A. Varma, B.A. Kairdolf, S. Nie, Anal. Chem. 82 (2010) 6237–6243.
[49] T.A. Schmitz, G. Gamez, P.D. Setz, L. Zhu, R. Zenobi, Anal. Chem. 80 (2008) 6537–6544.
[50] E. Betzig, G.H. Patterson, R. Sougrat, O.W. Lindwasser, S. Olenych, J.S. Bonifacino, M.W. Davidson, J. Lippincott-Schwartz, H.F. Hess, Science 313 (2006) 1642–1645.
[51] M.J. Rust, M. Bates, X.W. Zhuang, Nat. Methods 3 (2006) 793–795.
[52] J.M. Fonville, C. Carter, O. Cloarec, J.K. Nicholson, J.C. Lindon, J. Bunch, E. Holmes, Anal. Chem. 84 (2011) 1310–1319.
[53] F. Xian, C.L. Hendrickson, A.G. Marshall, Anal. Chem. 84 (2012) 708–719.
[54] Y. Li, B. Shrestha, A. Vertes, Anal. Chem. 79 (2007) 523–532.
[55] R. Lobinski, C. Moulin, R. Ortega, Biochimie 88 (2006) 1591–1604.
[56] L.A. McDonnell, R.M.A. Heeren, Mass Spectrom. Rev. 26 (2007) 606–643.
[57] T.L. Colliver, C.L. Brummel, M.L. Pacholski, F.D. Swanek, A.G. Ewing, N. Winograd, Anal. Chem. 69 (1997) 2225–2231.
[58] V.M. Doroshenko, V.V. Laiko, N.I. Taranenko, V.D. Berkout, H.S. Lee, Int. J. Mass Spectrom. 221 (2002) 39–58.
[59] Z. Takáts, J.M. Wiseman, B. Gologan, R.G. Cooks, Science 306 (2004) 471–473.
[60] L.S. Eberlin, X.H. Liu, C.R. Ferreira, S. Santagata, N.Y.R. Agar, R.G. Cooks, Anal. Chem. 83 (2011) 8366–8371.
[61] S.G. Boxer, M.L. Kraft, P.K. Weber, Annu. Rev. Biophys. 38 (2009) 53–74.
[62] A. Cabin-Flaman, A.F. Monnier, Y. Coffinier, J.N. Audinot, D. Gibouin, T. Wirtz, R. Boukherroub, H.N. Migeon, A. Bensimon, L. Janniere, C. Ripoll, V. Norris, Anal. Chem. 83 (2011) 6940–6947.
[63] S. Mounicou, J. Szpunar, R. Lobinski, Chem. Soc. Rev. 38 (2009) 1119–1138.
[64] B. Wu, J.S. Becker, Int. J. Mass Spectrom. 307 (2011) 112–122.
[65] D.S. Gholap, A. Izmer, B. De Samber, J.T. van Elteren, V.S. Selih, R. Evens, K. De Schamphelaere, C. Janssen, L. Balcaen, I. Lindemann, L. Vincze, F. Vanhaecke, Anal. Chim. Acta 664 (2010) 19–26.
[66] S. Matsuyama, M. Shimura, H. Mirnura, M. Fujii, H. Yumoto, Y. Sano, M. Yabashi, Y. Nishino, K. Tamasaku, T. Ishikawa, K. Yamauchi, X-Ray Spectrom. 38 (2009) 89–94.
[67] N. Erathodiyil, J.Y. Ying, Acc. Chem. Res. 44 (2011) 925–935.
[68] L. Strüdel, G. Lutz, P.L.P.L. Lechner, H. Soltau, P. Holl, X-Ray Detectors, in: K. Tsiji, J. Injuk, R. Van Grieken (Eds.), X-Ray Spectrometry: Recent Technological Advances, John Wiley and Sons, Chichester, UK, 2004, pp. 133–193.
[69] D.F. Smith, E.W. Robinson, A.V. Tolmachev, R.M.A. Heeren, L. Pasa-Tolic, Anal. Chem. 83 (2011) 9552–9556.
[70] J.S. Becker, Spectrochim. Acta B: At. Spectrosc. 57 (2002) 1805–1820.
[71] E.F. Cromwell, P. Arrowsmith, J. Am. Soc. Mass Spectrom. 7 (1996) 458–466.
[72] M. Resano, K.S. McIntosh, F. Vanhaecke, J. Anal. At. Spectrom. 27 (2012) 165–173.