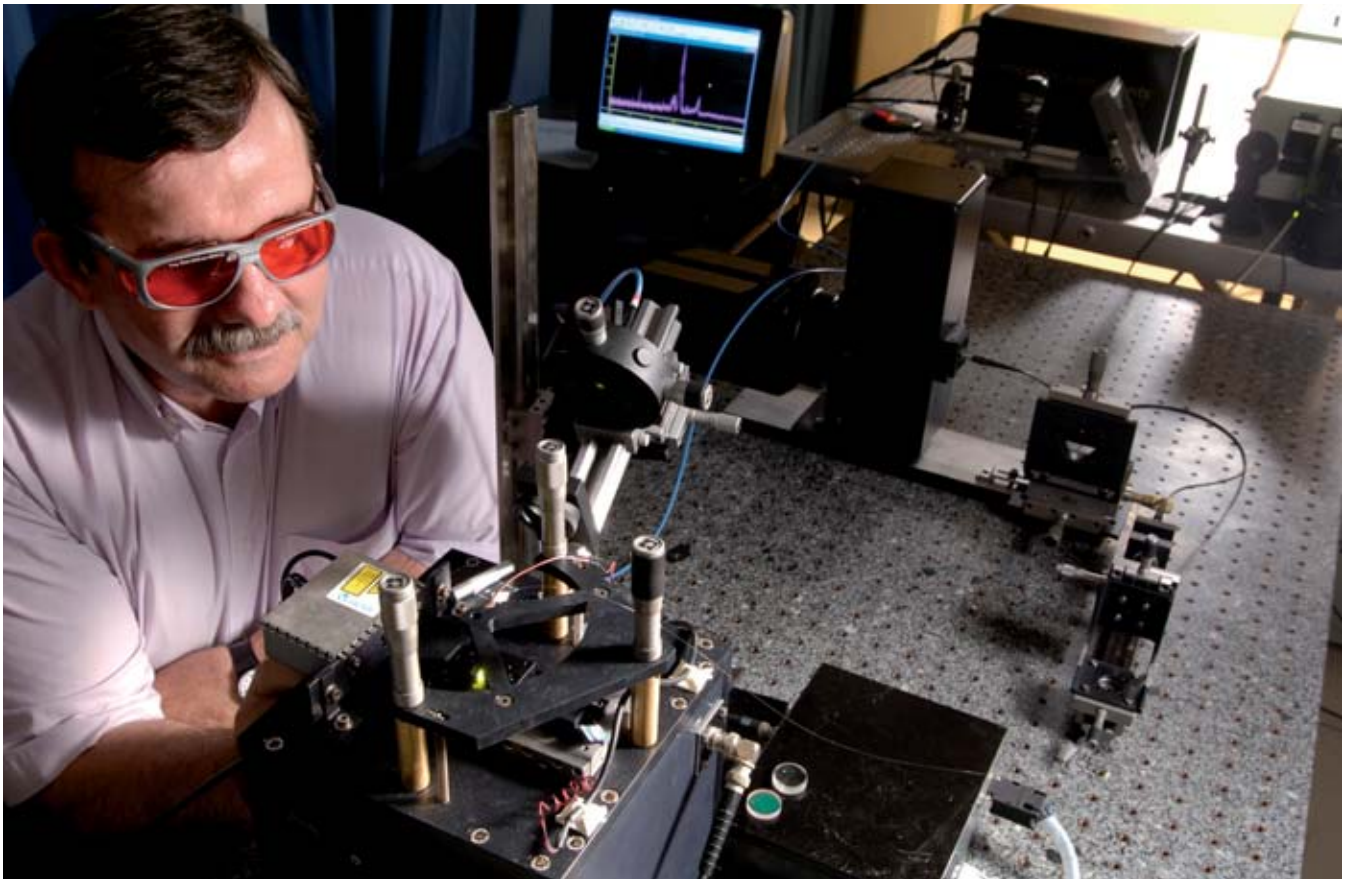
Nanoscale vision

The requirement to secure reliable predictive models, the advances achieved in modeling at the atomic and molecular scale, the need to gain a better understanding of interactions at the most detailed level entail a quest for analytical tools, in physical chemistry, at the nanometer scale. Among possible techniques for surface analysis, scanning near-field optical microscopy (SNOM), pushing back the limits of resolution as it does, stands out as being highly promising.



Philippe Stroppa/CEA

Overall view, showing the SNOM system developed at CEA. As an instance of the third branch in the near-field microscopy family, this microscope has the capability of yielding information at the nanometer scale.

In the mid-1980s, technological advances enabled Dieter Pohl, then a research worker at IBM Zurich Research Laboratory, to suggest the first SNOM (scanning near-field optical microscopy) configuration. This instrument is based on the detection (or emission), by a local probe, of evanescent optical waves, which, since they do not propagate, are not subject to the theoretical limit on **resolution** entailed by **diffraction** (see Box 1). That limit, set in this case by probe size – usually a metallized optical fiber, tapered to a tip – is then brought down to 10 **nanometers** or so. This is a technological limit, rather than a theoretical one, as is the case in so-called *far-field* optics.(1)

Near-field optical microscopy(1) has developed less swiftly than other kinds of local-probe microscopy,(2) such as electron microscopy, or atomic-force microscopy,(3) this being due, in particular, to tip fragility,

(1) Far-field and near-field optical microscopy: the *far-field optical microscope*, widely used in schools, for biology or geology instruction, in industry, and for research work, does not allow highly precise investigation of matter. Indeed, when the object to be investigated is illuminated, the use of a probe (the microscope objective) of a size larger than the wavelength of the light being used, allows to detect only details of a size larger than about half that light's wavelength. The resolving power of such a microscope is thus relatively low. On the other hand, for a *near-field optical microscope*, the probe, having a diameter much smaller than the wavelength of the light being used, yields information on details of the object that are much smaller than the wavelength of the light illuminating it.

(2) On this topic, see *Clefs CEA* No. 52 (Summer 2005), pp. 84–95.

(3) Atomic-force microscopy: this is based on measuring the force, or the force gradient that arises as the probe is moved along the surface of the object being investigated; this technique allows surfaces of all kinds of solids to be surveyed.

Propagating waves and evanescent waves

1

The experiment showing frustrated total reflection, as performed by Isaac Newton, provided evidence of the existence of evanescent waves. When a light beam propagates inside a prism of index n_1, and reaches a medium of index n_2 lower than n_1, at an incidence greater than critical incidence θ_c – defined by $n_1 \sin \theta_c = n_2$ – *total reflection* of the beam occurs at the interface of the prism and medium of index n_2. If the convex side of a lens is brought close to the prism face, as the distance between lens and prism gets smaller than the wavelength, part of the light is transmitted through the lens, into the medium of index n_2: *total reflection is frustrated*.

This process, well described by the wave theory of light, is due to the existence of an evanescent wave in medium n_2, where no propagating wave is found. The wave vector for this evanescent wave is a complex vector, having a wholly imaginary component k_z (the z-axis being perpendicular to the interface), this resulting in an exponential decrease in wave amplitude, along Oz. More generally, such properties, which are not encountered in classical far-field optics, are found in the **diffraction** spectrum of an object of subwavelength size l, illuminated by a wave. This spectrum includes, in the plane perpendicular to Oz, all spatial frequencies k_x, in the range from 0 to $1/l$. Thus, the smaller the object, the greater the amount of high spatial frequencies included in its spectrum, these remaining *confined* to the near vicinity of the surface, having no ability to propagate. To be detected, they must thus be sought where they occur, in other words by bringing in the detector as close as possible to the object. A probe of typical dimension $l << \lambda$, brought into the object's "near field," "frustrates" the evanescent wave, transforming it into a propagating wave, which may be detected in the far field.

The electric field E, propagating in direction z, is commonly expressed in the form:

$$E(z) = E_0 \exp[j(k_z - \omega t)],$$

where ω stands for the angular velocity of the electric field, and t for time.

The relation between ω and the wave vector is defined by:

$$\frac{\omega^2}{c^2} = k_x^2 + k_y^2 + k_z^2,$$

where c stands for the velocity of light in vacuum.

For the case where k_x and k_y are small, compared to the wavelength ($k < \omega/c = 2\pi/\lambda$), i.e. for low spatial frequencies and large details on the surface,

$$k_z = \sqrt{\frac{\omega^2}{c^2} - k_x^2 - k_y^2}.$$

The solution in z for the equation is an imaginary exponential, characteristic of a propagating wave.

In the contrary case, where k_x and k_y are large, compared to the wavelength ($k > \omega/c = 2\pi/\lambda$), i.e. for high spatial frequencies and small surface details,

$$k_z = i\sqrt{k_x^2 + k_y^2 - \frac{\omega^2}{c^2}}.$$

The solution in z for the equation is a damped exponential, characteristic of an evanescent wave.

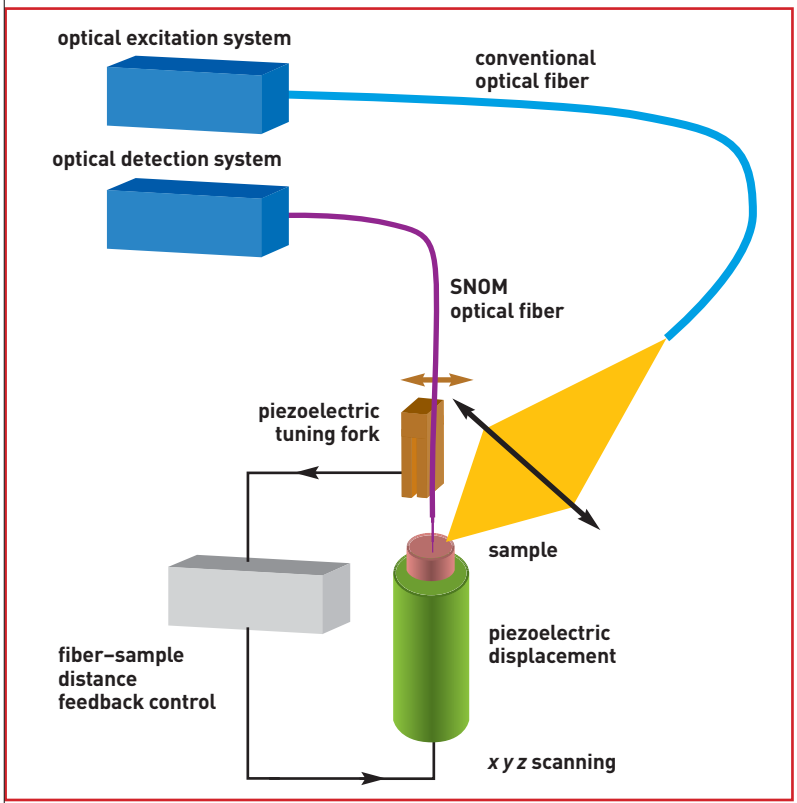


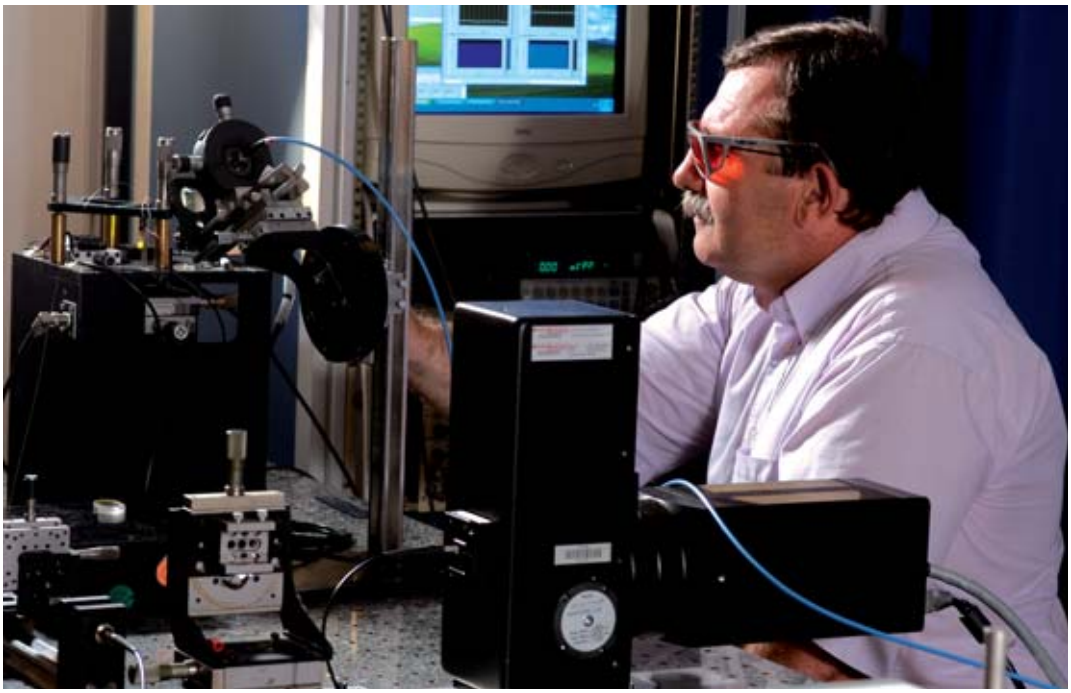
Figure 1.
Operating principle of a near-field microscope. A laser excitation source is directed to the near-field microscope by means of a conventional optical fiber. The beam is focused onto the sample. On the sample surface, an evanescent wave is generated (through scattering, diffraction, fluorescence...). The SNOM optical fiber probes, or "frustrates," this wave, transforming it into a propagating wave, which may be analyzed in the far field by a detection system, which may effect spectral or temporal discrimination. Fiber–sample distance must be sufficiently small to frustrate the evanescent wave. A feedback control system detects the fiber's vibration amplitude, and acts on sample height.

low signal level, and spectral response complexity. It is nonetheless currently undergoing heady growth, and devices are now being put on the market (after initial commercialization in 1994), most commonly derived from confocal[4] or atomic-force microscopes, offering a number of configurations, coupled with various analytical techniques (**fluorescence spectroscopy**, Raman effect[5]...). Many analysis or instrumentation laboratories, however, are opting to develop their own equipment, from atomic-force microscopes, or from basic components. This is the solution chosen by CEA, in order to have the ability, on the basis of one modular device, to explore all of this technology's potentials, in a variety of **spectroscopic** configurations, as appropriate for the situations considered.

The near-field optical microscope, a leading-edge instrument

The main constituent elements of the microscope are, on the one hand, the two optical excitation and detection systems, and, on the other, the near-field unit, comprising the probe positioning and feedback control system, and sample scanning system (see Figure 1).

Various principles have been implemented, for SNOM optical fiber positioning and feedback control, relative to the sample surface. The one that currently seems to offer best performance, and is most widely used, consists in making the fiber vibrate parallel to the sample surface, by exciting it at a resonance frequency, and measuring oscillation amplitude by means of a tuning fork, or a **laser diode** coupled to a two-quadrant **photodiode**. As the fiber comes closer to the surface, oscillation amplitude decreases, owing to shear forces, the



Philippe Stroppa/CEA

With its flexible, modular construction, the CEA-developed system allows all of the SNOM technique's potentials to be explored, in a variety of useful configurations. The spectrometer may be seen in the foreground, with the near-field unit at the rear.

strength of which varies steeply with distance from the surface. This information is used as an error signal, to control tip–sample distance, by means of a **piezoelectric** actuator, which keeps sample height to a fixed value, of the order of a few nanometers. The end of the tip thus remains in close proximity to the surface, in the optical near field, this being the region of evanescent optical wave confinement. If the sample is scanned horizontally, parallel to its surface, the error signal can be used to establish a topographic map. The scanning and positioning systems are made of piezoelectric ceramics,(6) with associated electronics to correct drift. It is indispensable to specify a dual (**micrometric** and nanometric) displacement system, to provide both the required displacement amplitude, and adequate precision. This unit allows topographic maps to be obtained, with a lateral resolution related to probe tip size (see Figure 2).

Adding optical excitation and detection systems allows local spectroscopic information to be accessed. The excitation system, as a rule, is a **laser**, the characteristics of which, whether spectral (fixed or tunable wavelength) or temporal (continuous or pulsed, pulse duration), depend on the sample, and the information being sought, on the surface subject to investigation. The detection system is likewise dependent on these self-same elements. One may, e.g. record the entire **fluorescence** signal, with or without temporal discrimination. The detectors exhibiting greatest sensitivity are photomultipliers or **avalanche photodiodes**, cooled as a rule, to suppress unwanted signals. Another option is to select just one wavelength, or even to record the entire spectrum emitted at the locus investigated. In this case, a spectrometer must be used, in conjunction with an intensified **CCD** (charge-coupled device) camera, pulsed if required.

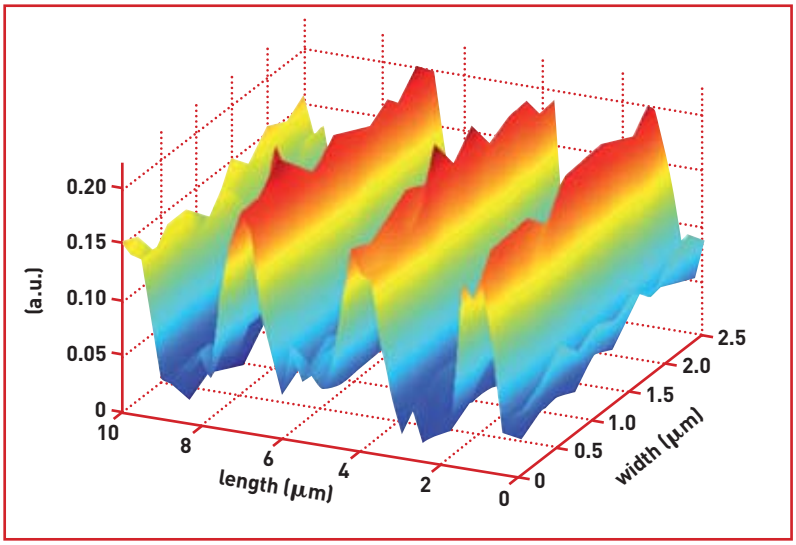


Figure 2.
This topographic map was obtained by scanning a test sample over a length of 10 µm, and a width of 2.5 µm. Distance between fiber tip and sample is kept, by feedback control, at a few tens of nanometers. Sample displacements are recorded during scanning. The equivalent of a map of the scanned region is thus obtained. In this case, the test sample exhibits parallel grooves 3 µm apart, and 2 µm deep.

How does a near-field optical microscope work?

There are various types of configuration, differing in particular as regards the location of the optical source,

(4) Confocal microscopy: through use of (focused) lasers, optical components, fast scanning devices, and computers to carry out digital image processing, this complex microscope architecture allows the interior of microscopic objects to be analyzed, and visualized in three dimensions.

(5) Raman effect: when radiation is scattered by molecules, very-low-intensity radiation, of different frequencies than that of the incident radiation, is found to appear. The changes in frequency observed are essentially linked to molecular vibrations. The diagram plotting scattered radiation intensity as a function of frequency exhibits low-intensity spectral lines, around a high-intensity maximum, corresponding to Rayleigh scattering. Analysis of this diagram allows information to be accessed on the structure of the molecules being analyzed.

(6) Ceramic: inorganic, nonmetallic material, featuring atomic bonds as a rule ionic (i.e. bonds characterized by electrostatic interactions between charge-bearing assemblies, with no sharing of **electrons**), or covalent (i.e. bonds set up when two **atoms**, in order to saturate their outermost orbitals, share one or more electron pairs).

and the role played by the probe itself. Depending on the nature of the sample (transparent, or opaque and reflecting), and on the positions of the emission and detection systems, relative to the sample, the configuration is said to be *in reflection mode*, or *in transmission mode*. This of course entails different optical layouts. Most commercial models may be set up in either mode.

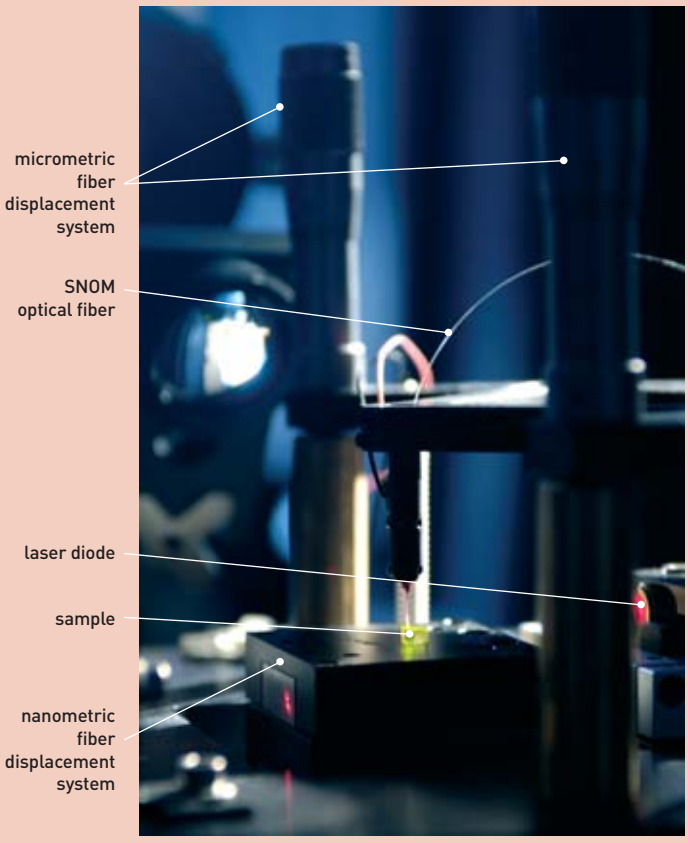
A threefold role for the probe

Depending on the role assigned to the probe, three functioning modes are possible: emission, detection, and perturbation. In every case, the signal analyzed is very low, owing to the smallness of the surface facing the probe tip.

In *emission* mode, the probe is used as a nanosource. Part of the scattered signal from the object results from the conversion of evanescent waves into propagating waves, collection of which may be effected in the far field by means of a conventional optical system. In *detection* mode, the probe is used as a near-field collector, and emission is carried out in the far field, through a conventional optical system. In either type of functioning, the collected signal is quite small, owing to the probe's low transmission (10^{-5}–10^{-7}), and the limited power available at the tip, due to its small size. Nevertheless, the probe may be used in both emission and reception modes. In either case, the probe, referred to as an *aperture probe*, consists either in a tapered, metallized optical fiber, or a hollow point, with a tip offering an emission (or collection) surface of about 50 nm diameter. The *perturbation* mode, on the other hand, involves a so-called *apertureless probe*. The probe is then used neither to emit nor to collect light, rather its presence in the vicinity of the sample, receiving illumination from the far field at the same time, perturbs the electric field, by modifying boundary conditions. A periodic perturbation may be detected in the far field, by synchronous detection. Spatial resolution is then higher, since the probe is a tapered metal point, the tip of which may be of smaller size than for an aperture probe. However, the signal is harder to interpret.

Broad operational conditions

The conditions for implementation of the SNOM technique are similar to those for atomic-force microscopy. It may be used in air, at the solid–liquid interface. No special preparation is required for the samples, such as might perturb observation. The surface, however, should be sufficiently flat, and of adequate regularity (local roughness of 10 nm, with possible vertical excursions up to a few micrometers). Being neither intrusive nor destructive, the system allows monitoring over time of the evolution of a process. It may be made suitable for nuclear conditions, and, since the volumes investigated are small, reactivity from the environment remains limited.



Philippe Stroppa/CEA

Figure 3.
Near-field unit of the SNOM device constructed at CEA (DEN/DPC), in collaboration with LCPME (CNRS–Henri-Poincaré - Nancy-I University). The optical probe may be seen, together with the micrometric and nanometric displacement systems, allowing probe positioning and feedback control, and sample scanning. This compact unit, about 15 centimeters wide, and its electronics form the core of the SNOM system. Optical excitation and detection systems must be associated to it, as appropriate for the kind of analysis to be carried out.

A SNOM system developed at CEA

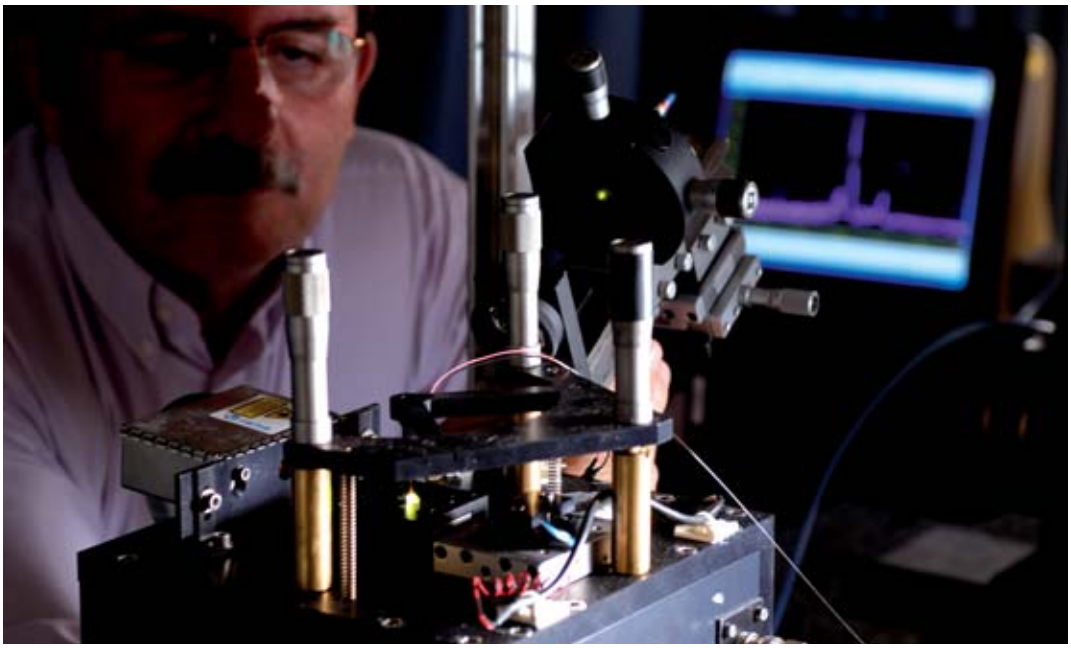
Working in collaboration with the Environmental Physical Chemistry and Microbiology Laboratory (CNRS–Henri-Poincaré - Nancy-I University/LCPME: Laboratoire de chimie physique et microbiologie pour l'environnement), the Physico–Chemistry Department in the Nuclear Energy Division (DEN/DPC: Département de physico-chimie), at CEA/Saclay, constructed, in 2006, its own near-field microscope (see Figure 3). The first investigation to be carried out using this microscope concerned glasses supplied by the Long-Term Behavior of Conditioning Materials Research Laboratory (LCLT: Laboratoire d'étude du comportement à long terme des matériaux de conditionnement), at CEA/Valrhô–Marcoule. These aluminate glasses(7) are **doped** with Eu^{3+} **ions**, which may serve as a fluorescent probe for the local surface chemical environment. This is a good example of the use of near-field microscopy for time-resolved fluorescence spectrometry.

The glass sample is illuminated by a frequency-doubled (λ = 532 nm), pulsed (4-**ns** duration) Nd:YAG laser, or by a continuous argon laser (λ = 457 nm), focused onto the region being investigated. The sample exhibits two distinct structures, visible to the naked eye, or through a conventional microscope: a relatively homogeneous amorphous(8) region, and a region appearing as poly**crystalline**.(9) A topographic map of a

(7) Aluminate: featuring the AlO_4^- group.

(8) Amorphous: a state characterized by complete loss of the crystalline order.

(9) Polycrystalline: consisting of a number of crystals, each crystal consisting in an assembly of atoms, ions, or molecules arrayed in regular, periodic fashion over all three space dimensions.



Philippe Stroppa/CEA

The near-field unit of the SNOM device constructed at CEA. Its operating principle consists in setting the SNOM optical fiber vibrating parallel to the sample's surface, while measuring oscillation amplitude by means of a tuning fork, or a laser diode.

4-µm-by-4-µm area, on the boundary between the two types of region, was recorded using the near-field microscope (see Figure 4). Two sites, noted as site A (amorphous region) and site B (polycrystalline region), were observed. The dot corresponds to the area probed by the near-field microscope, while the dotted circle overlays the area probed by a conventional microscope.

When the sample is illuminated by an Nd:YAG laser, or an argon laser, the Eu^{3+} ion is excited, swiftly falling back to level $^5D_0$, then deexcites by fluorescence to levels ^7F_J (with $J = 1 \ldots 4$). The structure of the fluorescence lines, and their relative intensity are strongly dependent on the environment of the ions being investigated, and most particularly on the symmetry exhibited by that environment. Fluorescence duration may likewise be modified by the ion's environment. The two spectra, from regions A and B, are markedly different (see Figure 4). Lines from region B (polycrystalline region) exhibit a more structured shape, owing to the larger **Stark effect** generated by the crystalline field in the Eu^{3+} ions' environment. Likewise, the intensity ratio for the lines shows that central symmetry is very low in region A (amorphous region), indicating a greater degree of disorder. Near-field measurement of fluorescence durations at a fixed wavelength ($\lambda = 615$ nm) yields a decay time standing at $\tau_1 = 640$ ns in the amorphous region, $\tau_2 = 960$ ns in the polycrystalline region. Such different fluorescence times are also signs of different environments, however they are more difficult to interpret. Measuring the selfsame fluorescence times in the far field, using a conventional microscope, yielded distinctly longer durations, $\tau_1 = 980$ ns, and $\tau_2 = 2{,}300$ ns. Such variation may be accounted for by the fact that, since the near field offers very low field depth (a few nanometers), fluorescence times are being measured for ions that are close to the surface, certainly lying in a more hydrated environment, water from the ambient atmosphere not being absorbed to any depth in glass. In the literature, it is a known fact that a larger number of water **molecules** in the

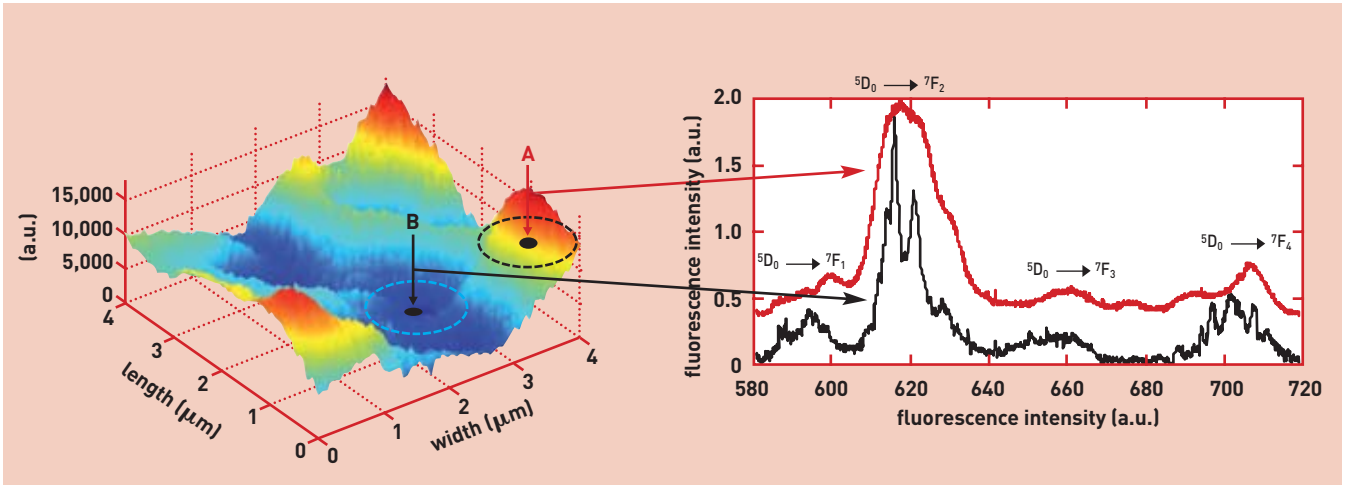


Figure 4.
At left, topographic map, as recorded with a near-field microscope, of the region located at the boundary of amorphous region A, and polycrystalline region B of a glass sample. At right, fluorescence spectra from regions A and B. The dotted circles bound the areas probed by a conventional microscope, whereas the black dots correspond to the areas probed by the near-field microscope.

Philippe Stroppa/CEA

The fluorescence signal recorded as the sample is illuminated by the laser yields information on the region subject to investigation.

immediate environment of Eu^{3+} ions lowers fluorescence time for this transition.

Wide-ranging areas of application

SNOM is a technique that is currently experiencing heady growth, and may find applications in many fields, such as physical chemistry, biology, or nanotechnology. The various applications being considered cover a large number of programs, being conducted at CEA/DEN. SNOM may contribute to the building up, and validation of **models** in the various areas of nuclear technology, such as the investigation of **radionuclide** retention and migration mechanisms in heterogeneous solids, or the investigation of corrosion initiation mechanisms, and the impact of corrosion on the properties of materials, and investigation of alteration processes in glasses.

In biology, optical microscopy is a tool seeing widespread employment, particularly by way of the ensemble of labeling methods involving a fluorescent **tracer**. Confocal microscopy allowed considerable improvements in spatial resolution, this reaching a fraction of a micrometer. By its very principle, near-field microscopy has brought further advances in resolution, down to 10 nanometers or so, thus allowing, for example, investigation of the fluorescence of a single molecule, directly observed in its own environment, with no prior dilution operation. Fluorescence images of **proteins**, of cell membranes involved in **photosynthesis**, of **genes** on **DNA** strands have been obtained. In the same manner, spectra were obtained from single proteins, along with measurements of their fluorescence lifetimes. Many currently ongoing investigations are aimed at determining the methods most appropriate for the application considered.

Near-field optical microscopy, by extending the range of spectroscopy down to the nanometer scale, is thus opening up a whole gamut of prospective applications. Results achieved are remarkable, and manifest this technique's potentials. Its use for the purposes of any one particular application, however, requires expertise, both in the field of spectroscopy and in that of precision instrumentation, since the signal is very weak, and the artefacts are many.

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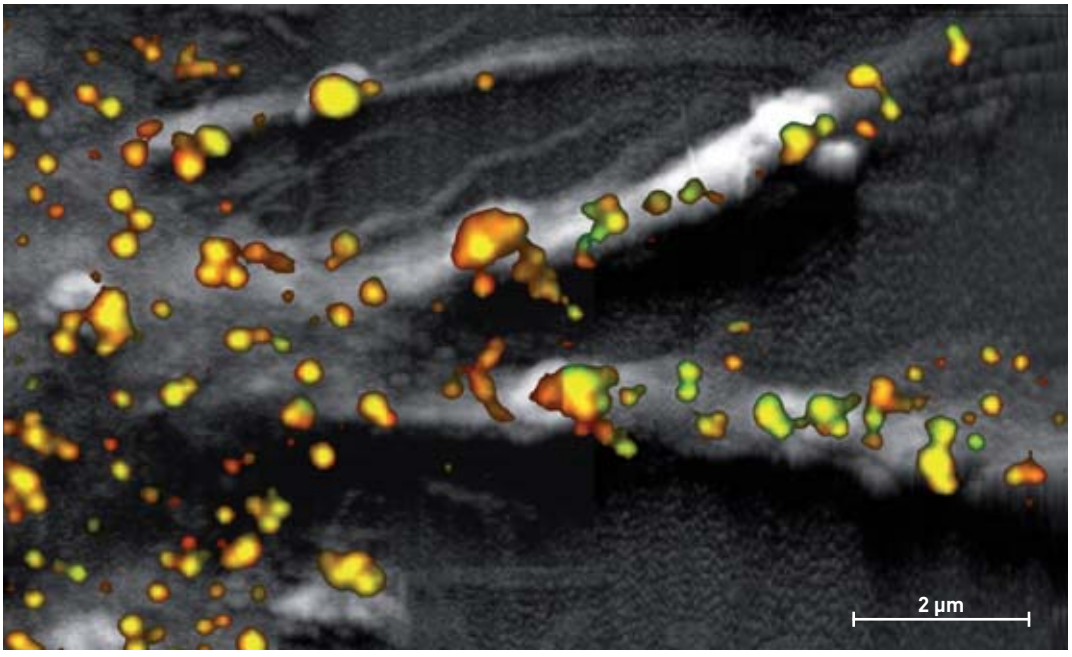


Combined near-field fluorescence and topography image of the distribution of a protein on the membrane of a cell.

(Taken from M. F. Garcia-Parajó, B. I. de Bakker, M. Koopman, A. Cambi, F. de Lange, C. G. Figdor and N. F. van Hulst, "Near-field fluorescence microscopy. An optical nanotool to study protein organization at the cell membrane", *NanoBiotechnology*, vol. 1, No. 1, July 2005, and M. Koopman, A. Cambi, B. I. de Bakker, B. Joosten, C. G. Figdor, N. F. van Hulst and M. F. Garcia-Parajó, "Near-field scanning optical microscopy in liquid for high resolution single molecule detection on dendritic cells", *FEBS Letters*, vol. 573, issues 1–3, July 2004, pp. 6–10.)

D Spectroscopy and spectrometry

Spectrometric methods are subdivided, as a whole, into two main categories, radiation spectrometry – itself comprising absorption spectrometry, emission spectrometry, Raman scattering spectrometry, and nuclear magnetic resonance spectrometry – and mass spectrometry.

Radiation spectroscopy and spectrometry[1] cover a ensemble of analytical methods allowing the composition and structure of matter to be ascertained, based on investigation of the spectra yielded by the interaction between atoms and molecules, and various types of electromagnetic radiation, emitted, absorbed, or scattered by the former.

Depending on their energy, photons interact selectively with the various electron shells, or levels, making up the electronic structure of the atom, or molecule. The electrons involved are *core electrons* (close to the atom's nucleus), for X-rays,[2] *peripheral electrons* (furthest from the nucleus, and involved in chemical bonds) for light absorbed, or emitted, in the near ultraviolet and visible region. In the infrared radiation region, it is the leap from one *molecular vibration* level to another that is involved, the switch from one molecular *rotation* level to another for microwave radiation, and atomic nucleus spin for NMR.

Absorption spectrometry

Those spectroscopy methods that rely on absorption make use of the Beer–Lambert law, setting out the proportional relation between the intensity of light absorbed, and the amount of absorbing matter:

$$A = \log (I_0/I) = \varepsilon \, l \, C,$$

where A stands for the absorbance of the medium traversed, I_0 for incident light intensity, I for transmitted light intensity, ε is the characteristic molar extinction coefficient, for a given wavelength, for the substance investigated – expressed in L mol^{-1} cm^{-1} – while l stands for the thickness passed through, expressed in centimeters, and C is the concentration, in moles per liter.

By measuring the medium's absorbance, for a given wavelength, the concentration of a substance, in a sample, may thus be determined.

In an absorption spectrum, as recorded by means of a spectrometer, *absorption peaks* correspond to the wavelengths the medium is able to absorb. Just as the spectrum from the Sun's light is obtained by making it pass through a prism, which breaks it up, spectrometers analyze the spectral distribution of the whole range of electromagnetic radiations, separating them out according to wavelength, by means of a reflection diffraction grating. Spectra exhibit peaks, each one corresponding to a specific wavelength.

Depending of the type of sample to be analyzed, and the performance level being sought, in the laboratory, absorption spectrometry is used either on molecules in liquid or gaseous phase, or on atomic vapor, obtained through thermal breakdown of liquid or solid samples.

Molecular absorption spectroscopy, in the UV–visible region, affords simplicity of use, however it is only applicable to samples of moderate complexity, since, owing to the width of *molecular absorption bands*, absorption spectra, as a rule, do not allow specific discrimination of every constituent, in a complex mixture.

In infrared (IR) spectrometry, absorption is the outcome of molecular vibration and rotation processes. Infrared absorption spectra thus allow the nature of chemical bonds to be determined, that make up a molecule, by ascertaining the bond's elasticity constant (influencing vibration frequency, as for a spring), thus confirming structural hypotheses.

As the number of atoms increases, the spectrum rapidly exhibits growing complexity, and interpretation becomes highly problematical, especially for organic compounds.

Atomic absorption spectrometry, in this respect, brings higher performance, since absorption by atoms yields very narrow absorption lines. Very precise measurements are thus feasible, even when the sample consists in a complex assembly of chemical elements. Atomic absorption is a reference technique for the analysis of trace elements in a wide variety of samples, in particular for biological samples.

Emission spectrometry

Atoms or molecules brought to an excited state may deexcite by emitting radiation, known as *emission radiation*. When the excitation is caused by selective absorption, by the atoms or molecules to be analyzed, of electromagnetic radiation, this represents a fluorescence emission (or a phosphorescence emission, depending on the electron excitation state involved).

As with absorption, fluorescence may be applied, in the UV–visible radiation region, to molecules, or atoms. X-ray fluorescence spectrometry, on the other hand, refers to the X radiation emitted by atoms excited by absorption of X-radiation. Fluorescence techniques are more complex to implement than is the case for absorption techniques, since they entail that the particle subjected to analysis be selectively excited by a monochromatic radiation. On the other hand, since the radiation emitted is likewise specific to the particle, fluorescence spectrometry involves a double selectivity, resulting in very low background noise, thus making it peculiarly well suited for the measurement of very low concentrations.

Emission of radiation may also occur when atoms are thermally excited, in an environment brought to high temperatures. Emission spectroscopy is based on the fact that atoms, or molecules excited to high energy levels deexcite to lower levels, by emitting radiation (emission, or luminescence). This differs from fluorescence spectrometry in that excitation is not applied selectively, rather it involves indiscriminately all of the particles making up the medium. Emission lines thus correspond to radiation directly emitted by a body brought to a high temperature, and the emission spectrum allows the detection, and quantification, of all atoms present in the emission source.

Raman spectrometry

Interactions between matter and electromagnetic radiation also give rise to scattering processes, such as elastic scattering, and inelastic scattering. Scattering may occur when the interface between

(1) The term "spectrometry," initially used only to refer to recording and measurement techniques, has tended to become synonymous with "spectroscopy," as the eye was supplanted, for observation purposes, by other receptors and instruments, while the visible region now only formed one special region, in analytical terms.

(2) It should be noted, at the same time, that X-ray crystallography is not deemed to be a spectroscopy method, in the strict sense of the term.

two media is encountered, or as a medium is passed through. This process, in most cases, is an "elastic" one, in other words it takes place with no change in frequency for the radiation forming the beam involved. Elastic scattering of solar radiation by the atmosphere is, for instance, responsible for the blueness of the sky, observed when the eye is not directed towards the Sun (*Tyndall effect*). Indeed, scattered intensity is all the greater, the shorter the radiation wavelength, which, in the case of the solar spectrum, corresponds to the color blue.

As regards spectrometry, the main use of scattering concerns *Raman spectrometry*. This involves the inelastic scattering of incident radiation by the molecules making up the sample. The difference between scattered radiation frequency, and incident radiation frequency allows the identification of the chemical bonds involved. Raman spectrometry is a technique that is widely used for structural analysis, to complement infrared spectrometry, and mass spectrometry.

Nuclear magnetic resonance spectrometry

The principle of nuclear magnetic resonance (NMR) is based on the fact that an atom has a *magnetic moment*, just like a spinning charge acting as a tiny magnet, governed by quantum mechanics, aligning in a magnetic field as the needle of a compass in the Earth's magnetic field. The principle of NMR consists in inducing, and detecting, the transition, for the nuclear magnetic moment, from the lowest energy level to the highest energy level, through absorption of electromagnetic radiation of a wavelength lying in the radiofrequency region: when the energy of the photon precisely matches the energy difference between the two levels, absorption occurs. Nuclei having numbers of protons, and neutrons that are both even exhibit zero spin. Carbon 12 and oxygen 16 atoms, which are very widespread in nature, thus have zero spin. On the other hand, hydrogen only has one single proton, and its nuclear magnetic moment equals 1/2: it may thus take on two possible energy states, corresponding to the two orientation states of its spin, relative to the magnetic field. Measuring the resonance frequency in the electromagnetic field allowing transition from one of these energy states to the other enables the molecules to be analyzed. This frequency is fixed, however the various nuclei in a molecule do not all resonate at the same frequency, since their magnetic environment is modified by their chemical (electronic) environment.

Many NMR spectra exhibit more peaks than there are protons in the nucleus, owing to the interactions between protons and their neighbors. Two nuclei may interact within the molecule, though they are separated by several chemical bonds: this is known as interatomic coupling. This interaction endows the NMR spectrum with a fine structure.

C. Dupont/CEA

Spectromètre de masse d'ions secondaires utilisé au CEA pour réaliser des mesures isotopiques rapides sur un échantillon par exemple prélevé sur une installation aux activités nucléaires suspectes.

Mass spectrometry

Mass spectrometry is a highly sensitive *detection* and *identification* technique, allowing determination of molecular structures, and thus of a sample's composition. This is not, strictly speaking, a form of spectrometry, since it is not concerned with discrete energy levels. What is its principle? A compound introduced into the device is vaporized, and subsequently ionized by an electron bombardment source (at 70 eV). The ion thus obtained, termed a molecular ion, allows the compound's molar mass to be determined. Breaking chemical bonds within the compound may yield characteristic fragment ions. These are then sorted according to their mass/charge ratio in an *analyzer*, through application of a magnetic and/or electric field, then collected by a *detector*, which amplifies the signal associated to the ions, which arrive with varying delays. A data processing system converts the information from the detector into a mass spectrum, readout of which, by comparing it with reference spectra, allows the identity details of the molecule to be drawn up. Through use of a high-resolution mass spectrometer, the exact mass of the compound may be determined, together with isotope percentages for each constituent atom.

Choice of ionization method is directly related to the nature of the sample, and the type of analysis. If mass spectrometry has gradually adapted to meet the growing demands from chemists, and biologists (separation of increasingly complex, highly polarized mixtures, determination of ever higher molecular masses on samples of ever more constricted sizes), this is essentially due to advances in *ionization techniques*, these including secondary ion mass spectrometry (SIMS), chemical ionization, thermospray ionization, and fast atom bombardment (FAB) sources, further comprising, from the 1980s, matrix-assisted laser desorption ionization (MALDI), and electrospray ionization (ESI), together with advances in *detection techniques*, from time-of-flight (TOF) measurement to "ion traps" (ITs), through quadrupoles (MS or Q).

In proteomics, for instance, only MALDI, ESI and SELDI (surface-enhanced laser desorption ionization) are employed.

Ion mobility spectrometry (IMS) is a chemical analysis technique in the gaseous phase, which consists in subjecting a gas to an electric field. Ionized molecules acquire a velocity that is characteristic for the ion, since this depends on mass, and charge. Arrival of the ions on one of the plates generating the field results in a current, which is recorded. The length of time after which a peak occurs can be related to the nature of the ion causing it.

Scientists often make use of a coupling of devices each belonging to one of the two main families of analytical techniques (see Box E, *What is chromatography?*), e.g. of a chromatograph with a mass spectrometer (or an electron-capture detector [ECD]), particularly for the investigation of trace complex mixtures.

B Fundamental interactions and elementary particles

The standard model of particle physics is the reference theoretical framework describing all known elementary particles (see Table 1) and the fundamental interactions these particles are involved in (see Table 2). The basic constituents of matter, known as fermions, are partitioned into two main categories, as determined by their participation in the fundamental interactions, or forces (the gravitational, electromagnetic, weak, and strong forces), which are mediated by vector bosons, the fundamental particles which carry out the transmission of the forces of nature[1] (see Table 2). Whether a particle belongs to the category of fermions, or to that of bosons depends on its spin (i.e. its intrinsic angular moment, or internal rotation moment), depending on whether it exhibits half-integer spin (fermions) or integer spin (bosons).

At the same time, to every constituent of matter is associated its antiparticle, a particle having the same *mass*, but the opposite *charge*. The positron is thus the positively charged antiparticle of the electron, which exhibits a negative charge.

Leptons and quarks

Fermions include, on the one hand, leptons, which may travel freely and do not participate in the *strong interaction*, which ensures the cohesion of atomic nuclei (it is consequently termed a *nuclear* interaction), and, on the other hand, quarks, which participate in all interactions but are not individually observed, enmeshed and confined as they are within hadrons, the particles susceptible to strong interaction, of which they are the constituents.[2]

In the lepton category, charged leptons participate in the *electromagnetic interaction* (which ensures the cohesion of atoms and molecules, and in the *weak interaction* (which underlies decay processes, in particular β radioactivity). Neutral leptons, or neutrinos, for their part, participate in the weak interaction only. Exhibiting very low mass, there is one type of neutrino for each type of charged lepton.

Independently from their involvement in interactions, the basic constituents of matter are classified into three *generations*, or *families*, of particles. From one family to the next, quarks and leptons having the same charges only differ by their mass, each family being heavier than the preceding one.

The electron, up quark (symbolized *u*) and down quark (symbol *d*), which belong to the first generation, are the lightest massive particles, and are stable. These are the sole constituents of normal matter, so-called baryonic matter (a baryon is an assembly of quarks), which is made up of protons and neutrons, this however only accounting for 4% of the Universe's energy content!

Particles in the other two families are heavier, and are unstable, except for neutrinos, which on the other hand exhibit non-zero mass, but are stable.

These latter particles may only be observed or detected in the final states resulting from collisions effected in accelerators, or in cosmic radiation, and rapidly decay into stable first-generation particles. This is why all the stable matter in the Universe is made up from constituents from the first family.

According to quantum mechanics, for an interaction to take place between particles of normal matter, at least one elementary particle, a boson, must be emitted, absorbed, or exchanged. The photon is the intermediate (or vector) boson for the electromagnetic interaction, the W^+, W^- and Z are the intermediate bosons for the weak interaction, and gluons are those of the strong interaction, acting at quark level.

As to the graviton, the putative vector for the gravitational interaction, it has not so far been empirically discovered. The gravitational force, which acts on all fermions in proportion to their mass, is not included in the standard model, due in particular to the fact that quantum field theory, when applied to gravitation, does not yield a viable scheme, as it stands. While gravitational effects are negligible in particle physics measurements, they become predominant on astronomical scales.

Interaction ranges

Quarks and charged leptons exchange photons. The photon having no electric charge, these particles conserve their electric charge after the exchange. Since the photon's mass is zero, the electromagnetic interaction has an infinite range. Having no electric charge, neutrinos are the only elementary fermions that are not subject to electromagnetic interaction.

In the electroweak theory (a unification of the weak and electromagnetic interactions), the weak interaction has two aspects: charged-current weak interaction, for which the interaction vectors are the W^+ and W^-; and neutral-current weak interaction, for which the mediator is Z^0. These two forms of weak interaction are active between all elementary fermions (quarks, charged leptons and neutrinos). The mass of these bosons being very large (about 80 GeV/c^2 for $W^\pm$, 91 GeV/c^2 for Z^0), the range of the weak interaction is tiny – of the order of 10^{-18} m. Since $W^\pm$ bosons have a non-zero electric charge, fermions exchanging such bosons undergo a change in electric charge, as of nature (*flavor*). Conversely, since the Z^0 boson has no electric charge, fermions exchanging one undergo no change in nature. In effect, neutral-current weak interaction is somewhat akin to exchanging a photon. As a general rule, if two fermions are able to exchange a photon, they can also exchange a Z^0. On the other hand, a neutrino has the ability to exchange a Z^0 with another particle, though not a photon.

Only those quarks that have a color charge[1] exchange gluons, these in turn being bearers of a color charge. Thus,

(1) The participation of basic constituents in fundamental interactions is governed by their *interaction charges* (electric charge, color charge), or "conserved quantum numbers." *Color charge*, a quantum number that determines participation in strong interactions, may take one of three values: "red," "green," or "blue" (these colors bearing no relation to visible colors). Every quark bears one of these color charges, every antiquark one of the three anticolor charges. Gluons are endowed with double color–anticolor charges (eight combinations being possible).

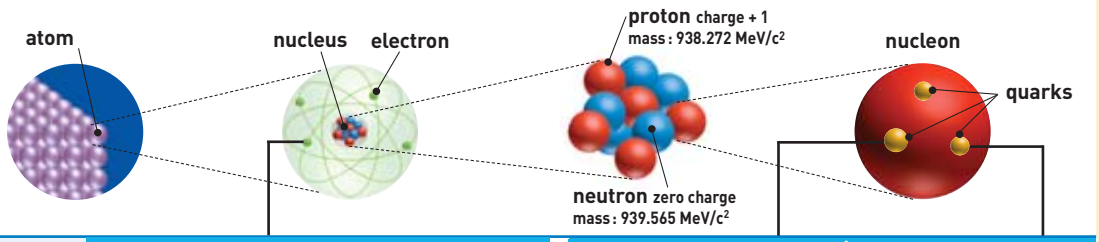
(2) To take e.g. nucleons: the proton holds two up quarks and one down quark, the neutron two down quarks and one up quark. A meson is made up of just two quarks (one quark and one antiquark).

B (cont'd)

when a gluon exchange takes place between quarks, the latter exchange their respective colors. Gluons have zero mass, however, since they do bear a color charge, they are able to interact together, which greatly complicates theoretical treatment of this interaction. The range of the strong interaction is consequently very restricted – of the order of 10^{-15} m.

The quest for unification

The theoretical framework for the standard model is quantum field theory, which allows a quantitative description to be made of the fundamental interac-



		leptons (able to move freely)		quarks (assembled into triplets, or quark–antiquark pairs, to form the many subatomic particles)	
Fermions Normal matter is made up of particles from this group. Most of these particles were around just after the Big Bang. Presently only to be found in cosmic rays, and around accelerators.	First family	**electron (e)** responsible for electricity and chemical reactions charge: - 1 mass : 0.511 MeV/c²	**electron neutrino (ν_e)** has no electric charge, and interacts very seldom with the ambient medium.	**down (*d*)** electric charge: - 1/3 the proton holds one, the neutron two mass : 4 – 8 MeV/c²	**up (*u*)** electric charge: + 2/3 the proton holds two, the neutron one mass : 1.5 – 4 MeV/c²
	Second family	**muon (μ)** a more massive companion to the electron. mass : 105.658 MeV/c²	**muon neutrino (ν_μ)** properties similar to those of the electron neutrino.	**strange (*s*)** a heavier companion to "up" mass : 80 – 130 MeV/c²	**charmé (*c*)** a heavier companion to "down" mass : 1.15 – 1.35 GeV/c²
	Third family	**tau particle (τ)** heavier still. masse : 1,776.99 ± 0.29 MeV/c²	**tau neutrino (ν_τ)** properties similar to those of the electron neutrino.	**beauty (*b*)** tau particle. mass : 4.1 – 4.4 GeV/c²	**top (*t*)** heaviest in the family (observed in 1995) mass : 171.4 ± 2.1 GeV/c²
Vector bosons Fundamental particles carrying out transmission of natural forces.		**photon** elementary grain of light, vector for the electromagnetic force	**gluon** bearer of the strong force between quarks	**$W^{\pm}$, Z^0** bearers of the weak force, responsible for some forms of radioactive decay	
Higgs boson?		responsible for "electroweak symmetry breaking"			

Tableau 1.
Table showing the twelve elementary constituents for which the standard model describes the interactions involved. The three charged leptons (electron e-, muon, μ^-, tau particle τ^-) are subject to electromagnetic and weak interactions, neutrinos (ν_e, ν_μ, ν_τ) are only affected by weak interaction, and the six quarks (up, charm, top – or *u*, *c*, *t* – bearing a charge of 2/3; and down, strange, bottom – *d*, *s*, *b* – bearing a charge of – 1/3) are subject to all three interactions. Every elementary constituent has its antiparticle, having the same mass, and algebraic quantum numbers (such as electric charge) of the opposite sign.

tions between elementary particles, while respecting the principles of *special relativity*, as those of quantum mechanics. According to the latter theory, if one seeks to observe a microscopic structure at high temporal and spatial resolution, this entails transferring to it an amount of energy–momentum, the greater, the higher the resolution being sought. However, according to the theory of relativity, such an energy–momentum transfer is liable to undergo transformation, yielding particles not present in the initial state: fermions may be generated, or annihilated, in particle–antiparticle pairs, while bosons may be so in any arbitrary number.

All processes involving one and the same fundamental interaction are interrelated. The quantum field theory approach, in which properties of **symmetry** play a fundamental part, seeks to describe all of the processes relating to each fundamental interaction, within overarching theoretical constructions.

The strong and electromagnetic interactions are formalized, respectively, in the theories of **quantum chromodynamics**, and **quantum electrodynamics**. The weak interaction, for its part, is not subject to a separate description, being described jointly with the electromagnetic interaction, in the unified formalism of **electroweak theory**. Theories of the *grand unification* of all fundamental interactions do exist, however they remain as yet lacking any experimental validation.

All the predictions of the standard model have been corroborated by experiment, except for just one, to wit, the existence of the **Higgs boson(s)**, which particle (particles?), it is hoped, will be discovered with LHC. The **Higgs mechanism** is thought to be responsible for the mass exhibited by elementary particles, the eponymous boson making it possible for zero-mass fermions interacting with it to be endowed with mass. This would allow the unification, at high energies, of the weak and electromagnetic interactions within the electroweak theory, while effectively accounting for the **breaking** of this **electroweak symmetry** at low energies, taking the form of two interactions, which may be seen as distinct at that energy level (see *The electroweak interaction from one accelerator to the next: the LHC roadmap and the yardstick of LEP measurements*, p. 23).

Going beyond, or completing the standard model?

The standard model features a set of parameters (such as the masses of elementary particles, or the intensities of fundamental forces) which are "anchored" in experimental findings. It is, in any event, a theory that is liable to be improved, or further elaborated, or even surpassed and left behind. It does not account in any way for the classification of the constituents of matter into three generations of particles, whereas it is precisely the existence of these three generations which makes it possible to account for **CP** (charge–parity) **invariance violation** (meaning that a physical process involving the weak interaction is not equivalent to its own mirror image), a violation that is in all likelihood the source of the matter–**antimatter** imbalance, running in favor of the former, in the primordial Universe. The model neither allows quantum treatment of gravitation, nor does it fully account for the fundamental property of *confinement*, which prevents quarks from propagating freely outside hadrons.

To go beyond, or to complete the standard model, research workers are mainly exploring two avenues:

– **supersymmetry** (widely known as SUSY) would associate, to every particle (whether a boson or a fermion) in the standard model, a partner from the other series, respectively a fermion or a boson. Supersymmetric partners would, at first blush, be highly massive, the lightest of them being a particle interacting very weakly only. This would be an ideal candidate to account for the **hidden matter** (or **dark matter**) in the Universe, accounting as it does for some 21% of the Universe's energy content, the remainder (close to 75%) consisting in a **dark energy**, the nature of which likewise remains to be determined. These WIMPs (acronym for "weakly interacting massive particles") are actively being sought (see *EDELWEISS II, the quest for dark matter particles*);

– the **substructure** path assumes there could be a new level of elementarity, underlying the particles in the standard model (or some of them). This would lead to a veritable blossoming of new, composite particles, analogous to hadrons, but exhibiting masses two to three thousand times heavier.

It should be noted that, whereas supersymmetry theories yield predictions that agree with the precision measurements carried out at LEP, the theories propounding substructures (or their simpler variants, at any rate) fail to do so. As for the more complex variants, these are encountering difficulties at the theoretical level.

fundamental interaction	associated particles (messengers)	actions
gravitation	graviton?	having an infinite range responsible for the mutual attraction of any two masses and for the law of falling bodies
electromagnetic interaction	photon	having an infinite range responsible for the attraction between electrons and atomic nuclei, hence for the cohesion of atoms and molecules
weak interaction	W^+, W^-, Z^0	responsible for β^- and β^+ radioactivity, reactions involving particles as neutrinos
strong interaction	gluons (there are 8 gluons)	ensures the cohesion of the atomic nucleus

Tableau 2.
Fundamental interactions, their vectors, and effects.

E What is chromatography?

Chromatography, together with the various forms of spectroscopy and spectrometry (see Box D, *Spectroscopy and spectrometry*), represent the two major basic analytical techniques, the former serving for the separation, the latter for the identification of the constituents of a substance.

Chromatography (from the Greek *khrôma*, "color," and *graphein*, "to write"), allows the *separation* of the constituents of a mixture in a homogeneous liquid or gaseous phase, as blotting paper might spread out in concentric rings a liquid poured onto it.

A chromatograph comprises a sample injection device, a *column*, a detector, and a recording and analysis system. Its principle is based on the equilibrium of compound concentrations, between two phases coming into contact: the *stationary phase*, in the column, and the *mobile phase*, which moves across it. Separation relies on the differential displacement of constituents inside the column, passing through in times that are proportional to their size, or depending on their structure, or affinity for the stationary phase (polarity...). As they reach the far end of the column, a *detector* measures, on a continuous basis, the quantities of each constituent.

The most common form of chromatography is **gas chromatography**, carried out on gaseous samples, or samples that may be vaporized without incurring breakdown. The mobile phase is a gas (helium, nitrogen, argon, or hydrogen), constantly sweeping through the column, which is placed in a thermostat oven. Detectors allow the selective analysis and identification of highly complex mixtures.

If the stationary phase is a nonvolatile, or not highly volatile liquid, exhibiting solvent properties for the compounds to be separated, the process is termed **gas–liquid chromatography**, or *partition chromatography*. If the stationary phase is an **adsorbent** solid (silica, alumina, zeolites, or **polymers**), this is **gas–solid chromatography**. Within this same family, of **adsorption** chromatography processes, **liquid–solid chromatography** is characterized by its stationary phase, this being a polar solid.

In **high-performance liquid chromatography** (**HPLC**), the sample must be wholly soluble in the mobile phase (elution solvent). The latter must be kept at high pressure (hence the alternative name of *high-pressure* liquid chromatography), to ensure a constant flow rate inside the column, and preclude any loss of head. HPLC involves solute–mobile phase–stationary phase exchange mechanisms, based on partition or adsorption coefficients, depending on the nature of the phases in contact.(1)

A chromatographic analysis yields a **chromatogram**, this being a graphical representation of the evolution of a parameter (intensity of the detector signal), related to instantaneous solute concentration, as function of time. This exhibits *peaks*, rising above the *baseline*, which obtains in the absence of any compounds (see Figure).

(1) There are two further types of liquid chromatography, *ion chromatography*, and *exclusion chromatography*.

N.B: This Box reproduces a number of excerpts from a presentation by Pascale Richardin, head of the Datation Group at the Research and Restoration Center of the French National Museums Administration (Musées de France), taken from the pages dealing with analytical methods, as posted on the site : ttp://www.culture.gouv.fr/culture/conservation/fr/biblioth/biblioth.htm

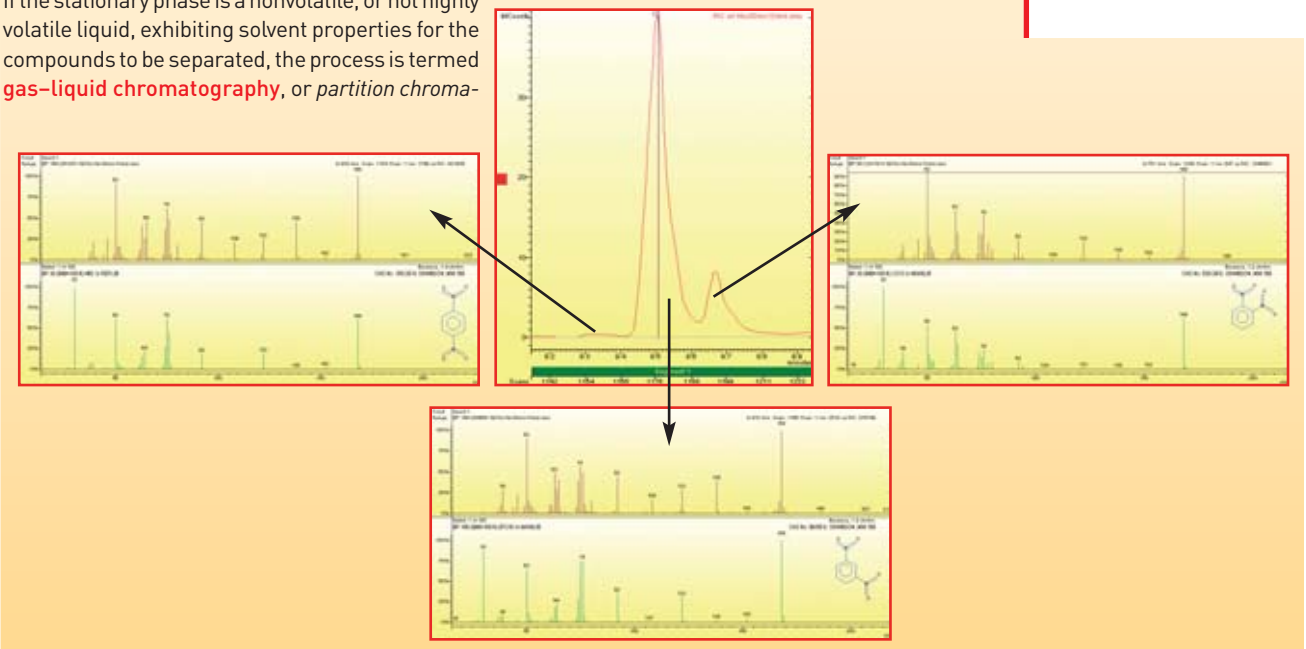


Figure.
An example of the combined use of mass spectrometry and chromatography: the separation of isomers ("sister molecules") of an explosive molecule (dinitrobenzene [DNB]), after solid-phase microextraction sampling, by gas chromatography, and their detection by mass spectrometry (SPME–GC–MS).