Scanning photoemission spectromicroscopy of neurons

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(Received 9 December 1992)

Photoemission spectroscopy now reaches a lateral resolution in the submicrometer range. It is possible, therefore, to perform chemical analysis in the microscopic domain required for life-science experiments. We present a successful test of this approach, including photoemission spectra taken on microscopic areas of a fixed neuron culture, and photoelectron two-dimensional microdistribution maps of specific elements. The test demonstrates that scanning photoemision spectromicroscopy can be successfully applied to biological specimens, although one cannot automatically connect results on fixed cultures and the *in vivo* properties.

PACS number(s): 87.80.+s, 07.80.+x, 79.60.−i

We present a successful test of scanning photoemission microscopy to the chemical analysis of biological specimens on a submicrometer scale. The test, performed with the spectromicroscope Maximum [1,2] at the Wisconsin Synchrotron Radiation Center, produced spectra of elements on the surface of a fixed neuron culture over 1×1-μm^2 areas, and photoelectron micrographs of the spatial distribution of the same elements. It should be noted that biological conclusions on *in vivo* properties cannot be directly derived from the analysis of fixed specimens. The success of our tests demonstrates nevertheless that scanning photoemission spectroscopy can be extended to the analysis of submicrometer areas of biological specimens, thus gaining access to the life sciences [2].

Until recently, this possibility was confined to the realm of dreams, since photoemission spectroscopy was typically performed on areas of the order of 0.1×0.1 mm^2 or larger [3,4]. This means that one of the most powerful probes for materials sciences [4] was nearly useless in the life sciences [2,5]. The latter requires indeed analysis over microscopic areas, similar to the size of cell features: 0.5 μm or less.

The situation began to rapidly evolve in recent years [1–3]; pioneering photoemission microscopy work on biological specimens is described in Ref. [6]. The progress accelerated with the advent of high-brightness sources of synchrotron radiation—an evolution that is expected to be accelerated by the near-future commissioning of ultrabright sources at Trieste (Elettra) and Berkeley (Advanced Light Source). In 1987, signal-level evaluations indicated the technical feasibility of photoemission experiments on a submicrometer scale and beyond. This stimulated the development of adequate instruments to exploit this opportunity [3]. In particular, it led to the construction of the Wisconsin scanning photoemission spectromicroscope Maximum [1,2] that currently holds the record for space and energy resolution: better than 900 Å and 350 meV.

Preliminary tests had already shown [2] that the Maximum spectromicroscope was capable of producing microscopic images of neuron networks, revealing cell bodies and also finer features such as axons and dendrites. Before the recent upgrade of the Maximum system, however, the performances were not sufficient to move from mere imaging to true spectromicroscopy—for example, to the use of photoemission spectra from small areas for local microchemical analysis. The present experiments show that spectromicroscopy is now becoming reality.

Cerebellar granule cells [7] from seven-day-old rats were seeded on a gold substrate (approximately 1.5×10^5 cells/cm^2), previously treated with 5 μg/ml of poly-L-lysine solution. The cells were obtained [8] by enzymatical and mechanical dissociation of the cerebellar tissue and plated in basal medium (Eagle's salt), containing 10% fetal calf serum, and allowed to grow in an incubator at 37 °C in a 5% CO_2 humidified atmosphere. After seven days the neuron cultures were fixed with *para*-formaldehyde and dehydrated.

The neuron specimens so produced tend to assume a monolayer configuration on the substrate, and to form a neuron network. Their suitability for photoemission experiments was demonstrated by the previous tested described in Refs. [2,5,7].

The Maximum spectromicroscope and the general experimental system used in these tests have been discussed

in detail in Ref. [1]. This is a brief description of the essential characteristics of the system. The photon beam is generated by an undulator [9] on the Wisconsin storage ring Aladdin. The source is limited by a pinhole after the monochromator. The photon beam is focused by a multilayer coated Schwarzschild objective. The photoelectrons are collected and analyzed by a double-pass cylindrical mirror analyzer (CMA). The sample is mounted on an x-y scanning stage.

The Maximum spectromicroscope can work in several different modes [1], three of which were used in our spectromicroscopy tests. In the first mode, the CMA is set to collect photoelectrons of low energy. The signal is dominated [4] by secondary emission; by operating the x-y scanning stage, one can generate photoelectron micrographs that do not discriminate the different chemical components [1,2].

Two examples are shown in Fig. 1: the 80×80-μm^2 micrographs were taken at a photoelectron kinetic energy of 1.3 eV. They show two different regions of the same neuron network; we can observe cell bodies, and smaller structures: axons and dendrites interconnecting the cell bodies in the network. These pictures illustrate the neuron culture's capability to grow as a nearly monolayer architecture on a flat substrate [2,5,7]. The estimated lateral resolution of the images is $0.5-1$ μm.

Results like those of Fig. 1 show that photoelectron microscopy can achieve performances comparable to optical microscopy, but they are not a new result [2]. The novelty of the present experiments is the move from mere photoemission microscopy to real spectromicroscopy [3]. One of the first examples of this move is shown in Figs. 2 and 3.

The curves in these figures are photoemission spectra (photoemission intensity versus photoelectron kinetic energy [4]) taken in several different areas of different neuron specimens. In each case the probed area had microscopic dimensions of the order of 1×1 μm^2. The spectra, therefore, reflect the chemical composition and properties of very small portions of our specimen.

All spectra in Fig. 2 exhibit characteristic features related to the Ca $3p$, K $3p$, and Na $2p$ core levels, plus other features mostly related to oxides. The spectra in Fig. 3 show in more detail the peak due to the overlapping spectral contributions of Ca and Na.

The detection of Ca, K, and Na on the neuron surface is not surprising, since these elements are present in the ion channels of the cell membrane of live cells: they play a fundamental role in guaranteeing the homeostasis of each cell and in the nerve pulse transmission. The localization of the elements can of course be modified by the fixing procedure. Our data show, nevertheless, that even after fixing the above elements are found in cell areas and not on the substrate.

We note that, although the spectral features in Fig. 2 are always present, their relative intensity changes substantially from spectrum to spectrum. These spatial changes in the quantitative chemical composition can be quite dramatic—compare in particular curve a with the remaining spectra. The causes of such changes are not identified at the present time, and the results of Fig. 2 must be interpreted only as a test of our instrument's capability to detect them.

Calcium, potassium, and sodium in the specimen were independently detected in photoemission experiments on a more macroscopic scale and with higher-energy (Al $K\alpha$) photons, conducted at the Centre de Spectromicroscopie of the Ecole Polytechnique Fédérale de Lausanne with the Scienta ESCA 300 spectromicroscope [10]. Some of the results are shown in Fig. 4; specifically, Fig.

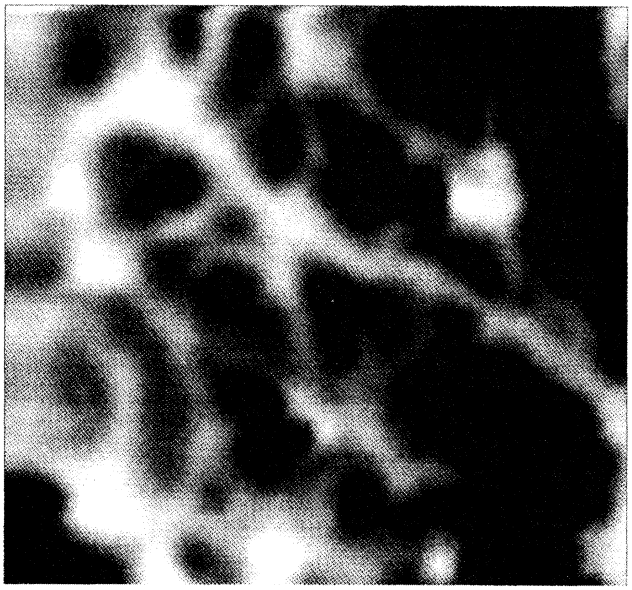

FIG. 1. Two different photoelectron micrographs revealing the structure of different portions of a fixed neuron culture. The images were obtained with a photon energy of 95 eV, by collecting photoelectrons of 1.3 eV kinetic energy, and correspond to 80×80-μm^2 areas. The color code shows the less emitting areas in light yellow. The estimated lateral resolution was $0.5-1$ μm.

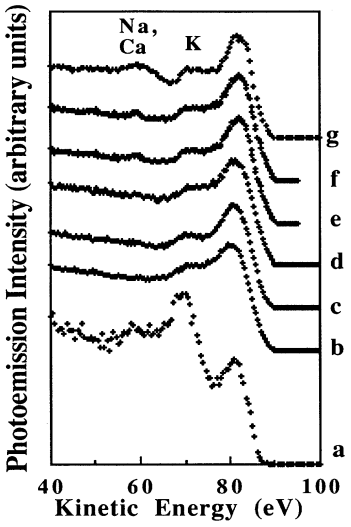

FIG. 2. A series of photoemission spectra taken with a photon energy of 95 eV in different 1×1-μm^2 areas of two different neuron specimens. Curves a–e correspond to the first sample, and the remaining to the second sample. The probed area changes from curve to curve, always remaining on cell bodies. The labels identify spectral features due to specific elements.

4(a) corresponds to a spectral region similar to that covered by Figs. 2 and 3, and whereas Figs. 4(b) and 4(c) show the Ca $2p$ and K $2p$ core level peaks.

Figure 4(a) shows that the spectral contributions observed in Figs. 2 and 3 become nearly invisible, and certainly much weaker than the dominating Au signal. This is due to several factors: to the change in cross section with the photon energy, and also to the fact that the spectrum of Fig. 4(a) covers a much wider specimen area than those of Figs. 2 and 3; it includes not only contributions from cells and their interconnections, but also from the gold substrate.

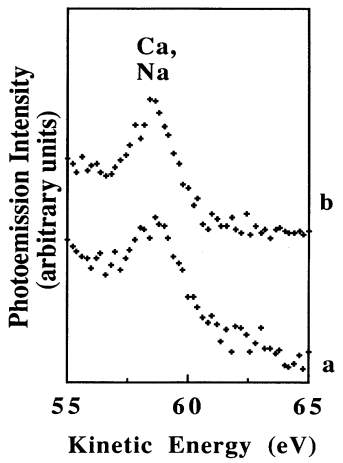

FIG. 3. Photoemission spectra similar to curves f and g in Fig. 2, but confined to the spectral region of the overlapping Ca $3p$ and Na $2p$ contributions.

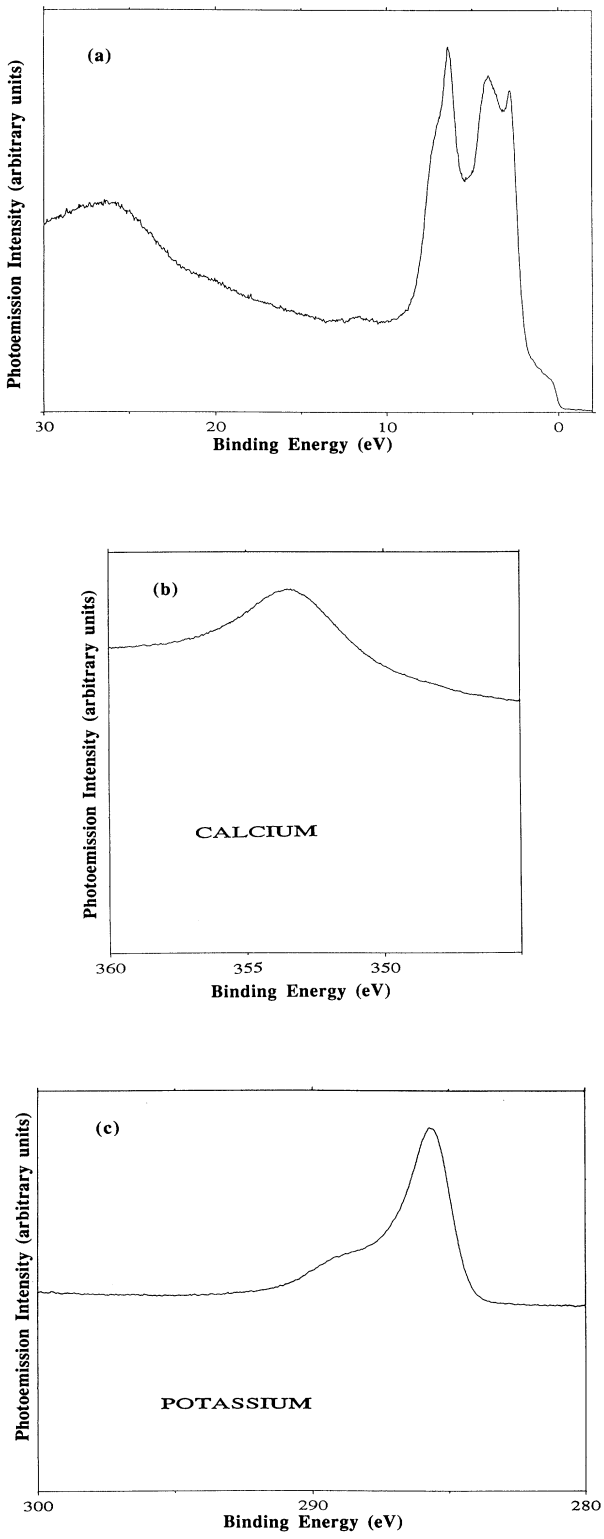

FIG. 4. Spectra taken with the Scienta ESCA-300 instrument in Lausanne, with Al $K\alpha$ photons, over a macroscopic area of a specimen similar to those of Figs. 1–3. Curve a shows the spectral region of the Au valence electrons and of the (not observed) Ca $3p$, K $3p$, and Na $2p$ electrons; curves b and c show the Ca and K $2p$ core-level peaks.

One could also hypothesize that the different relative intensity of the spectral features in Figs. 2, 3, and 4(a) is due in part to the different surface sensitivity of the two experiments [4]. The escape depth of photoelectrons is indeed extremely short—of the order of very few angstroms—for the kinetic energies of Figs. 2 and 3, resulting in very high surface sensitivity and therefore in the enhancement of the cell membrane signal. For Fig. 4(a), the escape depth becomes of the order of 50 Å, larger than the cell membrane thickness, and therefore the relative spectral contribution of the surface diminishes.

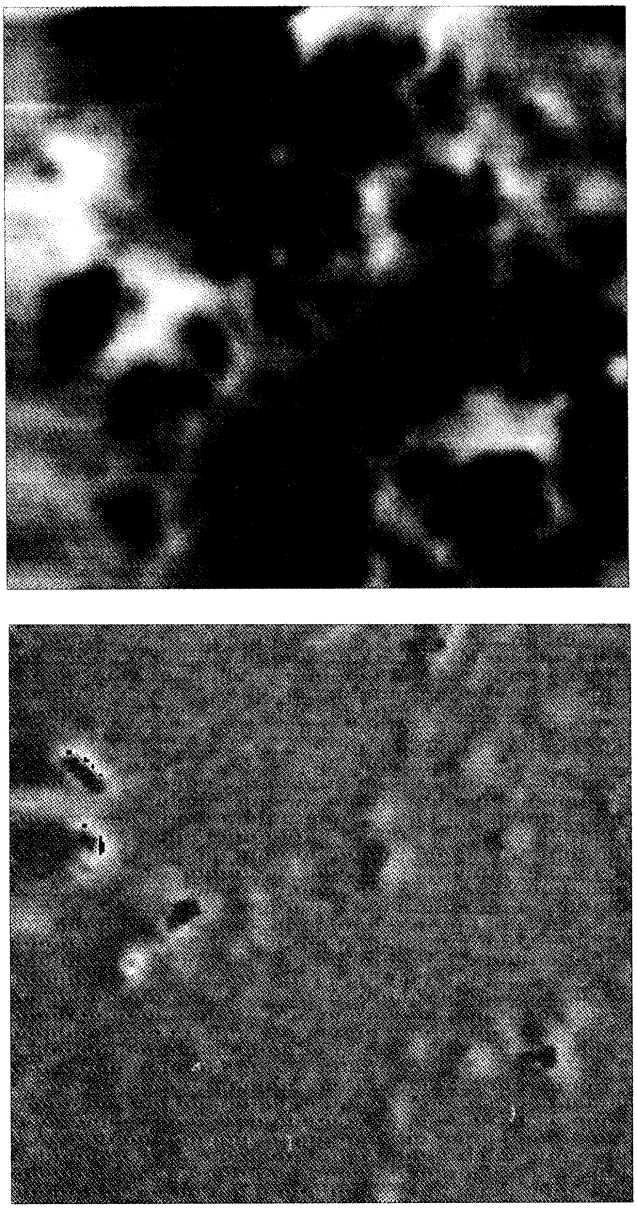

FIG. 5. (Top): photoelectron microimage similar to those of Fig. 1. (Bottom): the same area imaged by collecting photoelectrons at the energy corresponding to the K 3*p* core level, and therefore reflecting the spatial distribution of potassium.

The third mode of data taking in the present Maximum experiments also illustrates the move from the photoemission microscopy of Fig. 1 to spectromicroscopy. It is implemented by setting the CMA to the photoelectron energy of a given core level, and then measuring the photoemission intensity point by point while operating the sample scanning stage.

This produces microimages of the spatial distribution of the specific element corresponding to the core level. An example is shown in Fig. 5, that compares a global microimage similar to those of Fig. 1 (top), and the specific microimage of the potassium distribution. Note that there is a one-to-one correspondence between the features of the latter and those of the former—whereas not all of the global features are visible in the potassium-specific microimage, suggesting inhomogeneities in the distribution of the element.

Specifically, the potassium distribution appears concentrated in areas corresponding to the cell bodies of our fixed specimen. A similar conclusion is valid for sodium and calcium. The fixing procedure can dislocate, introduce, or remove all of these elements. We note, however, that on our fixed specimen we always found them in cell areas and never on the substrate.

Figure 5 provides, therefore, a successful test for the use of photoemission techniques in detecting chemical distributions on a micrometer and submicrometer scale. This approach can also be used to image not only a given element, but also the element in a specific chemical status [1–4]. The core-level energy, indeed, changes slightly with the chemical status [4]; with sufficient energy resolution, one can distinguish each chemical status from the others of the same element.

This last possibility has been successfully tested when using the Maximum spectromicroscope to investigate nonbiological samples [1]. Similar experiments appear possible for biological specimens, but with somewhat marginal signal level. This limitation will be removed in the near future by the advent of the ultrabright synchrotron sources at Trieste and Berkeley, scheduled to be commissioned in 1993.

These new sources will also greatly improve the overall performances of photoemission spectromicroscopy: for example, they will reduce the data-taking time and allow even better spatial resolution. These future possibilities further enhance, in perspective, the importance of the present tests.

These experiments are supported by the U.S. National Science Foundation (directly and through the Wisconsin Synchrotron Radiation Center), by the Wisconsin Alumni Research Foundation, by the U.S. Department of Energy, by the Fonds National Suisse de la Recherche Scientifique, by the Ecole Polytechnique Fédérale de Lausanne, and by the Italian National Research Council. We thank our colleagues J. H. Underwood, R. Perera, J. Kortright, H. Jotterand, P. Baudat, L. Perez, C. Coluzza, M. Marsi, M. Capozi, and T. dell'Orto for their participation.

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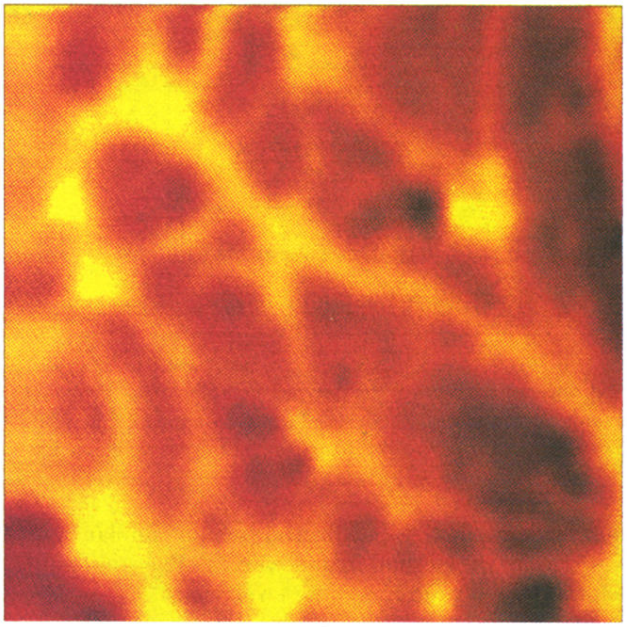
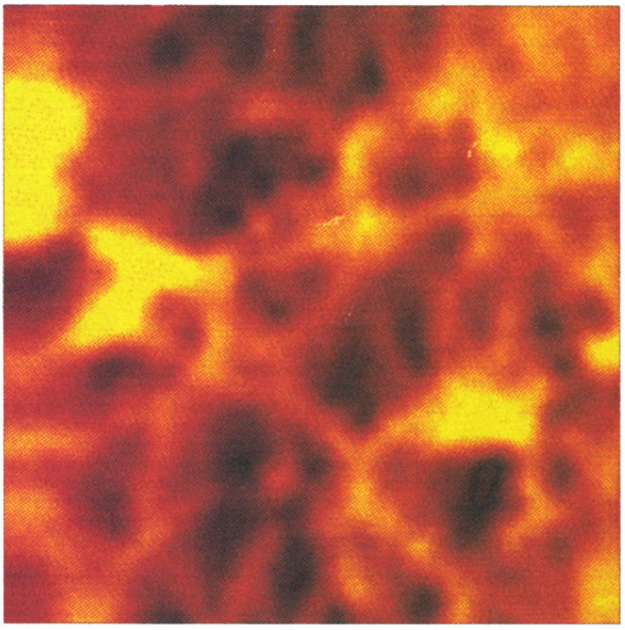


(a)



(b)

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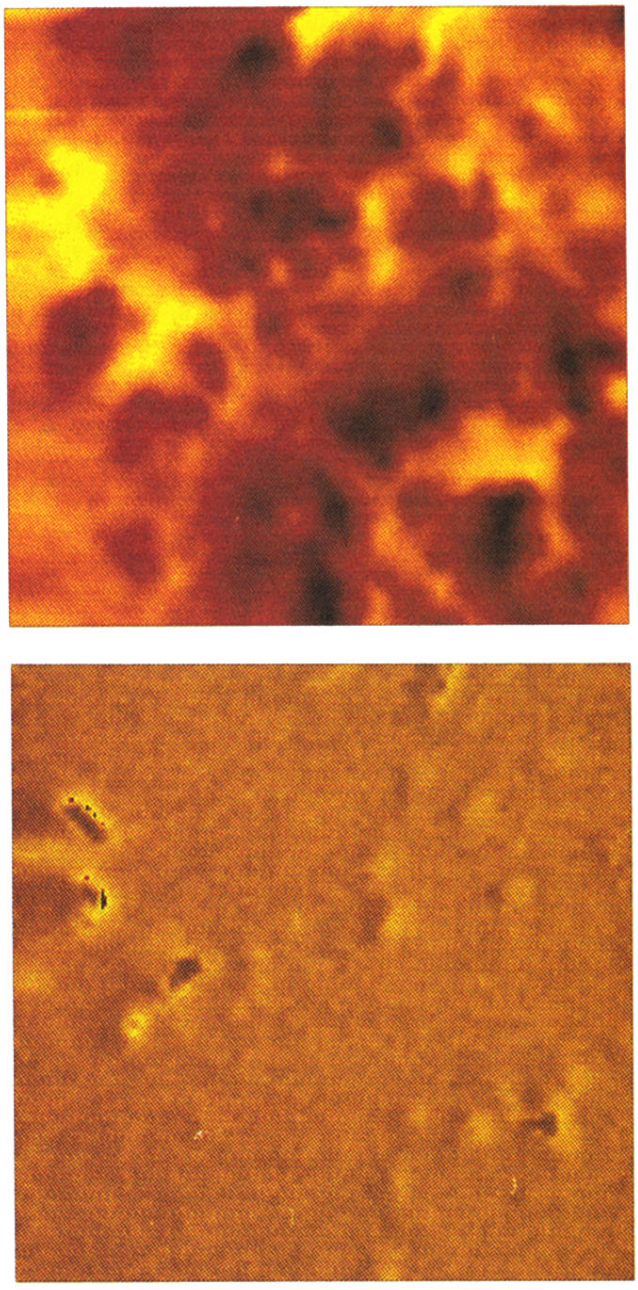

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