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SCANNING PHOTOELECTRON MICROSCOPY WITH UNDULATOR RADIATION: A SUCCESSFUL TEST ON UNCOATED NEURONS

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We present the results of the first test of scanning photoelectron microscopy of biological cells. The results were obtained with the recently commissioned X-ray photoemission microscope MAXIMUM at the Wisconsin synchrotron radiation center, based on an undulator source of photons and on normal-incidence focussing of the photon beam. Several photoelectron micrographs were taken of rate neurons, prepared without metal coating and without labelling agents. The results were compared with visible micrographs produced by the same instrument, and successfully tested for reproducibility over long periods of time.

The advent of bright and ultrabright sources of synchrotron radiation opens exciting new opportunities in X-ray microscopy [1,2]. In particular, it becomes feasible to achieve lateral resolution in traditional spectroscopic techniques such as photoemission [1]. In the case of biological specimens, photoemission microscopy eliminates some of the limitations of traditional electron microscopy. For example, in scanning-tunneling microscopy (STM) only very small (a few ångströms large) insulating objects can be imaged – whereas photoemission microscopy can image much larger insulating structures. The labellers required in other forms of electron microscopy are no longer necessary, thus the specimen is closer to its natural status [3]. Furthermore, photoemission *spectro*microscopy provides information on the chemical status of elements in the specimen, with a resolution that will eventually reach the sub-0.1 μm level. Thus, photoemission microscopy is emerging as an important surface-sensitive counterpart of other scanning X-ray microscopies [2].

These exciting opportunities have stimulated a number of photoemission microscopy projects at Stanford, Brookhaven, Wisconsin-Milwaukee and abroad [1,2]. At the synchrotron radiation center (SRC) of the University of Wisconsin-Madison, we recently commissioned [1,4] a prototype of the undulator photoemission microscope MAXIMUM (multiple-application X-ray imaging undulator microscope), a collaboration project also involving the center for X-ray optics of the Lawrence Berkeley Laboratory, the University of Minnesota, and the Xerox corporation. This instrument was used for preliminary test of scanning photoelectron micro-imaging of uncoated rate neurons. The successful results, described in this report, demonstrate the validity of the innovative technical solutions adopted for MAXIMUM, and the feasibility of using scanning photoelectron microscopy in life-science research as well as in the physical sciences.

Fig. 1 shows the overall structure of MAXIMUM [1,4]. The main components of the instrument are: (i) an undulator source of synchrotron radiation; (ii) a monochromator; (iii) an X-ray focussing system; (iv) a specimen scanning system; (v) a photoelectron collector; (vi) data-processing hardware and software. The key ele-

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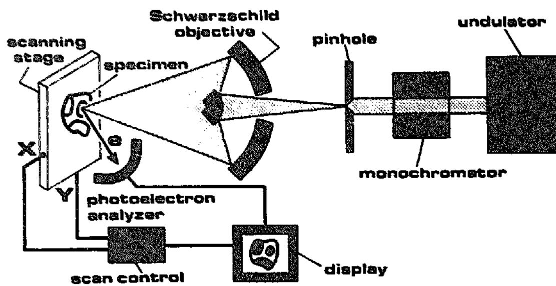

Fig. 1. An artist's view of the photoemission microscope MAXIMUM, developed in collaboration by the University of Wisconsin-Madison, the Berkeley Center for X-Ray Optics (CXRO), the University of Minnesota, the Xerox Corporation, and based on an undulator source developed by Berkeley-CXRO and by the Stanford Synchrotron Radiation Laboratory.

ment is the focussing device: this consists of a Schwarzschild objective [5] working at normal incidence; the efficiency of the objective's mirrors in reflecting X-rays is increased by the use of Mo–Si multilayer coatings, to ≈ 40% at the peak wavelength. The multilayer coating of the Schwarzschild objective used in this experiment has a photon energy bandpass centered at 72 eV.

The undulator is a permanent-magnet array with variable gap, inserted in one of the straight sections of the Aladdin electron storage ring at SRC [6]. This device, developed by the Stanford synchrotron radiation laboratory and by the Lawrence Berkeley Laboratory, emits high-brightness radiation over a narrow spectral band, whose photon energy position can be controlled by changing the magnet gap. The radiation is further monochromatized by a 6 m toroidal grating monochromator, and a pinhole is used to create a small-area source. Then the radiation is focussed by the objective onto the sample, extracting photoelectrons from a small area. The sample is mounted on a two-dimensional scanning mechanism with a coarse stage and a fine stage, based on piezo-actuators.

The present version of MAXIMUM is a prototype, with some temporary technical solutions adopted to shorten the construction time; such limitations are being eliminated in the construction of the final instrument [4]. Even with the present limitations, extensive tests have demonstrated that photoelectron-yield micro-images can be produced with a resolution of 2–3 μm. The test included imaging of gold structures on silicon and knife-edge studies of the lateral resolution [4]. At present, the focussing performances are limited by the relatively large roughness (28 Å rms) of the blank Schwarzschild objective, and by the alignment of the objective's components. The removal of such problems will enable us to reach submicron working resolution levels on Aladdin, limited by the source brightness. On ultrabright sources like ELETTRA at Trieste and the advanced light source (ALS) at Berkeley, the working resolution will become even closer to the Schwarzschild objective's diffraction limit, ≈ 300 Å at 120 eV photon energy.

The imaging of uncoated cells described in this article is a critical feasibility test for the use of MAXIMUM – and scanning photoelectron microscopy in general – in the life sciences. This test was conducted on neurons from eight-day-old rats [3]. The neurons were allowed to grow in a culture medium with serum, for approximately one week. The growth occurred on top of a polished (0.25 μm estimated roughness) stainless-steel plate, coated with a 2000 Å thick Au film. Neurons were bound to the substrate by means of a discontinuous poly-L-lysine intralayer. Then the neurons were fixed by polyglutaraldehyde, 2% in PBSS (phosphate buffer saline solution), and washed with bidistilled and de-ionized water. Finally, the specimens were dehydrated at a pressure of 10^{-3} Torr and at 26 °C for 24 h. This procedure appears very effective in eliminating the water – thereby making it possible to study the specimen in ultrahigh vacuum – without affecting the external structure of the cells. This important point is evident from the picture of fig. 2a, obtained with an optical microscope. Note, once again, that the preparation procedure does *not* include labelling or metal coating. This leaves the specimen in a status closer to the natural one than in the case of electron microscopy; furthermore, the energy density of the incident beam is much smaller than in electron microscopy.

Several photoelectron micrographs of neuron specimens were taken by detecting the total photoelectron yield while scanning the sample. Fig. 2b shows a typical result: the photoelectron micro-image of the region corresponding to fig. 2a, with several clusters of neurons. The reproducibility of the images was successfully tested by exploring the same region several times over a period of up to two days. The typical time required for taking a micrograph was 30–60 min.

The photoelectron microscopy images were further tested by comparing them with visible-radiation micrographs of the same area. For example, figs. 2a and 2c show visible micrographs corresponding to the the photoelectron micrograph of fig. 2b. Of these, fig. 2a was taken ex situ with conventional optical microscope, whereas fig. 2c was taken in situ with the same Schwarzschild objective that focusses the X-ray beam, combined with a beam splitter and with an eyepiece. This last micrograph illustrates one of the most useful characteristics of our focussing system: since the Schwarzschild objective works in the visible as well as for the X-rays (with multilayer coating), our X-ray micrographs can be immediately tested by examining the corresponding in situ images in the visible [1,4].

These tests demonstrated several important points. We obtained the first scanning photoelectron micrographs of cells, achieving a lateral resolution compara-

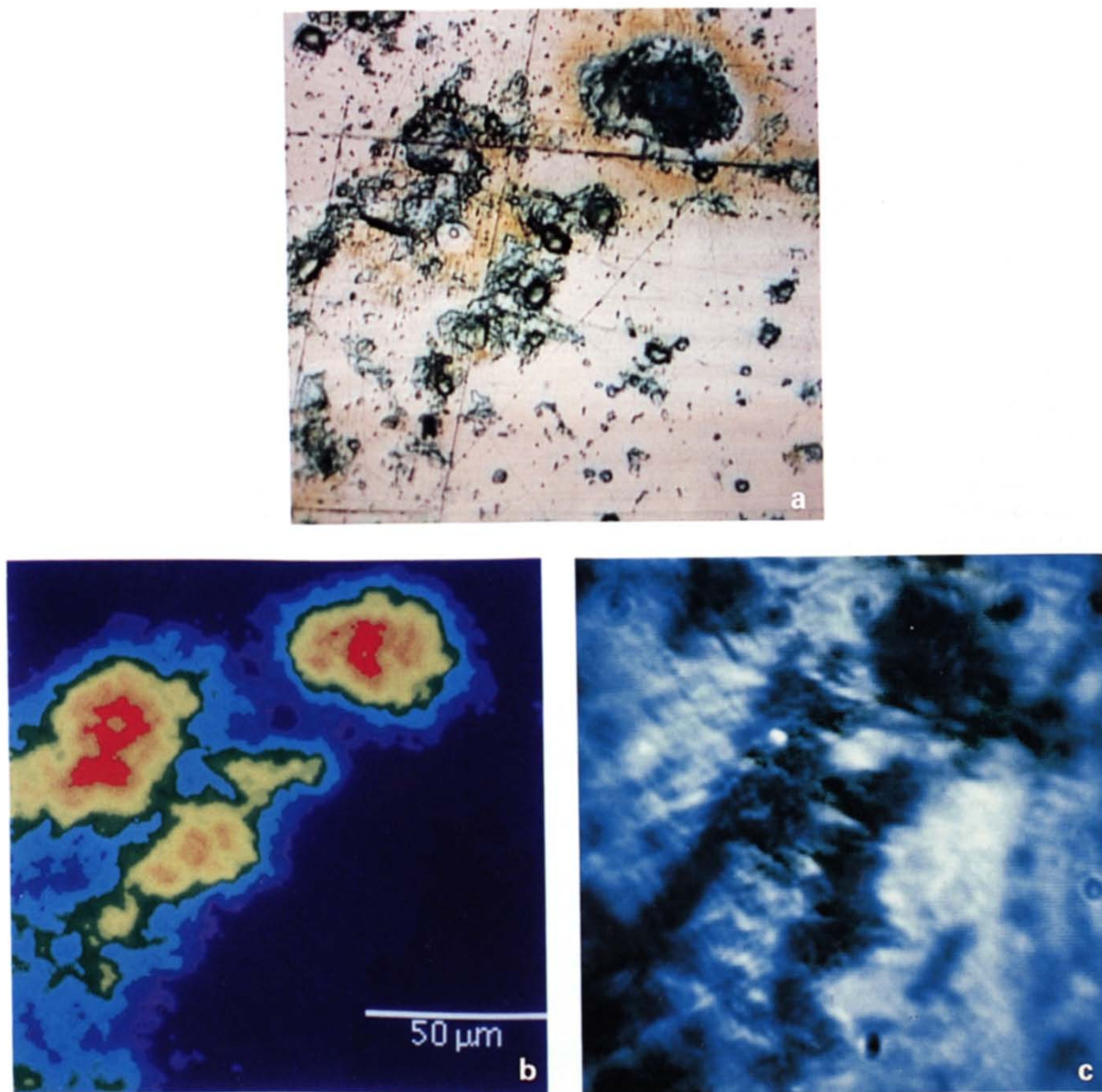

Fig. 2. (a) Visible micrograph of a specimen, including several clusters of neurons; this image was taken with a conventional visible microscope. (b) Photoelectron micrograph of the same region of the specimen, taken with 72 eV photons, showing an area of size 132 µm. The estimated lateral resolution was 2–3 µm. The dark background corresponds to the emission from the metal substrate. (c) Visible micrograph of the same region, taken in situ with the same Schwarzschild objective used for X-ray focussing, combined with an eyepiece.

ble to that obtained with nonbiological specimens. We demonstrated that our neuron preparation technique [3] is suitable for the development of specimens compatible with a ultrahigh-vacuum environment. The absence of metal coating or labelling agents leaves the system in a state close to the natural one, and visible and X-ray micrographs demonstrated that the structure of the neurons is not appreciably altered by the preparation process. Repeated images of the same region have shown no detectable alteration of the neuron structure, ruling out structural deterioration due to exposure to the X-ray beam.

The present results, although preliminary in nature, open the way to two possible future improvements. The first is an increase in lateral resolution, as previously discussed. The second is the energy analysis of the

photoelectrons, which will provide the information on the chemical status of elements – trace elements in particular. The signal levels obtained in the present tests indicate that energy analysis will be possible without increasing the time per micrograph to unrealistic levels. In conclusion, we have demonstrated the feasibility of scanning photoemission microscopy for biological specimens, and found evidence that scanning photoemission spectromicroscopy is also feasible.

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