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Role of Nuclear Analytical Probe Techniques in Biological Trace Element Research

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ABSTRACT

Many biomedical experiments require the qualitative and quantitative localization of trace elements with high sensitivity and good spatial resolution. The feasibility of measuring the chemical form of the elements, the time course of trace element metabolism, and of conducting experiments in living biological systems are also important requirements for biological trace element research. Nuclear analytical techniques that employ ion or photon beams have grown in importance in the past decade and have led to several new experimental approaches. Some of the important features of these methods are reviewed here along with their role in trace element research, and examples of their use are given to illustrate potential for new research directions. It is emphasized that the effective application of these methods necessitates a closely integrated multidisciplinary scientific team.

Index entries:

INTRODUCTION

Biomedical research requires the use of many different types of analytical instrumentation to properly investigate the many complex phenomena which occur in living systems. Nuclear analytical techniques (NAT) is a general term which describes a class of instrumentation based on the use of atomic or nuclear probes such as ions, neutrons, or x rays. Each nuclear and atomic probe is characterized by particular advantages and disadvanges. NAT includes a well-defined core of instrumentation and research approaches overlapping with many related areas such as elemental and isotopic analysis and imaging.

Examples of NAT core techniques which have been used for many years and are well established disciplines in their own right are analytical electron microscopy, x-ray imaging, and neutron activation analysis. More recently, the development of MeV proton beams for particle-induced x-ray emission (PIXE) trace element analysis (1,2,3) has emerged as the newest member of NAT. An example of a method which can be related to NAT because of the common goal in trace element detection with high spatial resolution is laser microprobe mass analysis (LAMMA) which is based on the production of finely focussed photons from a laser to produce ions which are analyzed and detected with techniques held in common with the core science of NAT.

An example of a new NAT-type technique is the application of x-ray beams produced by circulating electron beams in synchrotron light sources (SLS) to elemental analysis and imaging in biomedical experiments. Examination of this developing analytical tool shows how NAT can give special capabilities not provided by other methods and also stresses the closely woven interdependence of the several types of complementary analytical probes. Some of the material to be presented is based on the ongoing NIH Biotechnology Research Resource

X-Ray Microprobe Facility at the Brookhaven National Laboratory Synchrotron Light Source. The intent is to show why the x-ray microprobe is needed from the biological standpoint and to present brief examples of how it can usefully be employed in future years. Other examples of the ways that NAT has been applied to actual biomedical problems at several laboratories around the world are also included.

BIOLOGICAL REQUIREMENTS

Recent years have seen a growing interest in and awareness of the role of trace elements in biological systems. Trace elements are intimately involved in biological function and dysfunction at all levels of biological organization. At the molecular level, trace elements perform structural and catalytic roles in small molecules, macromolecules, and other cell constituents. At the cellular level, trace elements are necessary for maintenance and regulation of compartmentation of cell function, gene regulation, stimulus-response coupling, etc. At the organismal level, trace elements are required to integrate cell and organ function so that all physiological processes can occur. Biological systems have evolved elaborate and diverse control mechanisms to provide for trace element homeostasis at the subcellular, cellular, and organismal levels. These critical functions give trace elements profound influence on human health and disease states. Trace elements are well recognized as contributing factors in modifying oncogenesis, aging, development, and chronic diseases including cardiovascular. Trace elements are also of medicinal importance in that nearly 100 drugs contain trace elements (4).

The homeostasis and interaction of trace elements in biological systems is extraordinarily complex. In order for the biology of any biological system

to be fully understood, the role of trace elements in that system must be fully characterized and understood. Many new approaches have been developed in the last few years for studying the role of trace elements. The approaches include fluorescent indicators, ion selective electrodes, monoclonal antibodies, etc., and much progress has been made.

There are, however, several critical gaps in our understanding of trace elements. Much is known regarding trace element homeostasis at the cellular level through the use of radiotracers in cultured cells and cell-free systems. The gross trace element content of tissues is well characterized by conventional chemical analysis of bulk tissues. In contrast, relatively little is known about the trace element content of biological microstructures at microscopic scale. Even though we commonly think of normal biological function and disease processes in terms of cell-to-cell interaction and communication, with few exceptions trace element homeostasis is not characterized in this manner. The reason trace element metabolism is not generally studied at this level is simple--it is very difficult to do so. Many organs or organ structures, for example the hippocampus and cardiovascular system, are extraordinarily difficult to remove cleanly and reproducibly from the surrounding tissue matrix due to small size and/or complex shape. Thus, it is difficult to relate experimentally trace element content with the function and dysfunction of these structures.

The character of the distribution of trace elements in biological space is dependent upon the level of biological organization. It is useful to consider and classify trace element distributions in biological space because these distributions have an important role in the design of experiments, selection of the analytical techniques, and interpretation of the data. It can be seen from the generalizations presented in Table 1, that by definition,

trace elements are homogeneously distributed with a population of individuals, although there are, of course, many populations. Trace elements are, however heterogeneously distributed within an individual of a population, i.e., between the organs and tissues of an individual. Trace elements are more or less uniformly distributed within populations of cells (cell types) within organs, but are commonly compartmentalized among the structural and functional constituents of cells.

Two important generalizations may also be made about Table 1. First, the degree of heterogeneity of trace element content increases as we move from higher levels of biological organization to lower. A biologically significant difference in the trace element composition of two populations may be only a few per cent, while at the molecular level, ligands may show very high specificity for trace elements, and the trace element concentrations may easily reach parts per thousand (mass fraction) or higher. Second, the roles of time and chemical form of the trace elements become more important variables at lower levels of biological organization. To use the extremes of individuals and molecules as examples, the biological elimination halftimes for some elements are measured in decades, while the association-disassociation rate constants for some ligands may be seconds or less.

The biologist invariably wants to, and needs to, extrapolate data and conclusions obtained at one level of biological organization to higher and lower levels. It is necessary, therefore, to carefully consider the limitations imposed by the characteristics of the trace element distribution, the role of time, and the direction of extrapolation in the selection of the analytical technique and sampling proceeds. The optimal approach then, is to select the appropriate microprobe with adequate sensitivity and the appropriate spatial resolution to sample the appropriate biological structure.

MICROANALYSIS OF TRACE ELEMENTS

The need for sensitive trace element measurements in small volumes is one that is poorly met by currently available methods of analysis. Electron microprobes have very high spatial resolution and function very well for the analysis of very thin sections below the 1-μm level. At greater thicknesses multiple scattering of the beam degrades the spatial resolution to the micrometer level, and bremsstrahlung background reduces the sensitivity limits to a few hundred parts per million (ppm) by mass for many transition elements of biological interest. Secondary mass spectrometry (SIMS) or LAMMA have good spatial resolution, but are destructive, difficult to calibrate, and suffer from many molecular interferences. The use of synchrotron radiation-induced x-ray emission (SRIXE) or PIXE is an effective way of meeting some of the problems.

In order to illustrate the challenge of the trace element measurements in biological systems, it is instructive to plot the number of atoms of the element of interest as a function of "tissue" volume for a specific element. This is done for 1 ppm zinc in a biological matrix which is defined to have a density of 1 g/cm^3 (Fig. 1). Inspection of the figure shows that to make subcellular measurements of the Zn content will require the detection of 10^4 to 10^6 atoms. The number of atoms are also indicated for the range of biological structures and for macroanalysis where, in all cases, a tissue section 10 μm in thickness is considered. The analysis of the distribution of a trace element within a single cell can thus be very hard because of the small numbers of atoms at ppm levels.

NUCLEAR ANALYTICAL TECHNIQUES

Some of the biological requirements for microanalysis of trace elements are well matched to the attributes of several nuclear analytical techniques. In order to be specific, in several cases we have chosen to illustrate how new analytical capabilities can drive research into new areas by discussing several applications of synchrotron produced x-ray beams. Examples are also presented that show how other types of methods using ion beams can be applied. The short discussions of selected examples are not meant to be encyclopedic in scope. They serve, however, serve to illustrate the dual need for better analytical methods and for research groups combing skills in both the physical sciences and the biomedical sciences.

PROTON MICROPROBE

The proton microprobe is attaining the state of a well-developed instrument that is now in rather general use for biomedical experiments. The spatial resolution and sensitivity are coupled since the ion sources commonly used as a source of protons have a limited brightness. The small beam currents that are produced limit the detection sensitivity when measurements are made at the very best spatial resolutions attainable. A number of laboratories now work with beam resolutions of a few square micrometers and deliver enough beam to make possible measurements at the 10-ppm level.

A nice example of the use of the proton probe is the measurement of the composition of individual leukocytes in blood samples (5). The composition of the leukocytes are interesting to study because of the potential for noninvasive analysis of trace element status in humans.

SYNCHROTRON X-RAY MICROPROBE

Trace element microanalysis can be attacked very well by use of synchrotron-induced x ray emission (SRIXE) (6). The radiation damage caused by the synchrotron-produced x-ray beam passing through biological material is much less than for ionizing beams so that it is possible to use high x-ray fluences without destroying the target. Since x rays are relatively weakly absorbed, the experiments can be carried out in a wet environment and at atmospheric pressure, which is to say, in living biological systems. The flux of x rays that can be delivered to the target is very high so that a microprobe can be used with a very high spatial resolution combined with excellent trace element sensitivity (7,8,9).

To illustrate this point a SRIXE spectrum taken at the NSLS is shown in Fig. 2. This spectrum was obtained with a collimated beam of white radiation 20 μm × 20 μm irradiating a 10-μm section of rat kidney cortex for 300 s. The tissue volume of 4×10^{-9} cm^3 analyzed contained the approximate volume of two proximal convoluted tubule cells. The major trace elements, K, Ca, Fe, and Zn, are easily quantified, as are many minor trace elements including Cu and Se. These data show that operation of a x-ray microprobe at the ~ 1 μm resolution will be possible with a minimum detection limit of a few ppm for Zn using the simple detection system of a Si(Li) detector. The limits (7) of detection will be improved by x-ray focussing and use of wavelength dispersive x-ray detectors.

Analysis of Wet, Living Samples

High-energy x rays and heavy-ion beams, such as protons, can be used to measure the elemental composition of wet samples at atmospheric pressure. The relatively slow attenuation and scattering of these beams by the material through which they pass is the key factor. Electron probes are more prone to

multiple scattering that degrades the spatial resolution and to bremsstrahlung that impairs the detection sensitivities. The energy loss of the x-ray beam in the sample is less than for the charged particle beams, and they are thus the probe to be preferred when analysis of a cell or tissue in a living condition is attempted (10,11,12). While, it remains to be seen how closely it will be possible to actually approximate a living condition, it does seem obvious that analysis under the conditions described will be a valuable capability in many ways.

A wet cell that was used for an initial feasibility test was designed and tested at the NSLS in a BNL-Smith Kline & French collaboration that was headed by R. Beeuwkes (13). The cell used 7 μm polyimide films for entrance and exit windows for the x-ray beam and for the fluorescent x rays. The thickness of the wet cell chamber was fixed at 15 μm by a thin spacer film. A Kreb's balanced salt solution was circulated through the cell by an external pump system. A schematic diagram of the cell is shown in Fig. 3.

A first demonstration of the efficacy of the system has been made. The test biological cell was an isolated cat cardiac myocyte. Living cells were produced in the sample-containing cell and analyzed with the x-ray beam. Trace elements were successfully observed from a single myocyte and, it is believed, the myocyte was not killed during the measurement because of the preservation of the proper cell shape during the experiment. The success of the prototype experiment is satisfying and demonstrates that the technique should be generally useful.

Chemical Speciation

The chemical state of elements in biological systems is of considerable importance in elucidating the role of trace elements in biological processes. A new NAT-type method that may be of importance in the future is the application of synchrotron radiation to the problem.

In this case a monoenergetic beam of x rays with narrow bandwidth is used to produce characteristic K- or L-shell vacancies in the element of interest. Careful measurement of the position and shape of the absorption edge shows energy shifts which are related to the oxidation state and bonding of the element in question. The synchrotron source is extremely valuable for this because it is the most effective way to produce a x-ray beam with sufficient intensity, energy resolution, and small beam size to make the method useful for biological applications. The feasibility of this technique reviewed by Gordon (14) has been demonstrated on compounds of selected elements including V, Mo, Fe, Cu, and S. It remains to be seen whether chemical speciation using synchrotron radiation will develop into a generally useful analytical method or whether it will be of use only in limited situations (14).

ION BEAM ANALYSIS

Ion beam analysis is a term that includes a rather diverse collection of nuclear reaction and scattering methods that are used to make isotopic or elemental identifications and to do sensitive depth profiling determinations. The reactions depend to a great extent on the type of accelerator that can be used for the work.

The accelerators that are generally available for applied research are those of relatively small size that are no longer primarily used for nuclear physics experiments, or those that have been commissioned explicitly for analytical work. The majority of the applications to date have been in materials sciences, but there is increasing activity in the earth sciences and in life sciences as well.

The main application of ion beam analysis tends to be in the region of low atomic number, i.e., below Z = 20. The methods and technology relevant to

the work constitute all of low energy nuclear physics and are too long and complex to summarize in a few words. The advanages are generally those of other NATs in general—to reiterate, small sample and beam size and good spatial resolution, coupled with minimal sample preparation as well as the ability to work with wet samples at atmospheric pressure (in some cases). The disadvantage, compared to PIXE, is that the nuclear cross sections are generally much smaller than the atomic cross sections which are the basis of PIXE. Hence, in most cases, sensitivity is less than for PIXE. The potential of ion beam analysis has been explored for some time, and it is ready for widespread application.

It will suffice here to illustrate these points with a single example. Inelastic scattering or nuclear reactions can be used to produce characteristic gamma rays which are uniquely identified with particular isotopes. Particular examples are ^{7}Li, ^{10}B, ^{19}F, ^{23}Na, and ^{27}Al. Deconninck and Bodart (15) used the special properties of NAT to make in vivo measurements of the fluorine content of the enamel in a tooth. A proton beam was collimated and then brought into the laboratory where it impinged on the tooth surface of a subject who had been carefully placed in the appropriate position. Gamma rays resulting from the $^{19}F(p,\alpha\gamma)^{16}O$ reaction were detected and used to estimate the number of fluorine atoms bombarded by the beam. This is perhaps a somewhat exotic type of application, but it vividly demonstrates the power of NAT in solving unusual analytical problems.

STABLE ISOTOPE TRACERS

Stable isotope tracers can be used to advantage in cases where radioisotopes present problems in terms of the radiation dose to the patient or when suitable radioisotopes are not available. Stable tracers have not

been as widely used as radioactive tracers which is due, in part to a lack of detection methods that provide high spatial resolution and sensitivity (16).

This general idea was of interest to a Brookhaven group some time ago and was pursued far enough to show that nuclear techniques could be used and that there were biological applications of some interest (17). In brief, Slatkin et al. (18,19,20) proposed to use deuterium as a stable tracer in place of tritium, for example, for work that involved children or pregnant women as patients. Deuterated thymidine could be administered and the following incorporation in cells and tissue studied in order to ascertain the rate of tumor growth.

The nuclear detection method employed the $D(t,n)^4He$ reaction produced with a triton beam at the Brookhaven Research Electrostatic Accelerator Laboratory (REAL). It was possible to detect individual labelled lymphocytes in blood with a spatial resolution of about 15 μm and a minimum detection limit of around 10^8 deuterium atoms. A microphotograph of the nuclear track detector with clusters of tracks from such a labelled cell is shown in Fig. 4. It was possible to differentiate between labelled and normal cells and from the background deuterium in the sample backing. Estimates of enrichment factors showed that the sensitivity attained should be sufficient for the proposed applications.

Following this original study there have been several developments in position-sensitive nuclear particle detectors and ancillary systems which could be of use in improving the experimental technique. It is timely to consider implementing either the detection scheme outlined here or some other new method, such as LAMMA, to extend the use of deuterium as a tracer to an extensive laboratory and clinical test.

The use of Accelerator Mass Spectrometry (AMS) should also be worth looking at in the context of stable tracers (21). Presently AMS is known for

its extremely high sensitivity and small sample size requirements. It is thus eminently suited for experiments where spatial resolution is of minor concern. In the future, if it is possible to develop ion sources that have micron-sized sputtering beams, it conceivably can become a supremely useful new device for biomedical research.

CONCLUSIONS

A number of applications of nuclear analytical techniques to biomedical problems have been surveyed briefly. From this survey it can be seen that there is a wide spectrum of techniques available to solve problems that cannot be dealt with effectively by more conventional analytical methods. It is also clear that NAT requires specialized equipment and staff. Multidisciplinary groups, working as a recognized part of an accelerator laboratory or even at a laboratory which is entirely dedicated to this type of work and containing an accelerator that has no other function than to be used for analytical workare essential. This requirement is increasingly being recognized, and there are a number of groups that are effectively constituted in this way. Future NAT work will require an even closer working relationship between the biological community and the physicists and chemists working on technique development.

The last important requirement is recognition of the multidisciplinary nature of NAT by the administrators of science. The usual breakdown of an organization along scientific disciplines is not entirely satisfactory and could well be modified to promote the formation of interdepartmental groups to work on the type of research discussed here. At the same time, it will be necessary to worry about a more flexible funding policy that can make it easier to give support to projects that fall outside the usually well-defined funding directions.

It seems clear that NAT are now eminently usable for many important types of biological experiments. Many types of NAT are still in their infancy so that more development, improvement, and extended applications can be expected in the near future. NAT can be considered as an established specialty at this time, and it should achieve greater visibility and importance as it becomes more available to the biomedical community.

ACKNOWLEDGMENTS

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Table 1

CHARACTERISTICS OF TRACE ELEMENT DISTRIBUTIONS AT DIFFERENT LEVELS OF BIOLOGICAL ORGANIZATION

Biological level	Distribution	Volume
- Population	- homogeneous	- $>10^{6}$ cm^3
- Individual	- heterogeneous	- 7×10^{4} cm^3
- Organ/tissue	- heterogeneous	- 10^{3} cm^3
- cell	- homogeneous	- 10^{-9} cm^3
- organelle	- heterogeneous	- 10^{-12} cm^3
- molecule	- heterogeneous	- 10^{-17} cm^3

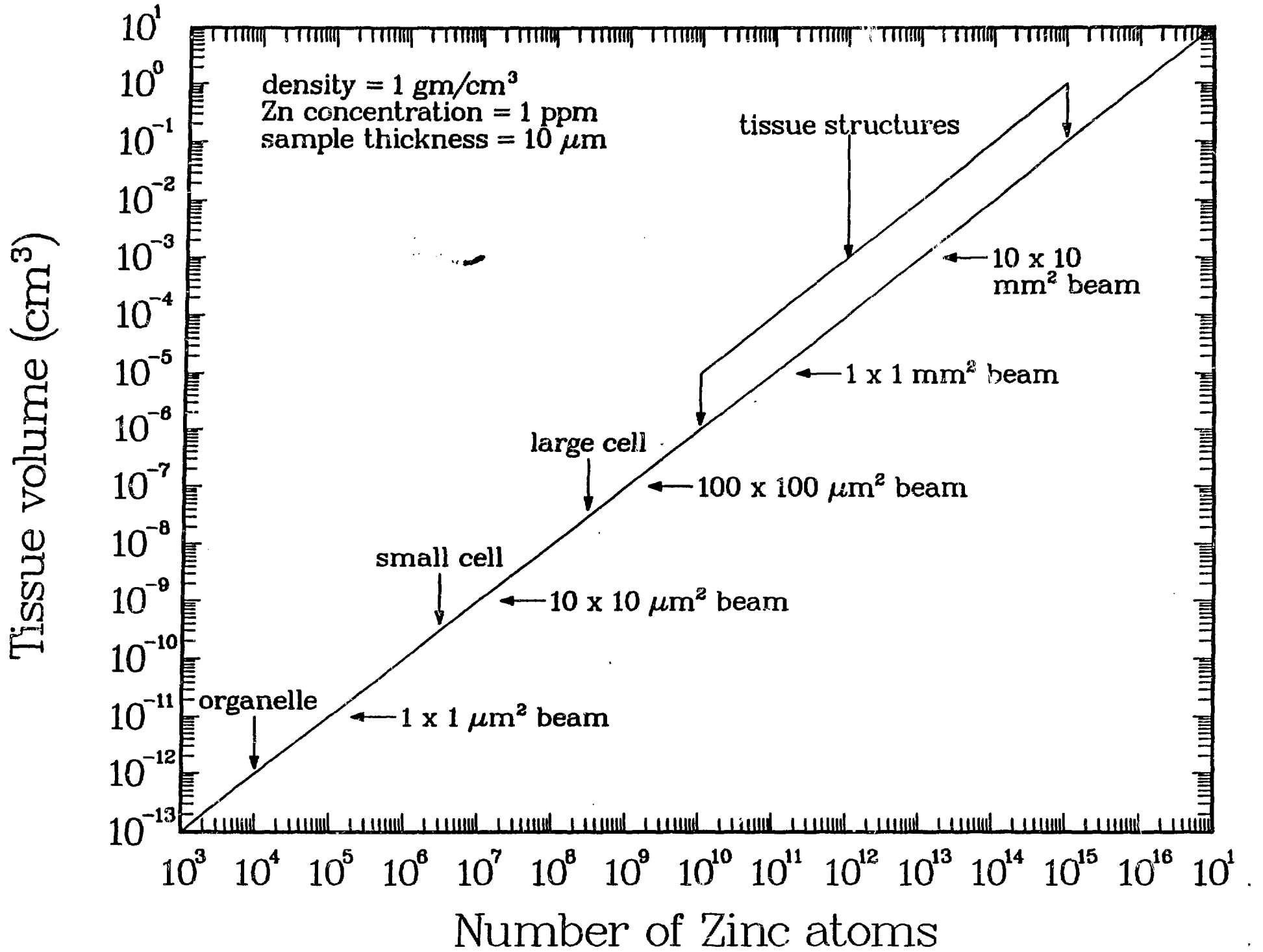
FIGURE CAPTIONS

Figure 1. The number of zinc atoms in a matrix with density one is given as a function of volume for a zinc concentration of 1 ppm. Sizes of typical biological structures are indicated and compared to typical ion and photon probe resolutions.

Figure 2. A SRIXE spectrum taken with a filtered white beam from the National Synchrotron Light Source. The sample was a 10-μm thick section of rat kidney cortex containing proximal convoluted tubule cells.

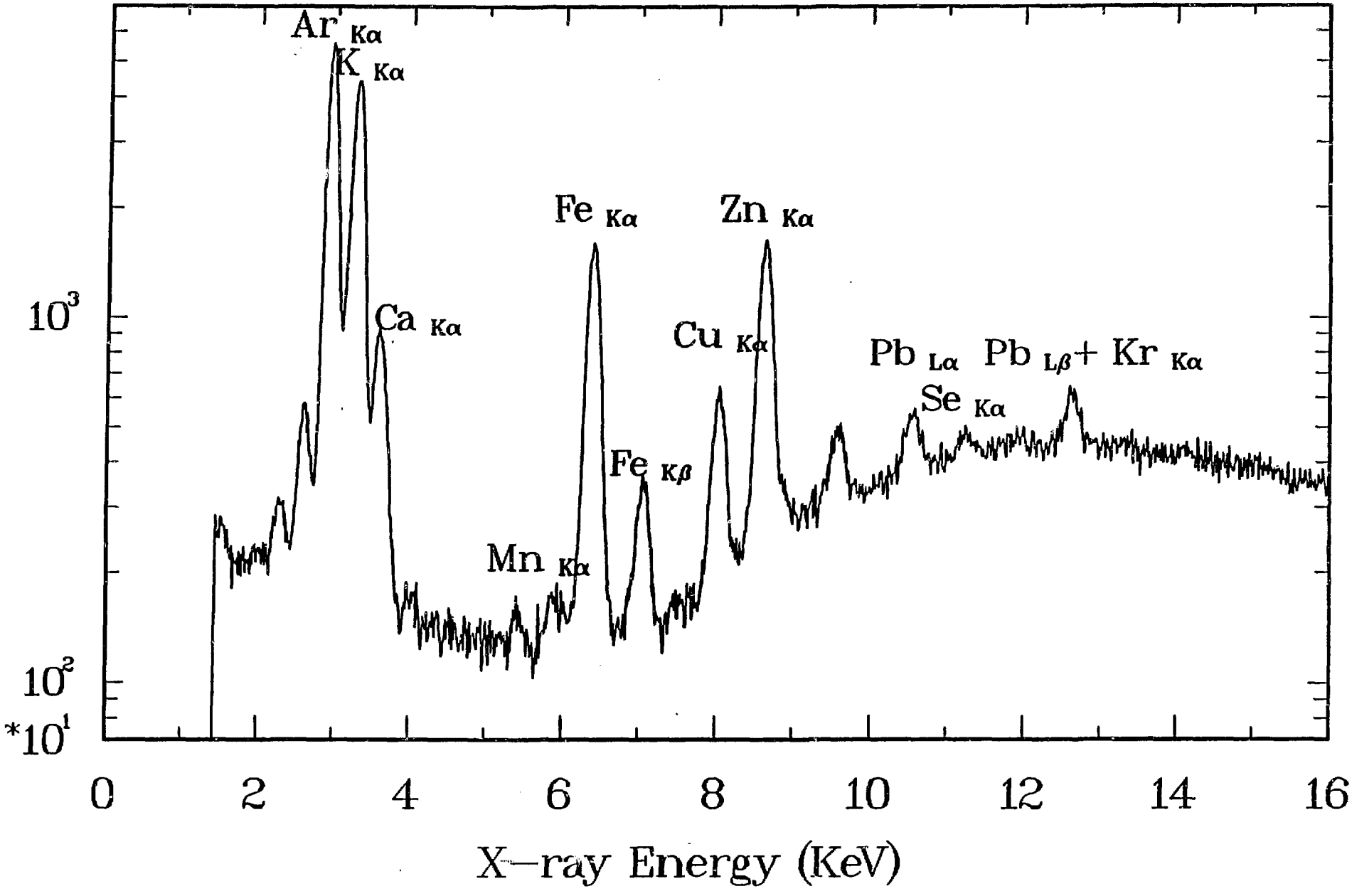
Figure 3. Schematic diagram of wet cell used for SRIXE trace element measurements.

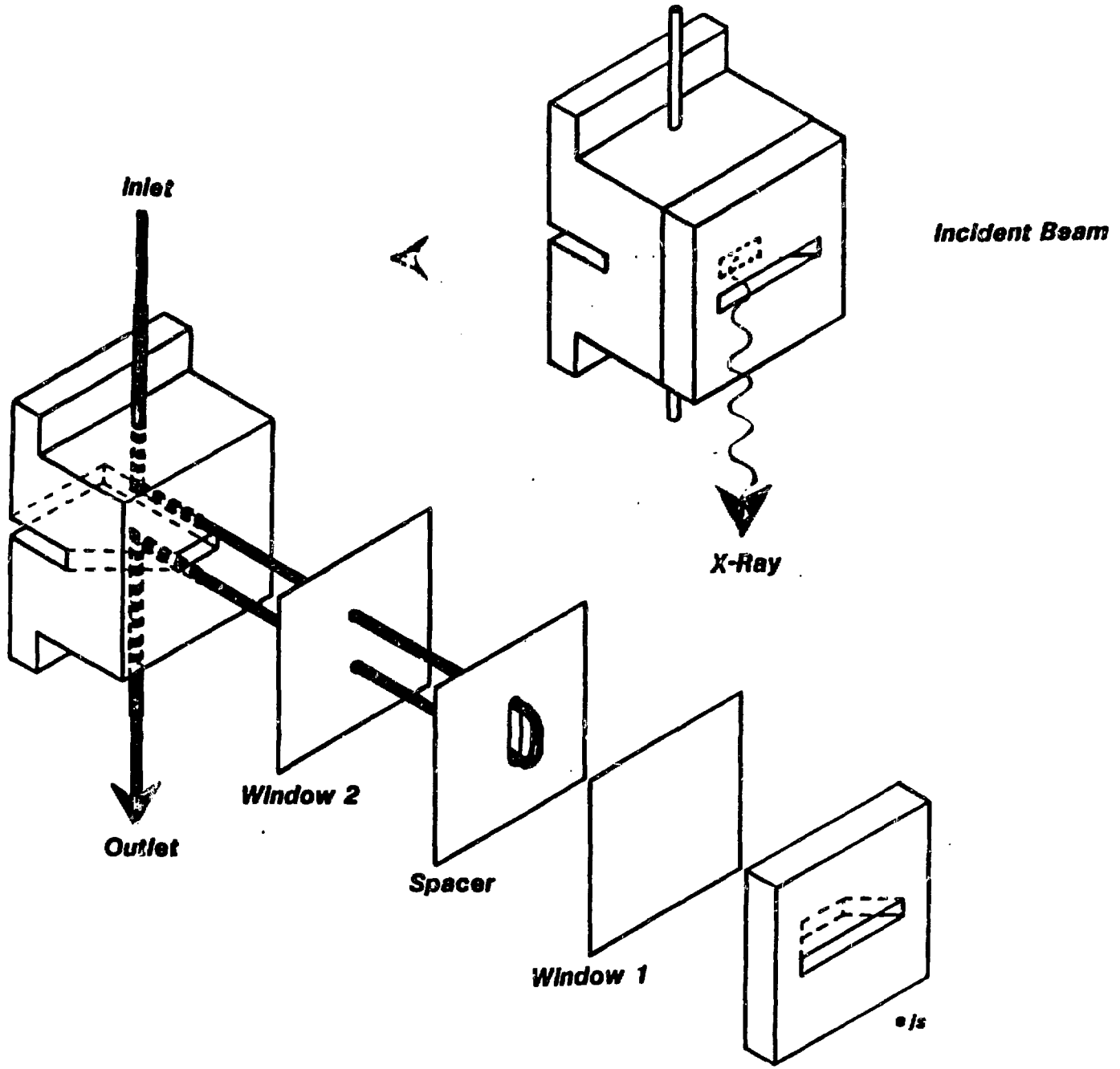
Figure 4. Microphotograph showing alpha particle tracks produced in a nuclear track detector during tritium bombardment of a deuterium-enriched leukocyte.


density = 1 gm/cm³
Zn concentration = 1 ppm
sample thickness = 10 μm
tissue structures
10 x 10 mm² beam
1 x 1 mm² beam
large cell
100 x 100 μm² beam
small cell
10 x 10 μm² beam
organelle
1 x 1 μm² beam
Tissue volume (cm³)
Number of Zinc atoms

KIDNEY (Cortex, 013–A, Pb–23)

Time (sec):	459.0	Structure:	proximal tubule	Aperture:	5 mm Ag
Ion chamber:	2.06	Radiation:	white	Beam Filter:	275 μm Al
IIC (scale):	namp	Slits(μm)	100 x 100	Detector Filter	7.3 μm Kaptor




Inlet
Incident Beam
X-Ray
Outlet
Window 2
Spacer
Window 1