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X-ray fluorescence (XRF) has long been used to make measurements of trace element concentrations in biological materials with very high sensitivity. It has not been previously possible to work with micrometer spatial resolutions because of the relatively low brightness of x-ray tubes. This situation is much improved by using synchrotron storage ring x-ray sources since the brightness of the synchrotron source is many orders of magnitude higher than is obtained with the most intense tube sources. These intense sources open the possibility of using the XRF technique for measurements with resolutions of approximately cellular dimensions. Developments in the synchrotron source and associated optical components over the next ten years should result in at least an order of magnitude improvement in resolution. A description of a current research project at Brookhaven which uses synchrotron radiation induced x-ray emission (SRIXE) is presented to illustrate a specific application of the method in biology. (The acronym SRIXE is used in analogy to PIXE, which refers to proton- (or particle-) induced x-ray emission.) The

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example is chosen from the biological area, but obviously SRIXE can be more widely applied.

Our SRIXE research is conducted on the X-26 bending-magnet beam port at the National Synchrotron Light Source (NSLS). Two separate facilities will be available. The first one, which is now operational, uses the white light from the synchrotron. The second one, presently being fabricated, uses a monochromator and an ellipsoidal focussing mirror, to produce an image of the x-ray source of approximately 30 μm. Imperfections in the mirror will result in aberrations about the focussed spot, so a pinhole will be required to produce a clean image.

The target area for the white light beam line is shown in Figure 1. The experiment must be carried out in a shielded enclosure (hutch) to protect personnel from exposure to the x rays. The x rays are transported to the hutch through a beam line which is isolated from the storage ring by a 254-μm beryllium window. The window modifies the x-ray spectrum, but eliminates the necessity to make measurements in a vacuum. A second 254-μm beryllium window is used in the hutch to separate a gas target region, used for atomic physics work, from the beam line vacuum. The photon intensity is monitored by a gas-filled ion chamber with 7.5-μm Kapton windows before and after stepper-motor-driven collimating slits which can be closed to 10-μm size. The photon beam is brought out into the laboratory air, helium, or vacuum, for final use. The samples are mounted on stepper-motor-driven x-y-z stages which can be adjusted in 1-μm steps. The sample can be mounted in a controlled wet environment if desirable. A ZnS scintillator screen is used to quickly position the beam by use of a microscope-TV sample-viewing system. Further measurements are then made relative to that fiducial mark.

Knowledge of the energy spectrum of the x rays is obtained by calculation from the basic theory of synchrotron radiation. Figures 2 and 3 show the results obtained from such a calculation for our white light beam line. We have done the calculation for an area of 1 mm^2 located in the plane of the beam and with a beam current of 1 mA. Since the NSLS is now operating with currents of around 150 mA at injection, the values shown in Figure 2 can be used to estimate sensitivities for trace element determinations in a given sample area. Sensitivities for trace element detection can be estimated and measured and are in the region of 10 to a few hundred parts per billion (ppb) depending on the exact assumptions made regarding detectors and x-ray energy spectrum.[1]

In biology, the interaction of toxic and essential elements is of considerable importance in elucidating the mechanism of action of toxic elements in biological systems. It is necessary to characterize and understand these interactions at the cellular level as interactions in particular cells may be obscured by the surrounding matrix when conventional bulk chemical analyses are used for trace element determinations. SRIXE is ideal for investigating the interaction between lead, a nonessential element of considerable health significance, and Ca, Fe, Cu, and Zn in brain and other target tissues for lead toxicity. Many brain structures are 0.01 to 1 mm in at least one dimension and have a complex topography. These structures are exceedingly difficult to dissect for conventional analyses, yet sections are easily prepared from frozen whole rodent brains. The full surface of the section is available for visual inspection, and the selected brain structure can be identified and its trace element content measured. The trace element content of the cerebellum is heterogeneous at the microscopic level. The

molecular cell layer, the granule cell layer, and the fiber tracts have a characteristic trace element content which can be related to the biochemical function of these structures. Figure 4 shows SRIXE trace element spectra for several locations on a mouse cerebellum. The spatial resolution is about 50 μm. The characterization of trace elements in these structures provides the framework for investigating the role of trace element interactions in neurobiology and disease processes.

References

1. See the summary given by A. L. Hanson et al., "Mapping of trace elements with photon microprobes: x-ray fluorescence with focused synchrotron radiation," Microbeam Analysis--1985, 227-229, and references cited there.

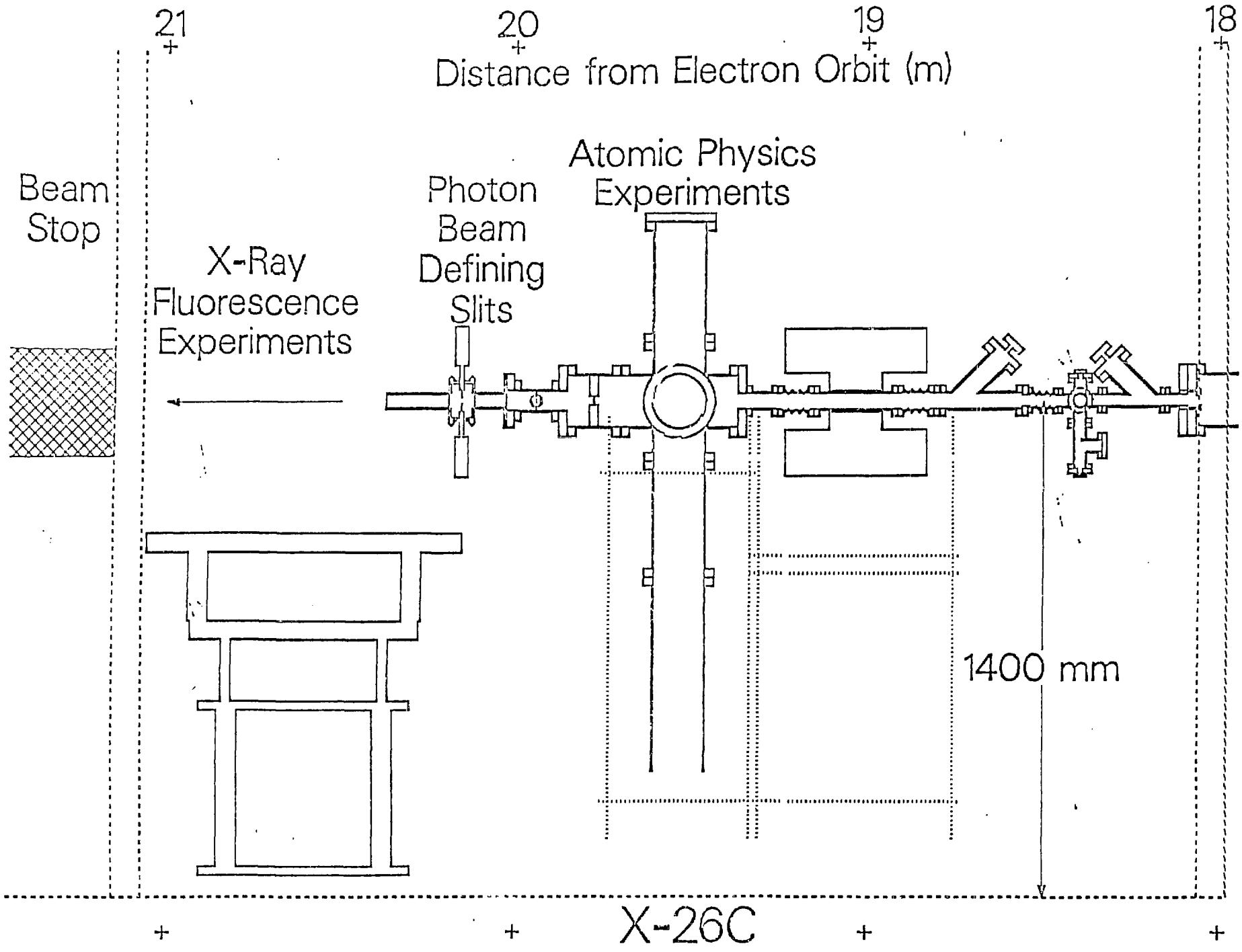
Figure Captions

FIG. 1--Schematic diagram of experimental enclosure.

FIG. 2--Energy spectrum of photons for: unfiltered beam, atomic physics beam, and x-ray fluorescence beam. Unfiltered beam is at top, atomic physics beam in middle, and x-ray fluorescence beam on bottom. Parameters are listed above.

FIG. 3--Total number of photons/mm^2-mA with energy above E, 20 m from electron orbit.

FIG. 4--SRIXE spectrum from 20-μm section of rat cerebellum. 150-μm spot beam size was used.


21
20
19
18
Distance from Electron Orbit (m)
Beam Stop
X-Ray Fluorescence Experiments
Photon Beam Defining Slits
Atomic Physics Experiments
1400 mm
X-26C

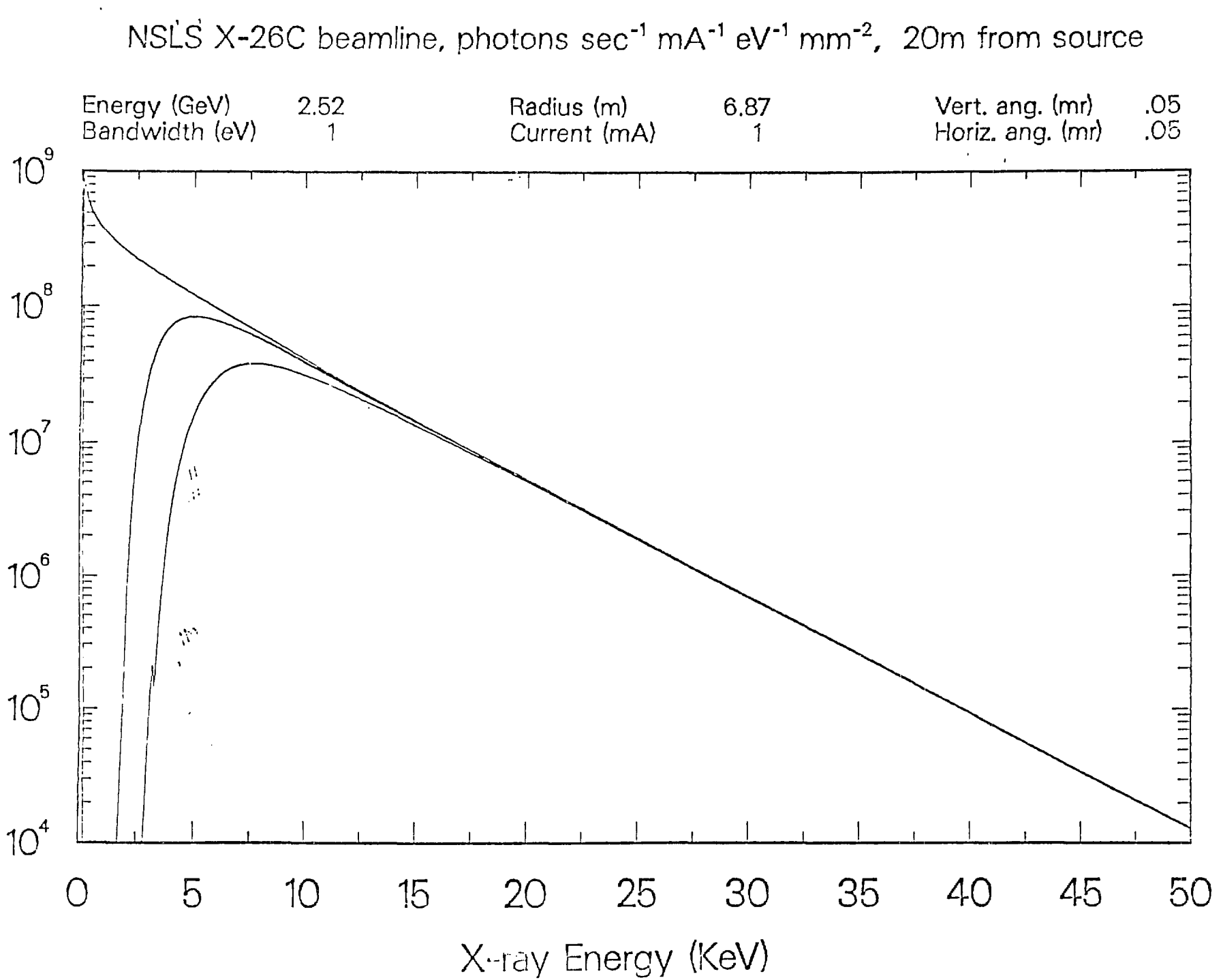

NSLS X-26C beamline, photons sec^{-1} mA^{-1} eV^{-1} mm^{-2}, 20m from source
Energy (GeV) 2.52
Bandwidth (eV) 1
Radius (m) 6.87
Current (mA) 1
Vert. ang. (mr) .05
Horiz. ang. (mr) .05
10^9
10^8
10^7
10^6
10^5
10^4
0
5
10
15
20
25
30
35
40
45
50
X-ray Energy (KeV)

FIGURE 2

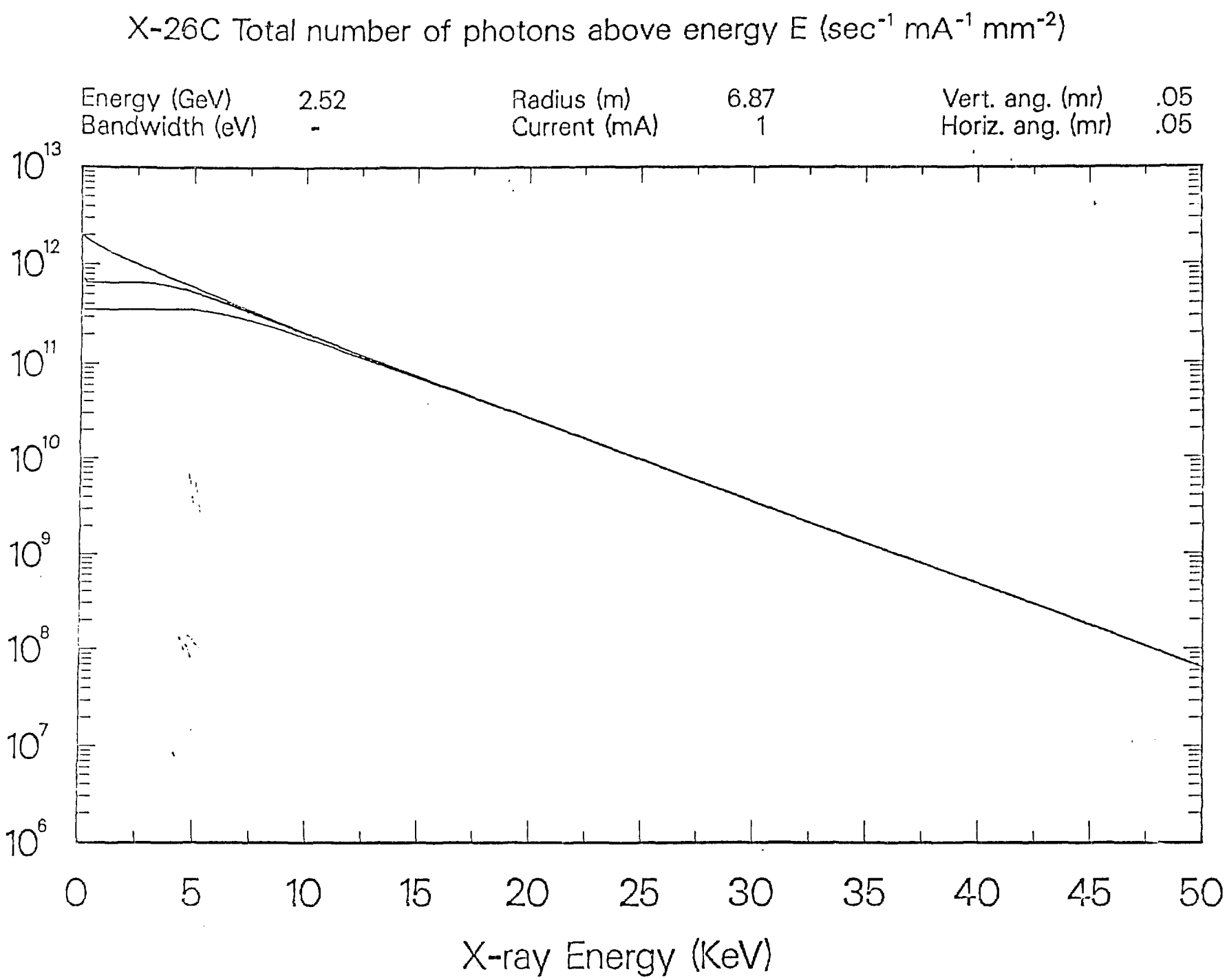

X-26C Total number of photons above energy E (sec^{-1} mA^{-1} mm^{-2})
Energy (GeV) 2.52
Bandwidth (eV) -
Radius (m) 6.87
Current (mA) 1
Vert. ang. (mr) .05
Horiz. ang. (mr) .05
10^{13}
10^{12}
10^{11}
10^{10}
10^{9}
10^{8}
10^{7}
10^{6}
0 5 10 15 20 25 30 35 40 45 50
X-ray Energy (KeV)

FIGURE 3

NIH03.23 CEREBELLUM (White Matter, 103–A, Pb–21)

Time (sec)	332.0	Detector	Si(Li)	Aperture (mm)	5
Ion chamber	2.28	Slits (mm)	0.15, 0.15	Primary Filter	16 mil Al
Energy (KeV)	white	Distance (mm)	40	Detector Filter	Kapton

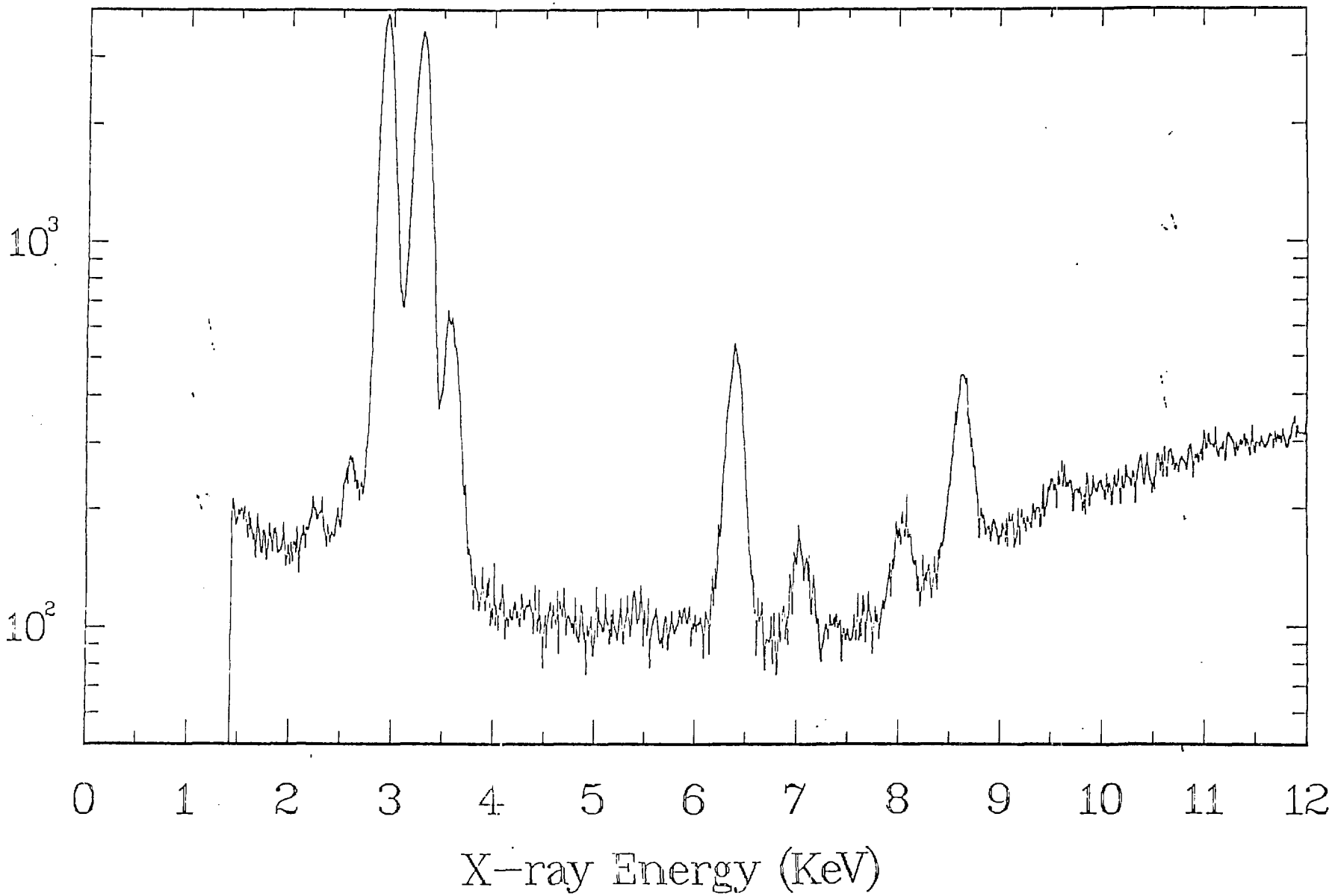


FIGURE 4