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# FT-IR MICROSCOPICAL ANALYSIS WITH SYNCHROTRON RADIATION: THE MICROSCOPE OPTICS AND SYSTEM PERFORMANCE

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When a Fourier transform infrared (FT-IR) microspectrometer was first interfaced with the National Synchrotron Light Source (NSLS) in September 1993, there was an instant realization that the performance at the diffraction limit had increased 40 - 100 times. The synchrotron source transformed the IR microspectrometer into a true IR microprobe, providing high-quality IR spectra for probe diameters at the diffraction limit. The combination of IR microspectroscopy and synchrotron radiation provides a powerful new tool for molecular spectroscopy. The ability to perform IR microspectroscopy with synchrotron radiation is still under development at Brookhaven National Laboratory, but several initial studies have been completed that demonstrate the broad-ranging applications of this technology and its potential for materials characterization.

## Introduction

IR microspectroscopy (IMS) is the union of microscopy and spectroscopy for purposes of microanalysis. Light microscopy provides a means by which to generate magnified images, resolve microstructural detail, and record images to facilitate their interpretation. IR spectroscopy provides the means for analyzing the molecular chemistry of materials. Because of its longer wavelengths, IR radiation limits the spatial resolution that can be achieved, but its ability to resolve chemistry through the absorption of IR radiation is of great importance. Combining light microscopy and IR spectroscopy permits the correlation of microstructure with molecular chemistry to characterize materials.

The performance of the IR microspectrometer is increased with the use of synchrotron radiation. The NSLS provides a small, high-brightness source of IR radiation. Performance is metered by spatial resolution and spectral quality. When coupled with the IR microspectrometer, high signal-to-noise (S/N) spectra are obtained from sample areas at or below the system's optical-diffraction limit.

In addition to its high intrinsic brilliance (100 - 1,000 times greater than a traditional thermal-emission source), the synchrotron offers other advantages.[1-3] Because it is a non-thermal source, synchrotron radiation produces less source noise. Synchrotron radiation is also highly collimated, which improves both spectral and spatial resolution.

High brightness, low noise, and improved optical performance make the synchrotron an ideal source for IMS. The spatial resolution and spectral performance that resulted from coupling the IR microspectrometer with the synchrotron source illustrate the primary advantages of this union.

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In various analytical applications, FT-IR spectroscopy has been used in the biological, earth, and materials sciences for nearly a decade. Specific applications of IMS to biology, polymer characterization, forensic science, geology, and cultural materials are reviewed to illustrate the usefulness of this technology.

## Instrumentation

The IR microspectrometer that was interfaced with the synchrotron was an IRμs scanning IR microprobe (Spectra-Tech, Inc./Shelton, CT). This system consists of an integrated FT-IR spectrometer and microscope optical module. Interfacing with the synchrotron source required removing a source-collimating mirror and mechanically coupling the synchrotron source to the optical module. (A schematic of the IRμs microspectrometer is shown in Figure 1.) The present experiments were performed in the mid-IR region (2.5 - 15 μm, or 4,000 - 650 $cm^{-1}$). The microscope optics used were 32X, 065 NA, and 15X, 0.58 NA objective lenses. In transmission, the condenser used was a 10X, 0.71 NA. Both objectives and condenser were Schwarzschild reflecting designs.

The IRμs system contains a rapid-scan Michelson interferometer with a germanium-coated KBr beam splitter. Spectral resolution can be selected from 2 - 16 $cm^{-1}$. The detector used in all synchrotron experiments was a 0.25-$mm^2$ mercury-cadmium-telluride (MCT) element with a low-energy spectral-sensitivity limit of 600 $cm^{-1}$ (16.7 μm). This detector was cooled with liquid nitrogen.

## Performance

The primary interest in synchrotron radiation as a source for IMS is to improve the performance of the instrumentation so as to record high S/N spectra at or below the optical-diffraction limit for the microscope's optical system. There are three tests for improved performance: (1) determine the beam's profile in order to establish that diffraction-limited spot size has been achieved; (2) measure the S/N of spectra obtained at the diffraction limit; and (3) determine the spatial resolution of the microspectrometer system by determining the system's ability to resolve chemical features at the molecular level. While these preliminary studies demonstrate significant improvement in performance, it is believed that the full potential of synchrotron radiation has not yet been reached.

The profile of beam intensity in the sample plane established that the IR beam was focussed to a diffraction-limited spot size of less than 12.5 μm. The profile was measured by translating a 12.5-μm-diameter circular aperture in 10-μm steps across the sample plane. The center of the field of view was taken as the origin, and the horizontal field's diameter (the X axis) and the vertical field's diameter (the Y axis) were scanned in 10-μm steps, the intensity at each point being recorded as the peak height of the interferogram. The beam's intensity-profile data are reported in Figure 2. These data show that the maximum intensity is observed at the 0,0 position and that the beam's diameter at half of its maximum intensity is below 12.5 μm. When the apertured beam's intensity was ratioed to the un-apertured beam's intensity, it was found that 61% of the incident radiant energy passed through the 12.5-μm-diameter aperture. These data were collected with a 32X, 0.65 NA objective, which has a theoretical diffraction limit for 10-μm radiation of 9.4 μm. The experimental beam's profile is consistent with the diffraction-limited performance of the microscope's optics.

A further test of the beam's geometry and system performance is to measure the resolution by recording spectra of a material that contains well-defined compositional boundaries. A cross-section of a multi-layered, laminated, photographic emulsion was used to test this system's resolution. The test laminate selected was a 5-layered polymer film on a paper backing. The thicknesses of the 5 polymer layers were 6, 4, 4, 8, and 32 μm. A series of IR-absorption spectra were recorded across the layers of the film at 1-μm intervals using a 6 x 6 dual-confocal-apertured sample area. The variations of the chemical composition across the layers were readily detected. Spectra were collected over a range of 4,000 - 650 $cm^{-1}$ and a resolution of 8 $cm^{-1}$, and 16 scans were co-added for each spectrum. The total acquisition time was 5 seconds per spectrum. This series of spectra profiles the composition across a visibly-sharp boundary between these layers. In the IR region, the observed spreading equalled the calculated diffraction-limited beam's diameter.

A series of spectra showing the spatial resolution observed when "stepping" across a compositional boundary is shown in Figure 3. These spectra represent the visibly-sharp boundary between a 6-μm-wide gelatin layer and a 4-μm-wide ethylene-vinyl-acetate layer. The spectral region shown contains the amine bands of the protein and the carbonyl band of the vinyl acetate. The interval between the reported spectra is 2 μm. In the top spectrum, the amide 1 band (at 1650 $cm^{-1}$) is the strongest band, with a small shoulder in the 1740 $cm^{-1}$ region. The bottom spectrum, collected 6 μm from the position where the top spectrum was collected, has the strongest band (at 1740 $cm^{-1}$), with a weak shoulder in the 1650 $cm^{-1}$ region.

In this experiment, the synchrotron's radiation beam was apertured to define a 6-x-6-μm area in the sample plane. This sample definition was achieved by using dual-remote confocal masks. This experiment shows that traversing a sharp boundary between these two phases produces a chemically-specific resolution at or below the optical system's diffraction limit. Scanning across a sharp boundary with a 6-μm-diameter aperture would (theoretically) produce a maximum resolution equal to the 6-μm-diameter aperture; diffraction effects would increase this apparent width. Since these data show separation at the 6-μm level, it is clear that diffraction makes a minimum contribution in this experiment. With the synchrotron radiation, we have achieved superior resolution to that which can be obtained with a normal thermal-emission source.

The S/N performance of the IRμs system with a thermal-emission source at the diffraction limit is greatly improved by the use of the synchrotron source. For example, the spectra shown in Figure 3 were collected at the diffraction limit and have a S/N ratio of 250:1. These spectra were collected by co-adding 16 scans. For the same sampling geometry, the conventional thermal source would require co-adding 256 scans to produce a S/N ratio of 25:1. This represents at least a 40-fold improvement in S/N performance in this experiment. While this is an excellent improvement, it is believed that the system was not optimized and that the synchrotron source is capable of providing an order of magnitude of greater intensity. This improvement in S/N performance at the diffraction limit will greatly expand the areas of application for IMS.

## Applications

During the past decade, IMS has been applied in various scientific disciplines to explore the molecular structure of materials at the microscopic level. These applications have crossed the boundaries of many scientific disciplines. Examples of applications in various fields are as follows:

*Biological Science*

The microstructural characterization of biological systems is a primary method for investigating structure. Throughout this past decade, several researchers have applied IMS to gain more insight into the chemical composition of biological materials. For instance: Atherosclerosis is a disease of the large arteries, resulting from the accumulation of lipids and other components in the arterial wall. Kodali used FT-IR microspectroscopy to probe the sub-cellular composition of atherosclerotic arterial walls and to create 3-dimensional images representing changes in composition.[4] The presence of cholesterol esters and other ester-containing compounds was identified in the presence of protein. The data clearly showed that changes in the chemical composition of an arterial wall varied with the physiological and morphological changes within the artery.

Wetzel and Levine examined cross-sections of brains of rats that had been given a stereotactic injection of blood into their subcortical white matter.[5] This model mimicked the pathology of an extra-vasated blood lesion, which can be caused by various problems (such as head trauma and stroke). By IMS, the authors found that the chemical functional groups normally enriched in white matter were altered at the lesions' site and obtained evidence that lipid peroxidation was the mechanism responsible for these chemical changes. These changes in composition were recorded in 3-dimensional maps of the intensity bands as a function of their geometrical position within the tissue section. Wetzel and Levine have extended their brain studies to the examination of white matter from Shieverer mice, which have a genetic defect in the myelin basic protein gene that results in a deficiency in the deposition of myelin. Through IMS mapping, the authors found that the functional groups characteristic of white matter in normal animals were significantly reduced in these mice.

The single human erythrocyte was studied by Dong using FT-IR microspectroscopy.[6] The spectrum of a single red blood cell was comparable to spectral data obtained by isolating red blood cells and preparing them for macro-analysis. The single-cell spectra contained all of the pertinent information about both the characteristic protein's secondary structure and bound carbon monoxide. Single-cell IR spectroscopy permitted the qualitative and quantitative determination of differences among individual cells. Further studies of single red blood cells showed that different mutant forms were identifiable through IMS.

*Polymer Science*

Diffusion profiles of additives in polymers were studied by Lin-Vien using the IR microprobe to study the diffusion of Cyasorb UV531 in polypropylene.[7] By measuring the IR intensities of bands specific to UV531, concentration profiles were analyzed at different temperatures to determine diffusion coefficients. In these studies, a "sandwich" of polypropylene plaques with the Cyasorb powder placed between and heated for specified periods of time created concentration gradients that could be studied by IMS. The plaques were cross-sectioned and concentration profiles measured by IMS. The quantitative agreement of the diffusion coefficients determined by IMS was in excellent agreement with prior data. The advantage of IMS was that the experiments could be performed rapidly and more precisely.

Polymer-dispersed liquid-crystal films were studied by FT-IR microspectroscopy to compare the polymer and liquid-crystal droplet regions.[8] Spatially-resolved functional-group maps of a

droplet showed characteristic textures corresponding to the visually-observed morphology of the droplet. These studies also showed the effects of applied electric fields on changes in the orientation of functional groups within liqud-crystal droplets. Using microspectroscopy, it was possible to correlate the microscopic texture with the chemistry and with the changes that occur when electric fields are applied. IMS applications for the elucidation of molecular structure and order of polymers were reviewed by Chalmers.[9] In other recent work, spatially-resolved IMS has been used to study the changes in polymer molecular structure through welds in polypropylene and in the profiling of polymer aging.[10-12] The applications of IMS in the polymer industry are extensive, particularly in the area of food-packaging materials, which are generally made of polymeric composites. The studies of cross-sections are used to establish the chemistry and the nature of bonding between various layers in the polymer laminates. These packaging materials are used extensively throughout the food, drug, and cosmetic industries.

*Earth Sciences*

Measurements of hydrogen in $MgSiO_3$ perovskite by Meade is an example of recent work using synchrotron with IMS.[13] Synchrotron IMS provided the high S/N necessary to quantitate the trace amounts of hydrogen in the nominally anhydrous perovskite. Microscopic single crystals were examined that had been grown directly from $H_2O$-rich melts of $MgSiO_3$ at a pressure of 27 GPa and at a temperature of 1830°C. The quantitative analysis of the hydrogen in these materials led to the conclusion that, when integrated over the volume of the lower mantle, this concentration is comparable to 12% of the mass of hydrogen in the earth's atmosphere.

Individual hydrocarbon fluid inclusions in intracrystalline cavities can be observed in minerals such as feldspar, quartz, halite, fluorite, and carbonate. Methane, carbon dioxide, liquid water, aromatic ester, and linear (or branched) alkanes were identified by Barres using IMS.[14] Hydrocarbons in aqueous inclusions give valuable information regarding the chemical and thermal regimes during the evolution of sedimentary basins; thus, it is especially important to study these inclusions. IMS techniques have been used to document the evolution of coal chemistry from lignite to anthracite and to determine chemical variations of mascerelles in coals. Mastalerz was able to determine variations in the chemistry between and within macerals, depending upon the coal rank using IMS.[15] In this study, IMS was combined with electron microprobe data, the latter providing elemental information to supplement the analysis of the organic constituents in the coal by IR spectroscopy.

Silicates, carbonates, phosphates, sulphates, and nitrates are readily analyzed by IMS. Since these are common functionalities of geological materials, the applications of IMS in earth sciences will be extensive.

*Forensic Science*

IMS has become a primary technique for the analysis of trace evidence. The technique is used to identify fibers, paint fragments, cosmetics, hair treatments, plastics, gunshot and explosive residues, inks, copy toners -- and the list would go on and on. The light microscope has been the primary tool for the trace forensic analyst, but IMS adds a new dimension to the evaluation of evidence. A new approach to forensic analysis with IMS was recently reported by Bartick.[16] In this preliminary study, the application of microscopical internal-reflection spectroscopy for the analysis of forensic evidence was reported. The micro-ATR technique is an extension of IMS that permits the analysis of materials' surfaces with little or no sample preparation. Micro-ATR

with synchrotron radiation has not yet been explored; however, it promises to be a useful technique for intractable samples.

Another interesting application of IMS to forensic science is the work of Kalasinsky on the deposition and visualization of drugs of abuse in human hair.[17] IMS is a unique method for analyzing drugs of abuse in hair, since it has the ability to analyze only the central core (medulla region) of a cross-sectioned hair. Kalasinsky has found that drugs of abuse are concentrated in the hair's medulla. With longitudinal cross-sections of hair, the deposition of the drug can be monitored as a function of the hair's length, providing a historical record of drug use. This promises to be a unique technique for evaluating an individual's habitual use of drugs of abuse.

*Cultural Materials*

IMS is being used to identify objects of art and archaeological artifacts. The composition and layered structure of paints in a work of art can be as characteristic of the artist and of the time period as can the style in which it was painted. Detailed studies of paintings often include microscopic analysis of paint layers' cross-sections as well as pigment identification. IMS has been shown to be extremely useful in characterizing binding media and pigments in very small paint cross-section samples. Derrick has used IMS to characterize many works of art and has compiled a library of reference spectra of artists' materials.[18] The analysis of paints and pigments is extremely important to art conservationists. One of the most important documents examined by the Getty Conservation Institute was the Dead Sea scrolls. The dyes on archaeological textiles from the Paracas Necropolis (400 B.C. - 400 A.D.) in Peru and from Etowah Mound C (c. 1200 A.D.), located in northwestern Georgia, were analyzed by Martoglio using IMS.[19,20] The analysis of these dyes provided specific identification that allowed insights into the histories of these peoples.

## Conclusions

IMS is a basic technique for the molecular characterization of materials that can be extended to new levels of performance and resolution by the use of synchrotron radiation. With synchrotron radiation, IMS can be performed at the diffraction limit of the IR radiation, and spectra with high S/N quality can be obtained. An improvement in performance of at least 40 times has been achieved with the promise that, when the system is refined and optimized, perhaps an improvement of 1000 times is realizable. The synchrotron is an ideal source for IMS, and the combination of these two techniques will extend the technology to ever-increasing applications.

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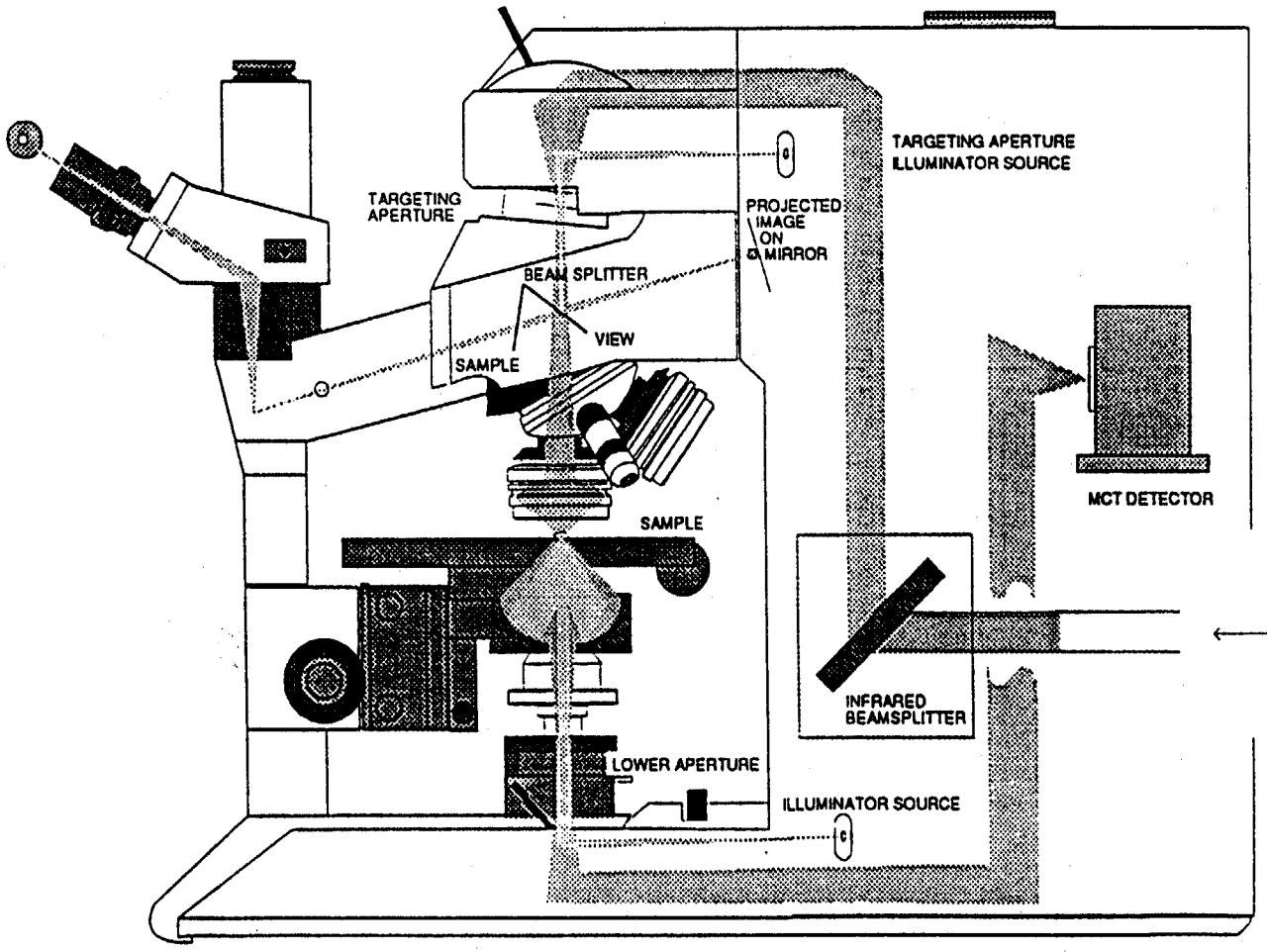
## Figure Captions

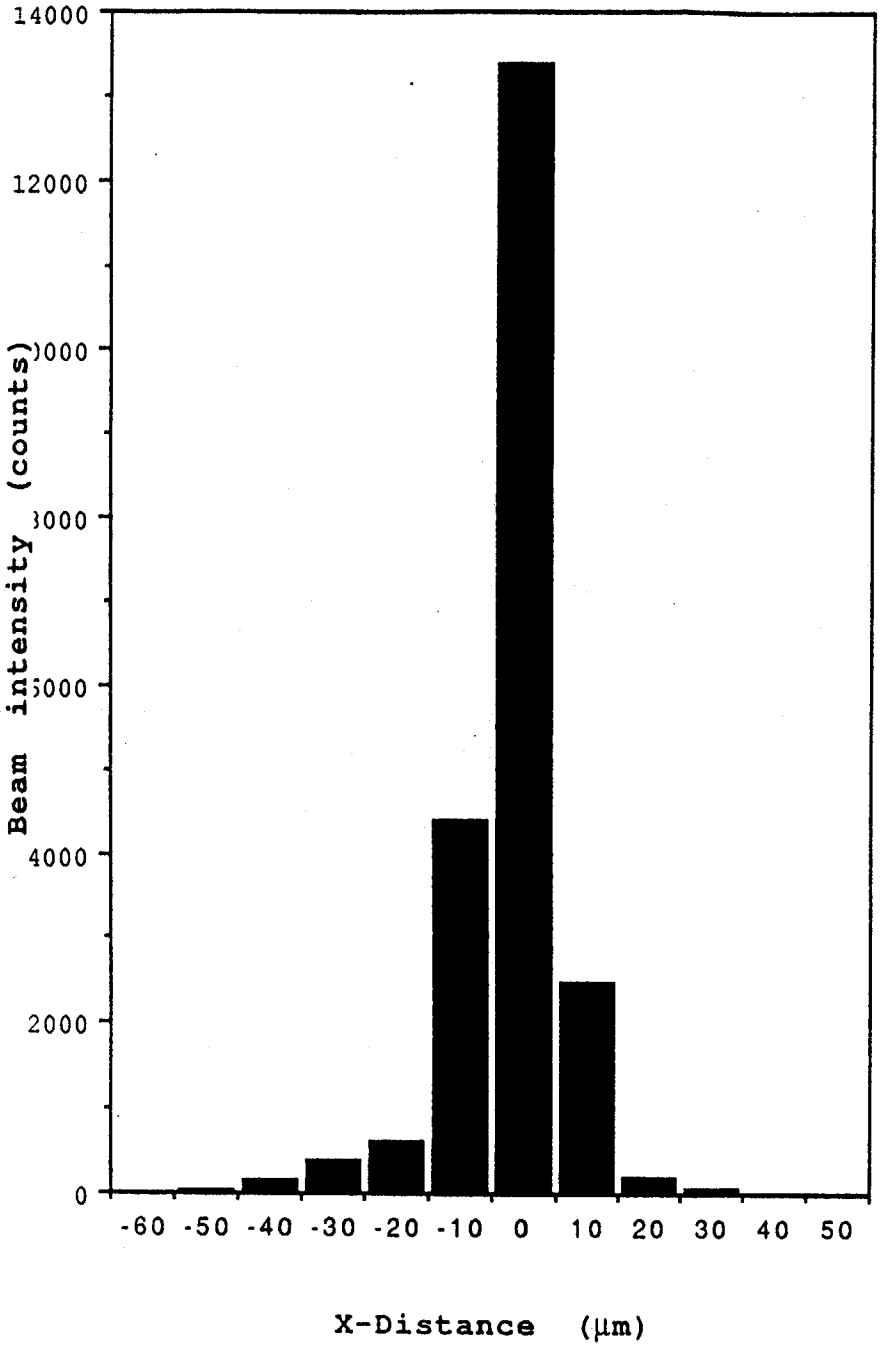
Figure 1. Schematic diagram of the IRμs scanning infrared microspectrometer.

Figure 2. Histogram profiles of infrared beam intensity showing the integrated intensity through a 12.5-μm-diameter circular aperture stepped in 10-μm intervals.

Figure 3. Spectra across a visibly-sharp interface of gelatin (protein) and polyethylene (vinyl) acetate, recorded with a 6-x-6-μm aperture. The sample was stepped 2 μm between each spectrum.

# *IR μs*™ Molecular Microspectroscopy System

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12000
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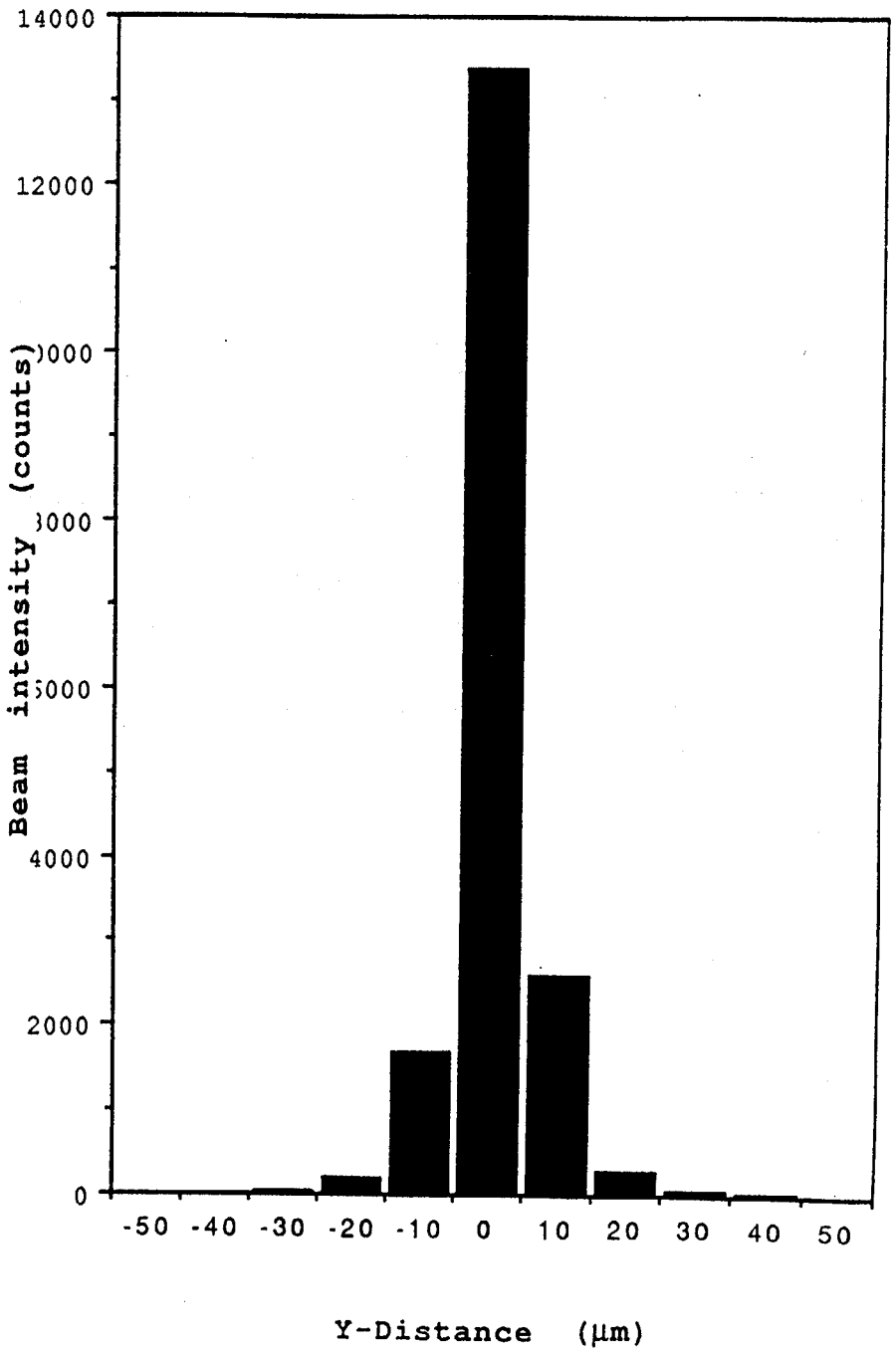
X-Distance (μm)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12000
Beam intensity (counts)
4000
2000
0
-50 -40 -30 -20 -10 0 10 20 30 40 50
Y-Distance (μm)

MULTY LAYERED LAMINATE ANALYSIS WITH SYNCHROTRON RADIATION - NSLS

6 X 6 um CONFOCAL APERTURES, 16 SCANS, 8 cm-1
TWO MICROMETER STEPS