V. 1. Micro-beam Analysis System at Tohoku University

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Introduction

The nuclear microbeam has proved to be a powerful analytical tool by combining various analysis techniques such as PIXE, RBS, NRA, CT and STIM¹,². A microbeam system was constructed at the Tohoku University Dynamitron Laboratory for biological applications. The microbeam line was installed in July 2002 and has since been optimized to obtain a beam spot size smaller than 1 μm. In microbeam analysis of biological specimens, simultaneous measurement of structural and elemental properties is very important³,⁴ and can be obtained by combining PIXE, RBS and STIM analysis. This paper describes performance of the microbeam system and presents typical results of biological applications.

Microbeam Analysis System

The microbeam system is installed at the 4.5 MV Dynamitron accelerator at Tohoku University. Technical details of this system have been given in a previous paper⁵. The system was designed for a spatial resolution less than $1 \times 1 \, \mu m^2$ using the second order calculation code “TRANSPORT”⁶. This beam spot size is obtained with object-to-lens distance of 6 m and working distance of 30 cm when the energy resolution of the beam is $\Delta E/E = 1.3 \times 10^{-4}$ and beam divergence is limited to 0.2 mrad with object size set to $30 \times 8 \, \mu m^2$. The microbeam line consists of a doublet quadrupole lens and a system of microslits, divergence-defining slits mounted on a heavy rigid support to isolate from vibrations. Three turbo-molecular pumps evacuate the beam line and target chamber.
The quadrupole lens core is cut from a single iron piece. The bore radius is 5 mm and is machined with a tolerance less than 2 μm using a numerically controlled machine to reduce sextupole field contamination. The focused microbeam is scanned across the sample by using electrostatic deflection plates and HV amplifiers. Offset voltages are applied to deflection plates in order to position the microbeam on a selected sample region. The scanner is located downstream of the quadrupole doublet and the maximum scanning area is larger than 1 × 1 mm². The schematic diagram of the target chamber is shown in Fig. 1. The target chamber is a rectangular box and applicable to either in-vacuum or in-air PIXE combined with RBS and STIM experiments. In the analysis under vacuum, samples are set 25 mm downstream of the chamber center and working distance is 259 mm. Horizontal and vertical demagnification factors are 1/35.1 and 1/9.2, respectively. A sample holder can sustain a CaF₂ beam viewer and a total of 8 samples. Normally, we use a Si(Li) detector (Sensitive area of 80mm², effective thickness of 4000 μm and Be window thickness of 12 μm) for PIXE measurement and the detector is set at 115 degrees with respect to the beam axis. Since recoil protons deform the PIXE spectra, a Mylar filter is attached to the front of the detector. The minimum distance from sample to the detector is less than 10 mm, restricted by the size of the sample holder. An ion-implanted silicon detector (sensitive area: 100 mm², depletion depth of 100 μm) is attached at a distance of 60 mm for RBS measurements at an angle of 140 degree. To get structural and density information, scanning transmission ion microscopy (STIM) is used. Since transmitted ions are directly detected, a very low current is sufficient for structural mapping. Therefore, the beam current has to be reduced. The silicon detector is attached to a wheel off center from the beam axis and a Faraday cup is also attached to this wheel. The wheel is turned slowly until the detector is centered on the beam axis while monitoring the count rate of the detector and adjusting beam current accordingly. Using off-axis and on/off-axis geometry, one can perform simultaneous structural imaging in PIXE analysis. Off-axis geometry is obtained by rotating the wheel and respective scattering angle is determined from the counting rate of the particle detector. In on/off-axis geometry, a thin scattering foil with a collimator is set 10 mm downstream of the sample.

In in-air analysis, proton beams are extracted through a Mylar film (4 μm), which serves as backing film of the sample. Beam broadening through the film is estimated to be less than 0.01 μm and does not degrade beam quality significantly. Three samples are attached on a circular sample holder and can be changed without breaking the vacuum.
Sample position is 65 mm downstream compared to the in-vacuum sample position. Demagnification factors are 1/28.5 (horizontal) and 1/8.2 (vertical), respectively. To reduce sample damage through beam and energy loss in STIM measurement, He gas is blown on the sample. X-ray detector and RBS detectors are set in vacuum at 125 and 140 (or 162.7) degree with respect to the beam spot on the sample, respectively. STIM measurement can be performed in vacuum and in air as well. To minimize the energy loss in gas, the sample to detector distance is kept below 5 mm. Total energy loss in He gas is ~10 keV and the short distance and finite detector size make it impossible to carry out off-axis and on/off-axis STIM measurements.

The data are acquired by a multi-parameter data acquisition system which accepts 4 ADC inputs of energy pulse signals in addition to two position signals and allows to combine 4 ion-beam analysis techniques in a simultaneous measurement\(^5,10\). The system can be extended more than 10 ADC inputs, which is sufficient for additional ion beam applications. The beam is continuously scanning across the sample and data is taken whenever one of the ADCs detects a pulse signal. The data is acquired event-by-event (list mode) and elemental maps and energy spectra of selected regions can be generated on-line, while the system is acquiring data.

**Results**

The beam spot size is measured by beam scanning across mesh samples (Ni, Cu and Au mesh, 1000 or 2000 lines/inch), and measuring X-rays. Figure 2 shows the typical line profile of a 2000 Ni mesh, measured in vacuum fitted by symmetric double Gaussian convolution. The line profile is well reproduced by the symmetric double Gaussian convolution. This implies that the beam profile can be assumed to be of Gaussian shape. Beam spot sizes of less than 0.9 \(\times\) 0.9 \(\mu\)m\(^2\) are obtained at a microslit gap of 50 \(\times\) 15 \(\mu\)m\(^2\) in vacuum with a beam current of \(\sim\)40 pA. These results are slightly better than those expected from the calculations. However, when we measured beam spot size with 1000 Cu mesh, the spot size rapidly converged and then saturated at 2 \(\mu\)m\(^5\), which hinted at a possible system problem. Off-axis STIM analysis provided the solution, as shown in the spectrum on the Cu 1000 mesh in Fig. 3. Two peaks appear in the STIM spectrum, which implies the existence of two different mesh thicknesses. Figure 3 also shows the visual images corresponding to these two peaks. It is obvious that the mesh edges exhibit a step, which is observed in the SEM image. The edges of Ni and Au 2000 mesh are very sharp,
however, and these mesh are ideal for the measurement of the beam spot size\textsuperscript{11}.

The intensity of the beam halo is $\sim 1/500$ fraction of the central part, strongly depending on vacuum pressure. The stability of beam position on target sample is maintained during several hours of measurements. However, if we heat the rigid support of the beam line by heat gun for a few minutes, the beam spot moves by about 6 $\mu$m. It takes 30 minute for the beam spot to return to the original position after heating is stopped. If we blow cool air on the rigid support by air conditioner, the beam moves gradually and takes a long time to return to its original position after cooling is stopped. As well as temperature control, local heating/cooling, such as lightning by a light bulb and air blowing by air conditioners, on the system should be take care during measurements.

First, we applied the system to the study of radiation damage of polymer foils, whose behavior under beam irradiation is comparable to that of practical biological samples\textsuperscript{12}. Measurements are made in-vacuum and in-air for various beam conditions. Typical results of samples irradiated in-vacuum are shown. Before and after irradiation we accumulated on/off-axis STIM data. Irradiation was carried out with 3 MeV proton beam, beam currents of $\sim 400$ pA, beam spot size of $3.7 \times 3.7 \mu m^2$ and scanning area of $300 \times 300 \mu m^2$. Total accumulated charge was 2.9 $\mu C$ and RBS and off axis STIM data were measured. Figure 4 shows RBS and off-axis STIM spectra at three specific times (0-0.3 $\mu C$, 1.3-1.6 $\mu C$ and 2.4-2.7 $\mu C$) of the entire data set divided into eleven parts. Changes in RBS spectra are clearly seen, which correspond to elemental loss of oxygen. About 40 % of oxygen elements are lost during the irradiation. Density changes are also seen in the off-axis spectra. Peak energy is increased $\sim 10$keV at the end of irradiation, which is consistent with RBS data. Low energy peak ($\sim 500$channel) in the off-axis STIM spectra is formed by protons which are scattered from hydrogen and can be used to monitor elemental loss of hydrogen.

Next, the microbeam system was used for the elemental analysis of bovine aortic endothelial (BAE) cells, which were cultured in a bromodeoxyuridin(BrdU) containing medium\textsuperscript{13}. Figure 5 shows typical elemental maps and the optical image of BAE cells obtained in the vacuum condition. These images were obtained before the STIM system was in operation. Data accumulation took two hours to obtain these images with beam currents of $\sim 60$ pA, beam spot size of $1.5 \times 1.5 \mu m^2$ and scanning area of $50 \times 50 \mu m^2$. Distributions of P, S, Cl and K elements are clearly seen and correspond to the shapes of BAE cells. Trace Br elements, which are absorbed into their nuclei during DNA synthesis,
are also seen in the image.

These results demonstrate that the present microbeam analytical system is applicable to biological studies.

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References

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Figure 1. Schematic diagram of the Target Chamber.

(a) in-vacuum arrangement.  
(b) in-air arrangement.  
(c) close-up of in-air arrangement.

Figure 2. X-ray line profile of the Ni Mesh (2000 lines /inch).
Figure 3. STIM spectrum and images of the Cu mesh (1000 lines/inch).

Figure 4. RBS and off-axis STIM spectra of Mylar Film (--- 0-0.3 μC, ---: 1.3-1.6 μC, ---: 2.4-2.7 μC).

Figure 5. Elemental maps and optical images of bovine aortic endothelial (BAE) cells.