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Analytical possibilities of highly focused ion beams in biomedical field

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ABSTRACT

At the Centre for Ion Beam Applications (CIBA), a 3.5 MV HVEE Singletron™ accelerator serves to provide MeV ion beams (mostly protons or He^+) to six state-of-the-art beam lines, four of which are equipped with Oxford triplet magnetic quadrupole lens systems. This facility is used for a wide range of research projects, many of which are in the field of biomedicine. Here we presented a discussion of currently ongoing biomedical work carried out using two beamlines:

(a) The Nuclear Microscopy (NM) beamline is mainly used for trace elemental quantitative mapping using a combination of Particle Induced X-ray Emission (PIXE), to measure the trace elemental concentration of inorganic elements, Rutherford Backscattering Spectrometry (RBS), to characterise the organic matrix, and Scanning Transmission Ion Microscopy (STIM) to provide information on the lateral areal density variations of the specimen. Typically, a 2.1 MeV proton beam, focused to 1–2 μm spot size with a current of 100 pA is used.
(b) The high resolution single cell imaging beamline is equipped with direct STIM to image the interior structure of single cells with proton and alpha particles of sub-50 nm beam spot sizes. Simultaneously, forward scattering transmission ion microscopy (FSTIM) is utilized to generate images with improved contrast of nanoparticles with higher atomic numbers, such as gold nanoparticles, and fluorescent nanoparticles can be imaged using Proton Induced Fluorescence (PIF). Lastly, in this facility, RBS has been included as an option if required to determine the depth distribution of nanoparticles in cells, albeit with reduced spatial resolution.

1. Introduction

The research work at CIBA largely depends on the use of ion beams with precisely controlled phase space characteristics, as delivered by a 3.5 MV Singletron (High Voltage Engineering Europa B.V., Netherlands), and the possibility to form highly focused nano-sized beam spots via quadrupole lens systems (Oxford Microbeams, UK). The Singletron produces high brightness light ion beams with a typical long term (~5 h) energy stability of a few tens of eV [1].

Here we discuss two beamlines involved in biomedical research applications, the Nuclear Microscopy line and the Single Cell Imaging line. These beamlines differ in the techniques employed.

The minimum beam-spot size that can be realized in a magnetic quadrupole lens system depends on the size of the object apertures, and these in turn will, for a given beam brightness, determine the available beam current on the sample. The Nuclear Microscopy beamline is optimized for analytical experiments, and for the analysis modes of PIXE and RBS, a beam current around 100 pA is required in order to be able to generate images within acceptable run times, typically 0.5–2 h. These conditions require an object aperture dimension of a few tens of micrometers, for which the minimum achievable beam-spot size is then around a third of a micrometer [2,3]. On the other hand, the Single Cell Imaging line employs techniques which do not involve such relatively high beam currents, and is optimized for techniques where the probability for an ion to be detected approaches unity. It is then possible to work with object apertures of a few microns, for which spot sizes of 20 nm have been achieved, for ion counts rates of around 10,000 ions per second. This allows techniques such as

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direct STIM (on-axis STIM), FSTIM (forward off-axis STIM), PIF (proton induced fluorescence) and AIF (alpha induced fluorescence) to be carried out at nano-resolutions [4].

It was recognized a long time ago that MeV light ions traverse matter along well-defined straight paths, therefore they are unique probes for relatively thick (microns to tens of microns) tissue sections and single whole cells with good spatial resolutions, and at ppm detection limits [5].

2. Description of the nuclear microscope beam line

The CIBA nuclear microscope beamline [2,3] incorporates an Oxford Microbeams OM2000 end station and an OM50 target chamber with a 150 mm working distance between target and the OM50 quadrupole lens configured in the high excitation triplet mode. This beamline is equipped with an Oxford Microbeams OM50 target chamber (shown schematically in Fig. 1) which has eight ports: one for the beam pipe, one for a long working distance zoom optical microscope with a glass covered port for viewing the target and the other ports for fitting various detection systems such as PIXE, RBS, STIM, ERDA and ion induced fluorescence measurements with a photo multiplier tube (PMT). A Faraday cup is mounted together with STIM setup behind the sample for beam current measurement when the target is a thin biological specimen. For most of the measurements of biomedical samples carried out in this beamline, the target is tilted at an angle of 45° to the beam and to the PIXE detector positioned at 90° to the beam. The optical microscope is mounted at 45° to the beam (see Fig 1). This geometrical arrangement allows the PIXE detector to be positioned close to the target, which results in large PIXE detection solid angles, thereby maximising trace element detection. The RBS detector is mounted above the incoming beam, at a scattering angle of 160°. The STIM detector sits behind the sample on a vacuum sealed rotary shaft, and can be rotated between 0° and 20°, thereby facilitating both on-axis (direct) STIM or off-axis STIM measurements. For thin samples, a Faraday cup can be used to measure the beam charge via a vacuum feed-through and a charge digitiser module. All data acquisition functions are implemented via the OMDAQ 2007, a Windows 7 based DAQ system [6].

Biomedical applications typically involve the characterisation of tissue or cell specimens. In such studies, the Nuclear Microscopy beamline is typically operated with a 2.1 MeV proton beam and a beam spot size of 1–2 μm, at on-sample beam currents of 100–500 pA. The beam is scanned by a pre-lens magnetic scanning coil over a region up to 4 × 4 mm. The data acquisition system [6] allows the scanning of predefined areas as well as line scans. Furthermore, all experimental data and parameters can be saved in event by event list-mode files, so that off-line re-runs of the experiments is possible. This mode is advantageous for biomedical analyses, because biomedical specimen often have areas of interest which are not obvious before the experiment is carried out.

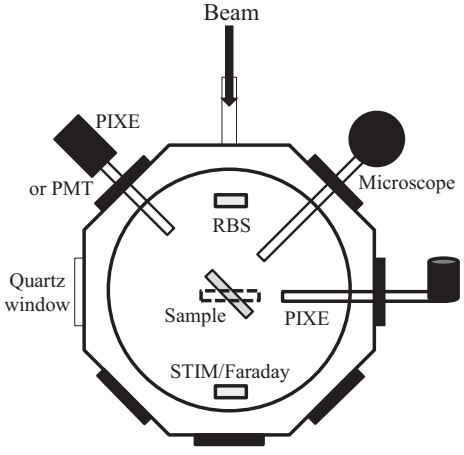

Fig. 1. Target chamber of nuclear microscopy beamline.

3. Description of the single cell imaging beam line

Recently, a Single Cell Imaging beam line [4] was set up at a position 30 degrees after the switcher magnet. This development was driven by the increasing interest in imaging techniques with resolutions substantially below the optical diffraction limit of 200 nm. Electron microscopy, which of course offers such resolutions, is however limited to surface analysis because the electron beams suffer large angle scattering as they pass through the sample. MeV light ions, in contrast, will travel with essentially straight trajectories [7] Therefore, to a good approximation, a finely focused MeV light ion beam will maintain its resolution as it traverses through a single whole biological cell. Using the Single Cell Imaging end station, we have demonstrated that it is possible to focus MeV proton beams to spot sizes well below 50 nm, and assemble STIM and PIF (proton induced fluorescence) images of whole cells, revealing interior structures (nuclei, organelles) of whole cells [8].

This single cell end station is mounted on an optical table, and incorporates a quadrupole lens system made up of 3 compact OM52 quadrupole lenses [9] operated in a triplet mode. An electrostatic scanning system is used to scan the beam over the target [10]. The target chamber has been constructed with in-built optical and fluorescent microscopes for sample imaging and for target positioning, and incorporates a highly stable sample stage driven by piezoelectric drivers allowing 25 mm movement in all the three spatial directions. Typically, an experiment is started by focussing the ion beam under optical control on a quartz target, and checking the resolution using a proton beam written free-standing nickel grid [11]. The focussing of beam spot sizes below 250 nm is usually done via STIM, using a Hamamatsu S1223 pin-diode or a conventional RBS detector and the Ni grid. An annular surface barrier detector is mounted at 180° scattering angle for quantitative measurements of high Z elements at ~300 nm resolution.

A Hamamatsu photomultiplier (model PMT R7401P) is used for ion induced fluorescence imaging. The data acquisition system used in this beam-line can generate up to 2048 × 2048 pixel imaging using the IonDAQ package [12].

The single cell imaging beam line is routinely operated at sub 100 nm resolution for imaging single cell specimen using techniques such as direct STIM using proton or alpha particles for cell interior structures, FSTIM for enhanced contrast for cells loaded with high Z elements such as gold nanoparticles, and proton and alpha induced fluorescence imaging as well as secondary electron imaging [13]. It is worth noting that alpha STIM gives much better contrast than proton STIM due to the higher energy loss of alpha particles in matter compared to that of protons. However, alpha particles often have much higher bleaching rates (quenching of optical emission) during fluorescence imaging compared to protons.

4. Biomedical applications of nuclear microscopy beam line

PIXE uses MeV protons to produce characteristic X-ray photons via inelastic Coulomb collisions. A large solid angle spectroscopy system is then used to measure the energies of X-rays, typically large surface area Si(Li) detectors are used. In recent years, these are increasingly being replaced by Silicon Drift Detector (SDD), which do not require LN_2 cooling. The energy of characteristic X-rays is unique to the parent atom, thus the identification of partic-

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