Available online at www.sciencedirect.com
ScienceDirect
Nuclear Instruments and Methods in Physics Research B 260 (2007) 124–129
NIM B
Beam Interactions with Materials & Atoms
www.elsevier.com/locate/nimb

Nano-imaging of single cells using STIM

Ren Minqin [a,b], J.A. van Kan [a], A.A. Bettiol [a], Lim Daina [c], Chan Yee Gek [c], Bay Boon Huat [c], H.J. Whitlow [d], T. Osipowicz [a], F. Watt [a,*]

[a] Centre for Ion Beam Applications (CIBA), Department of Physics, National University of Singapore, Singapore 117542, Singapore
[b] Department of Biochemistry, National University of Singapore, Singapore
[c] Department of Anatomy, National University of Singapore, Singapore
[d] Department of Physics, University of Jyvaskyla, P.O. Box 35 (YFL), FIN-40014, Finland

Available online 14 February 2007

Abstract

Scanning transmission ion microscopy (STIM) is a technique which utilizes the energy loss of high energy (MeV) ions passing through a sample to provide structural images. In this paper, we have successfully demonstrated STIM imaging of single cells at the nano-level using the high resolution capability of the proton beam writing facility at the Centre for Ion Beam Applications, National University of Singapore. MCF-7 breast cancer cells (American Type Culture Collection [ATCC]) were seeded on to silicon nitride windows, backed by a Hamamatsu pin diode acting as a particle detector. A reasonable contrast was obtained using 1 MeV protons and excellent contrast obtained using 1 MeV alpha particles. In a further experiment, nano-STIM was also demonstrated using cells seeded on to the pin diode directly, and high quality nano-STIM images showing the nucleus and multiple nucleoli were extracted before the detector was significantly damaged.

PACS: 78.70.−g; 82.80.Yc; 87.64.−t

Keywords: Nano-imaging; Nano-STIM; STIM; Scanning transmission ion microscopy; Single cell imaging; Nuclear microscopy

1. Introduction

There has recently been an upsurge in interest into new methods of imaging biological cells and tissue. Scanning transmission ion microscopy (STIM) which was demonstrated over 20 years ago [1] has the potential for producing structural images of cells and tissue, but has been infrequently utilised by only a few nuclear microprobe groups over the last two decades. STIM relies on measuring the energy loss of a beam of highly focused MeV ions as it passes through a sample, and because the transmitted protons in general maintain a straight path as they pass through a biological specimen (e.g. a tissue section or a cell), then a high quality structural image of a relatively thick specimen can be formed. The use of STIM has in general been limited to identifying specific regions of interest in a biological sample prior to elemental analysis by nuclear microscopy [2–14], but has also been used to construct tomographic structural images of specimens that are difficult to section [15–19].

Recently it has been possible to focus an MeV proton beam to below 100 nm, achieved in the proton beam writing facility of the Centre for Ion Beam Applications (CIBA), National University of Singapore [20]. The p-beam writing facility uses OM52 lenses arranged in a compact couplet triplet formation resulting in increased X and Y demagnifications of 228×60, respectively. Sub-100-nm beams are routine, with a current achievable best spot size of 35×75 nm for 10 000 protons per second [20]. The use of high energy ion beams focused to nano-dimensions raises the possibility of STIM nano-imaging of single cells and tissue. Since this resolution is well below the wavelength of light and is maintained through relatively

* Corresponding author. Tel.: +65 65162815; fax: +65 67776126.
E-mail address: phywattf@nus.edu.sg (F. Watt).

0168-583X/$ - see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.nimb.2007.02.015

thick tissue sections and whole cells, the nano-STIM technique could well have significant potential for structural imaging in biomedicine.

2. Experimental details

The CIBA proton beam writing line, which can routinely focus MeV ion beam to sub-100 nm dimensions, is not readily amenable for either STIM or nuclear microscopy investigations, since the target stage to lens distance is reduced to 7 cm compared to 15 cm in the CIBA nuclear microscopy line [20]. To adapt to the reduced working distance we have constructed a miniature assembly using a Hamamatsu pin diode S1223-01 (3.06×3.06 mm chip) [21] mounted in a miniature assembly fronted by a silicon nitride window of thickness 50 nm (see Fig. 1). The cells (MCF-7 American Type Culture Collection [ATCC] breast cancer cells) were successfully grown on the upper surface of the silicon nitride window, as indicated by the optical micrograph of the cells on the silicon nitride window shown in Fig. 2. The cells were prepared according to the protocol as described in Table 1, which is a procedure used in cell biology, to preserve the structural integrity of the cells. The STIM assembly was mounted on a computer-controlled Burleigh Inchworm EXFO XYZ stage which has a travel of 25 mm for all axes with a 20 nm closed loop resolution [22].

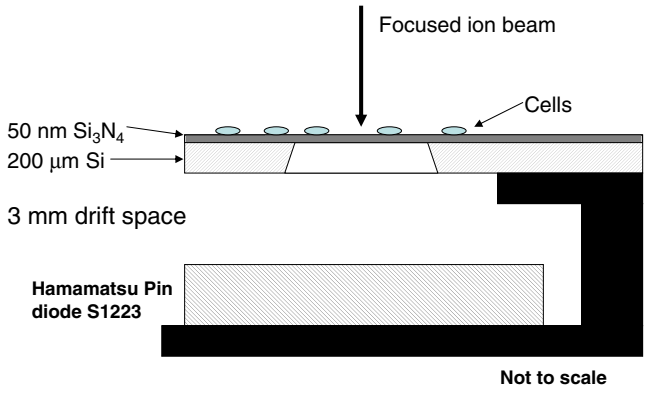

Fig. 1. Schematic diagram of the STIM assembly, showing the silicon nitride window and the pin diode. The cells are grown on the upper surface of the silicon nitride window.

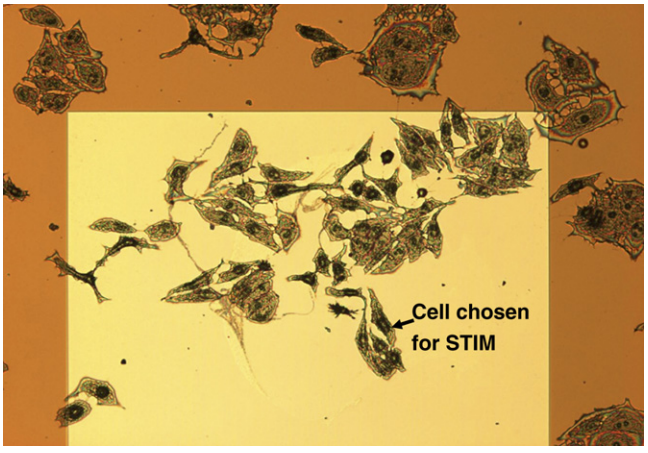

Fig. 2. Optical micrograph of the breast cancer cells grown on a 50 nm thick silicon nitride window (500 μm × 500 μm). The cell chosen for the STIM studies is marked in the figure.

3. Results and discussion

3.1. Results of STIM using the silicon nitride window as substrate

A 2 MeV H_2^+ beam (an H_2^+ ion is equivalent to two 1 MeV protons) was focused down to sub 100 nm, and scanned across a suitable cell located on the silicon nitride window as shown in Fig. 2. A H_2^+ beam rather than a 1 MeV proton beam was chosen because this represents the brightest beam from the accelerator, and also because the beam halo due to slit scattering is minimized (the H_2^+ beam splits into two protons on contact with the slit edges and therefore have a very different focal point). A one MeV alpha beam was also focused to sub-100 nm and scanned across the same cell. The STIM spectra from the cell for both the H_2^+ and the alpha beam were collected by the Hamamatsu pin diode positioned directly behind the window and operating at a 10 V bias voltage. It can be seen from the STIM energy spectra shown in Fig. 3 that the H_2^+ STIM energy spectrum displays relatively little energy loss compared with the alpha beam, expected because of the higher stopping power of the 1 MeV alpha particles compared with the 1 MeV protons (408 keV/μm for alpha particles and 52 keV/μm for protons in carbon). The energy resolution of the Hamamatsu pin diode, chosen at random from a batch purchased from the manufacturer, was measured to be 23 keV for the 1 MeV alpha particles and around 27 keV for the 2 MeV H_2^+. Estimates of the straggling of the protons and alpha beam traversing the 50 nm substrate window (1 keV for 1 MeV protons and 2.4 keV for 1 MeV alpha particles) as calculated by SRIM [23] were small compared with the detector energy resolution. The STIM images for both the H_2^+ and alpha beam were collected using the OMDAQ system [24] using a median energy fit, and are shown in Fig. 4. As expected, the STIM image using the alpha beam exhibits a higher contrast compared with the image using the H_2^+ beam, although in both cases the cell nucleus is clearly visible.

3.2. Results of STIM using the pin diode as substrate

In a second experiment, it was decided to investigate STIM imaging using the pin diode as substrate, thereby dispensing with the relatively fragile silicon nitride window. Two potential problems were investigated: (a) growing the cells on the pin diode and (b) damage incurred by the pin diode due to the incoming ion beam. As shown in Fig. 5, growing the cells on the pin diode by following the same