Nuclear Instruments and Methods in Physics Research B 348 (2015) 111–114

Contents lists available at ScienceDirect

Nuclear Instruments and Methods in Physics Research B

journal homepage: www.elsevier.com/locate/nimb

Development of a new light collection and detection system optimized for ion beam induced fluorescence microscopy

Sudheer Kumar Vanga *, Zhaohong Mi, Long Cheng Koh, Ye Tao, Andrew A. Bettiol, Frank Watt

Centre for Ion Beam Applications, Department of Physics, National University of Singapore, 2 Science Drive 3, Singapore 117551, Singapore

ARTICLE INFO

Article history:
Received 25 July 2014
Received in revised form 28 January 2015
Accepted 30 January 2015
Available online 20 February 2015

Keywords:
Ion beam induced fluorescence
Scanning transmission ion microscopy
Parabolic mirror
Fluorescence detection

ABSTRACT

Ion beam induced fluorescence microscopy is a new imaging technique which has the potential to achieve sub-50 nm spatial resolution fluorescence images. Currently the resolution of the technique has been limited to around 150 nm mainly because of inefficient collection and detection of emitted photons from the sample. To overcome this limitation, a new light collection system based on a custom made parabolic mirror is employed to enhance the fluorescence collection. The custom made mirror is designed so as to obtain both structural (scanning transmission ion microscopy) and ion beam induced fluorescence imaging simultaneously. The design and characterization of the parabolic mirror is discussed in detail.

1. Introduction

Ion beam induced fluorescence together with ion beam characterization techniques such as particle induced X-ray emission (PIXE) and Rutherford backscattering spectroscopy (RBS) has been used to determine defects in crystal structure in solid materials and to study the chemical bonds in organic materials [1–3]. More recently the technique has been applied for bio-imaging such as to observe the autofluorescence from cryosectioned skin tissues [4] and to study the non-fluorescent and fluorescent tissue stains in common pathological tissue [5]. In our previous studies, using a focused MeV proton beam, fluorescence images of N2A blastoma cells stained with Sytox green and A549 lung carcinoma cells, stained with EGFR and receptor α2b1 integrin, was obtained at the single cell level with resolutions 200 nm or better [6,7].

Although the state-of-the-art spatial resolutions achieved are of the order of 20 nm using MeV ions [8], the same spatial resolutions have not been achieved in ion beam induced fluorescence microscopy. Though the technique has been applied to single cell imaging, the ion beam induced fluorescence resolution is limited to 150 nm whereas in scanning transmission ion microscopy (STIM) spatial resolution of 25 nm is reported [9]. There are two main reasons for the difference in spatial resolutions between STIM imaging and ion beam induced fluorescence imaging, one is the lack of efficient radiation stable fluorescent probes and the other is poor light collection system. Because of these limitations, the imaging requires high ion beam currents for the fluorescence detection resulting in higher spatial resolution compared to STIM. The current system in our cell imaging facility at Centre for Ion Beam Applications at the National University of Singapore, utilizes a light collection detector placed behind the cell sample which allows the detector to collect only a portion of emitted light (forward scattered light). Overcoming this light collection limitation will allow for improved resolution fluorescence imaging well below the 100 nm level. Furthermore, combining ion beam induced fluorescence imaging with structural imaging using STIM will provide potentially new information which will improve our understanding of cell function at the sub-cellular level.

In this paper, we focus on improving the ion beam induced fluorescence light collection system with a custom made parabolic mirror.

2. Parabolic mirror design

A parabolic reflector is able to reflect light emitted from an isotropic point source positioned at its focal point into parallel rays traveling in the direction of the parabolic axis. These parallel rays of emitted light from the sample can be focused on to a detector which enables the collection of isotropic emission from the sample. However, a complete parabolic mirror can not be implemented in the set-up since the ion beam has to travel through the mirror and interact with the sample to emit the fluorescence. One of the major challenges is accommodating the parabolic mirror in the current system. The specific constraints in designing the parabolic mirror are listed here.

* Corresponding author.
E-mail address: physkv@nus.edu.sg (S.K. Vanga).

http://dx.doi.org/10.1016/j.nimb.2015.01.076
0168-583X/

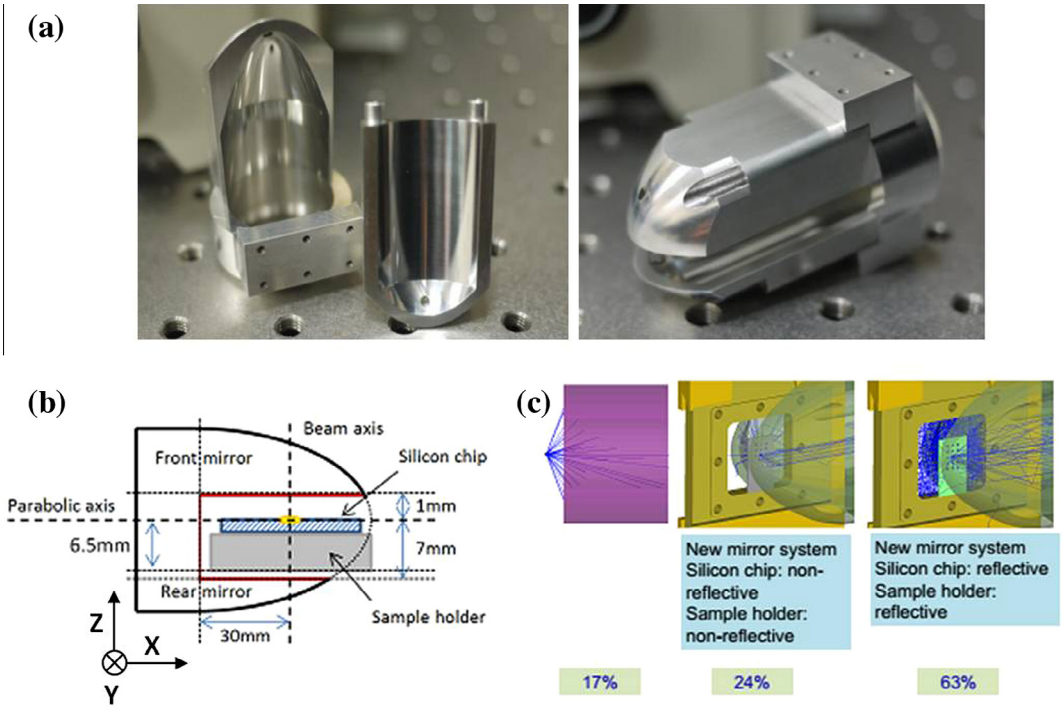

Fig. 1. Parabolic mirror design parameters (a) Final custom-made mirror showing the individual parts. (b) Schematic showing the design parameters and (c) comparison of the fluorescence collection efficiency of the existing set-up and custom-made parabolic mirror with non-reflective and reflective sample and target holder obtained from ray tracing simulations.

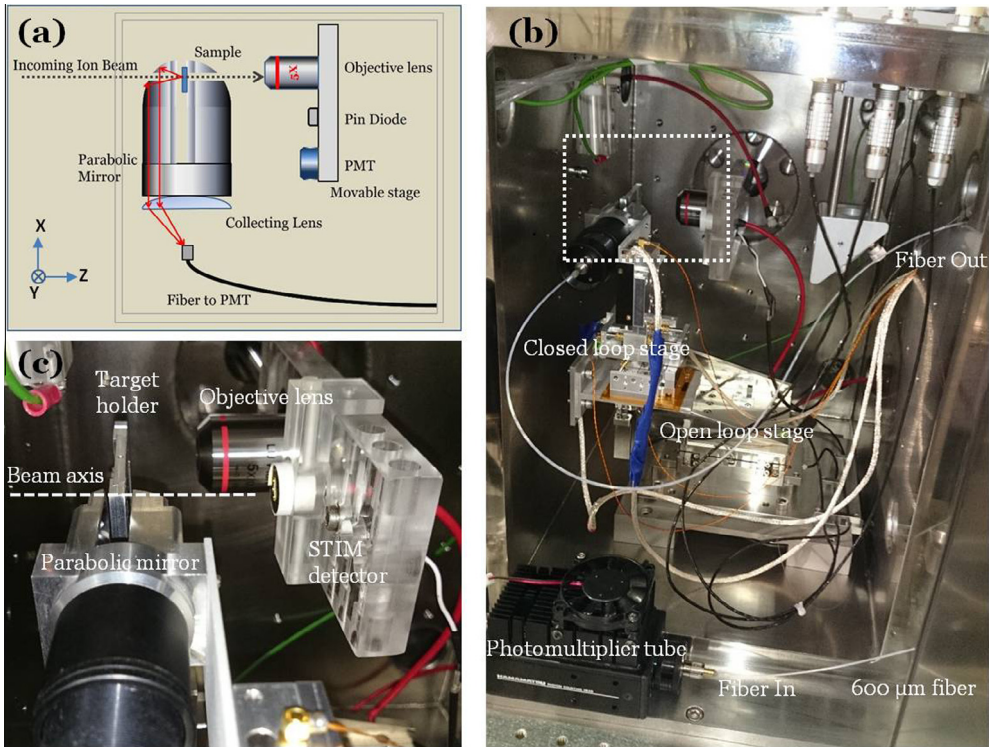

Fig. 2. Ion beam induced fluorescence microscope set-up. (a) Schematic of the set-up; (b) shows the experimental set-up indicating the individual components (c) shows the magnified view of the rectangular area indicated in (b).

- For high demagnification geometries, the target needs to be close to the lens and hence near to the target chamber wall. This sets a limit on the size of the parabolic mirror.
- The target needs to be positioned at the focal point of the mirror system, and this requires a suitable opening in the mirror.
- Since the focal point of the parabolic mirror lies in the principle axis, the position of the opening should be asymmetric.
- For STIM imaging, the focused ion beam passes through a 2 mm hole in the front of the mirror, transmits through the sample, and pass through a similar hole in the back portion of the mirror.