Ion beam induced fluorescence imaging in biological systems

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ABSTRACT

Imaging fluorescence generated by MeV ions in biological systems such as cells and tissue sections requires a high resolution beam (<100 nm), a sensitive detection system and a fluorescent probe that has a high quantum efficiency and low bleaching rate. For cutting edge applications in bioimaging, the fluorescence imaging technique needs to break the optical diffraction limit allowing for sub-cellular structure to be visualized, leading to a better understanding of cellular function. In a nuclear microprobe this resolution requirement can be readily achieved utilizing low beam current techniques such as Scanning Transmission Ion Microscopy (STIM). In recent times, we have been able to extend this capability to fluorescence imaging through the development of a new high efficiency fluorescence detection system, and through the use of new novel fluorescent probes that are resistant to ion beam damage (bleaching). In this paper we demonstrate ion beam induced fluorescence imaging in several biological samples, highlighting the advantages and challenges associated with using this technique.

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1. Introduction

Of the imaging modalities that are available in nuclear microscopy, fluorescence/luminescence imaging is by far the least exploited. Yet in the field of biological science, fluorescence microscopy is the most widely used technique and often the technique of choice for visualizing tissue and sub-cellular structure. In recent years, significant advances have been made in optical microscopy, in particular, the commercial development of super-resolution microscopy techniques such as Photo Activated Localization Microscopy (PALM), Stochastic Optical Reconstruction Microscopy (STORM) and Stimulated Emission Depletion (STED) [1,2]. These techniques have enabled the visualization of structure that has been previously inaccessible by light microscope techniques. These super-resolution optical fluorescence based techniques have been able to achieve spatial resolutions that break the optical diffraction limit of approximately 200 nm, or roughly half the wavelength used to excite the fluorescence.

The use of mega-electron volt (MeV) focused ion beams for biological imaging is well established [3,4]. For high beam current regimes (typically 100 pA or more) techniques such as Particle Induced X-ray Emission (PIXE) and to a lesser extent Rutherford Backscattering Spectrometry (RBS) have been successfully utilized for mapping trace elements in various biological samples [5]. In the low beam current regime where imaging with individual ions is

possible, Scanning Transmission Ion Microscopy (STIM) has become the most widely used technique for identifying structure and density variations in biological samples [6,7]. The advantage of STIM is the ability to visualize structure that would otherwise require the use of staining techniques which in turn contaminates the sample under investigation. In contrast, there have been only a limited number of studies into imaging fluorescence generated by MeV ions in biological samples. The most notable work in this area was by Rossi et al. [8] who looked at ion beam induced luminescence from various stains used in histological studies, and more recently work done in Singapore on single cells tagged with organic dyes [9,13].

This paper discusses the implementation of ion beam induced fluorescence imaging at the Centre for Ion Beam Application (CIBA), NUS Singapore [11]. The various challenges faced when trying to perform imaging at sub-optical diffraction limit spatial resolutions will also be discussed. Examples of fluorescence imaging in single whole cells and tissue will be presented.

2. Light collection and detection system

Efficient light collection and detection is important for achieving the sensitivities that are required for imaging fluorescence at the single ion level. The detector of choice for many years has been the photomultiplier tube (PMT). The PMT routinely used for in-vacuum fluorescence imaging is the Hamamatsu R7400P which is configured for photon counting and has a peak wavelength sensitivity at approximately 400–420 nm. This particular PMT can also be

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configured with a lens. PMT based systems are able to detect light over the entire visible spectral region with a relatively high quantum efficiency and gain (typically quantum efficiency is 40% or less). Furthermore, they can be operated in vacuum making it relatively straight forward to place them in close proximity to the sample for improved detection solid angle. This configuration has been the main method that has been used at CIBA for high resolution fluorescence imaging experiments. The main draw backs of this detection configuration are the inability to perform ion induced fluorescence imaging simultaneously with STIM, and the difficulty in performing monochromatic imaging due to the awkward placement of filters in front of the PMT. Furthermore, if the PMT is placed directly behind the sample, a beam stop is required to prevent the transmitted beam from hitting the PMT glass, thus generating unwanted background signal. The use of a beam stop results in a reduction of collection efficiency.

An alternative method for collecting light from the sample involves the use of a reflective objective lens (Schwarzschild Objective). These lenses are made from two curved mirrors, the rear of the secondary mirror acting as a natural beam stop. The advantage of using a reflective objective is that their construction is vacuum compatible and they have a relatively long working distance with a moderate numerical aperture. In addition, reflective objectives can also operate over an extremely broad spectral range making them suitable for both UV–visible and infra-red imaging. In the system at CIBA, we utilize two infinity corrected reflective objectives from Newport (36×, NA = 0.52, WD = 10.4 mm and 15×, NA = 0.4, WD = 24 mm). A third light detection configuration that has been recently developed in CIBA involves the use of a custom made off axis parabolic mirror collector. This new mirror system has been designed to fit into our existing target chamber and for the first time allows us to simultaneously collect fluorescence and transmitted ions. This is achieved through the inclusion of a

hole along the beam axis. More details of this system and its performance is discussed elsewhere [10]. A schematic diagram of the three possible light collection and detection systems utilized in CIBA is shown in Fig. 1.

In addition to improving the light collection system, more sensitive single photon detectors can also be used to achieve high resolution fluorescence imaging approaching single ion sensitivity level. Recent developments in avalanche photo diodes (APDs) and silicon photomultiplier technology has resulted in several new types of single photon detectors that are also suitable for fluorescence detection. These detectors potentially offer the gain and sensitivity of a PMT without any of the disadvantages which include the need for a high voltage bias supply, susceptibility to stray magnetic fields and ease with which they can be damaged by high intensity light. The main drawback of APDs is their reduced efficiency in the UV-blue part of the spectrum which is often the region of interest in fluorescence microscopy. The APD module utilized in CIBA is the Perkin-Elmar SPCM-AQRH module which has a quantum efficiency greater than 70% for wavelengths around 700 nm but drops off to below 10% at 400 nm where PMT systems usually are more efficient. In order to use these new types of detectors, or to perform spectroscopy, it is important to have the ability to extract the light from the vacuum chamber either through free space optics, or with the aid of an optical fiber. This capability has been built into the CIBA cell imaging beam line [11].

An additional consideration when designing a light collection system for ion beam induced fluorescence imaging is the excitation volume in which light is generated by the ion beam. The excitation volume produced by an ion beam for thin samples is roughly uniform in depth due to the almost linear electronic energy loss of the ion beam as it traverses the sample. For thick samples it roughly follows the ionization profile that can be calculated using Monte-Carlo software like SRIM [12]. For optical/laser excitation, the exci-
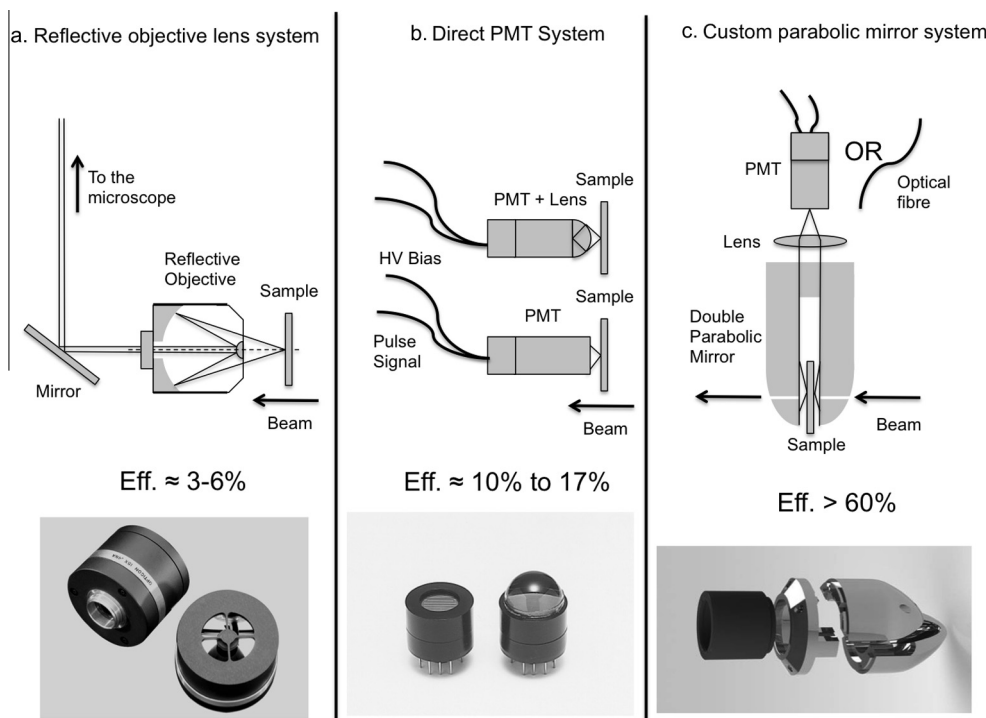

Fig. 1. Three light collection configurations for ion induced fluorescence imaging. (a) Light collected using a reflective objective lens (Schwarzschild Objective), (b) light collection and detection by directly using a PMT tube mounted behind the sample, (c) light collection using a custom made parabolic mirror with a lens. This system has the ability to either direct the light onto a PMT or to focus the light onto an optical fiber. The effective light collection efficiencies shown in this figure are estimated based on either numerical aperture data or optical ray tracing.