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Single particle tracking of fluorescent nanodiamonds in cells and organisms

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ABSTRACT

Ever since the discovery of fullerenes in 1985, nanocarbon has demonstrated a wide range of applications in various areas of science and engineering. Compared with metal, oxide, and semiconductor nanoparticles, the carbon-based nanomaterials have distinct advantages in both biotechnological and biomedical applications due to their inherent biocompatibility. Fluorescent nanodiamond (FND) joined the nanocarbon family in 2005. It was initially developed as a contrast agent for bioimaging because it can emit bright red photoluminescence from negatively charged nitrogen-vacancy centers built in the diamond matrix. A notable application of this technology is to study the cytoplasmic dynamics of living cells by tracking single bioconjugated FNDs in intracellular medium. This article provides a critical review on recent advances and developments of such single particle tracking (SPT) research. It summarizes SPT and related studies of FNDs in cells (such as cancer cell lines) and organisms (including zebrafish embryos, fruit fly embryos, whole nematodes, and mice) using assorted imaging techniques.

1. Introduction

Biological systems are dynamic in nature. Over the past few decades, several microscopic techniques have been developed to study biological dynamics at the molecular level, including single particle tracking (SPT), fluorescence correlation spectroscopy (FCS), fluorescence recovery after photobleaching (FRAP), and Förster resonance energy transfer (FRET), etc. [1]. Among these techniques, only SPT allows direct monitoring of the movement of molecules or vesicles in biological matrices [2,3]. The technique measures the trajectories of these entities and quantifies their Brownian diffusions, confined motions, and directed transports. It enables high-precision longitudinal tracking of the fate of the transported molecules or vesicles not only in single living cells but also in whole organisms. Since its first introduction in the late 1980's, SPT in combination with super-resolution imaging at the present has become a key technology for quantitative probing and analysis of intra- and inter-cellular processes at the nanometer scale [4].

A nanomaterial useful for SPT applications is fluorescent nanodiamond (FND) [5], which has received increasing attention in recent years for its excellent biocompatibility and superlative optical properties [6,7]. The negatively charged nitrogen-vacancy (NV^-) defect centers embedded deep in the diamond matrix are responsible for the fluorescence emission. The center is unique in that it can emit bright far-red fluorescence at ~700 nm wavelength when excited by green–yellow light, and nearly 70% of the emission lies in the near-infrared (NIR) window of biological tissue [8]. The fluorescence is perfectly photostable, without bleaching and blinking [9], making the nanoparticle an ideal probe for long-term imaging and tracking applications. It is especially suitable for use as a contrast agent for following intra- and inter-cellular communications by SPT.

In fluorescence-based SPT, laser illumination of biological samples always produces high autofluorescence background that can interfere with the detection and lower observed signal-to-noise ratios. One parameter that can be manipulated to enhance the image contrast of FND is the fluorescence lifetime. In nanoscale diamond, NV^- has a relatively long lifetime, which is up to 20 ns for FNDs in water and physiological medium (Fig. 1) [9–12]. This lifetime is substantially longer than that (typically 1–4 ns [13]) of cell and tissue autofluorescence. Therefore, time-gating fluorescence

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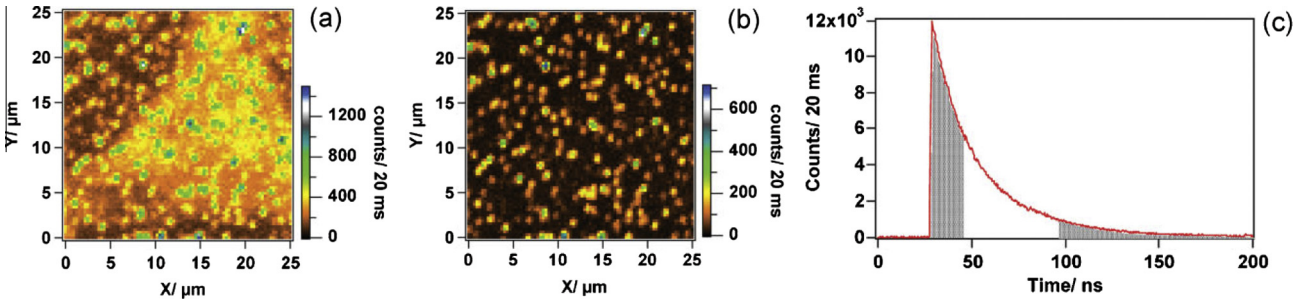

Fig. 1. Time-resolved confocal fluorescence images of a fixed cell containing FNDs. (a) Raster-scan image obtained by detecting all photons, displaying NV^- fluorescence together with cell autofluorescence. (b) Time-gated raster scan constructed from photons detected between 15 and 53 ns after pulsed laser excitation. Scan area: 25 × 25 μm^2. (c) Fluorescence time decay from one of the FNDs shown in (a). (From Ref. [10].)

imaging or fluorescence lifetime imaging microscopy (FLIM) [14] can be fruitfully applied to eliminate the background signals when probing single FNDs in cells and organisms.

Another approach that has been undertaken to enable background-free imaging is to utilize the magnetic properties of the NV^- centers based on a technique known as optically detected magnetic resonance (ODMR) [15]. The method takes advantage of the fact that the spin polarization of NV^- can be achieved by optical pumping with green lasers. A decrease of the fluorescence intensity results when the center is exposed to the microwave radiation in resonance with the $m_s = 0 \rightarrow m_s = \pm 1$ transitions of the ground electronic state at 2.87 GHz. In this method, confocal or wide-field fluorescence images are first acquired with or without the 2.87 GHz microwave irradiation (Fig. 2). Subsequent subtraction of the data between these two images at every pixel yields selective images of the FNDs even in high background [16,17].

The third strategy also exploits the spin properties of NV^- to establish background-free imaging. However, instead of using microwave radiation, a modulated magnetic field is applied to create a mixture of $m_s = 0$ and $m_s = \pm 1$ sublevels and thus induce a change in fluorescence intensity after spin polarization [18,19]. Only FND can display such fluorescence intensity modulation but not the surrounding environment. By lock-in detection, the signal of FNDs in a mouse tissue has been selectively retrieved with a 100-fold increase in signal-to-noise ratio compared to direct detection [19]. The efficient penetration of magnetic fields through tissues makes this approach particularly appealing for whole animal imaging.

This article provides a focused review on SPT of FNDs in biological matrices by confocal and wide-field fluorescence imaging in combination with advanced optical techniques including FLIM and ODMR. It is demonstrated and illustrated how these methods can be applied to probe the dynamics of the carbon-based nanoparticles in cells and organisms with high precision and resolution. Promises and potentials of this NV-enabled nanotechnology for imaging and tracking of single FND-labeled cells *in vivo* are also discussed.

2. SPT in cells

The first application of FNDs for SPT in cells appeared in 2007. Fu et al. [9] introduced the particles into HeLa cells (human cervical cancer cells) by endocytosis and managed to track the motions of single FNDs (35 nm in diameter) in the cells in two dimensions. The same team subsequently improved the SPT technology by mounting a microscope objective lens vertically on a *z*-motion piezoelectric translational stage to monitor the movement of the targeted FNDs within the depth of field in three dimensions (3D) [20]. Fig. 3 displays a typical result of the SPT for a bare FND in a HeLa cell. An analysis of the 3D trajectories for more than 200 s revealed a diffusion coefficient comparable to that of the quantum dots confined within endosomal or lysosomal compartments. The high brightness of the particles also allowed two-photon excited fluorescence imaging of single FNDs (~100 nm in diameter) inside the cells using a femtosecond laser set at 875 nm wavelength for illumination [20].

In addition to SPT, FNDs can be probed individually with high image contrast by using FCS, which analyzes the correlation of the fluorescence intensity fluctuation of the particles within a tightly focused region [21,22]. With 40 nm FNDs encapsulated in a lipid layer, Hui et al. [22] found that the diffusion of the particles in the cytoplasm of a HeLa cell can be enhanced by more than one order of magnitude, compared with that of bare FNDs. The technique together with SPT by one-photon or two-photon excitation has enabled researchers to probe both the short-term and

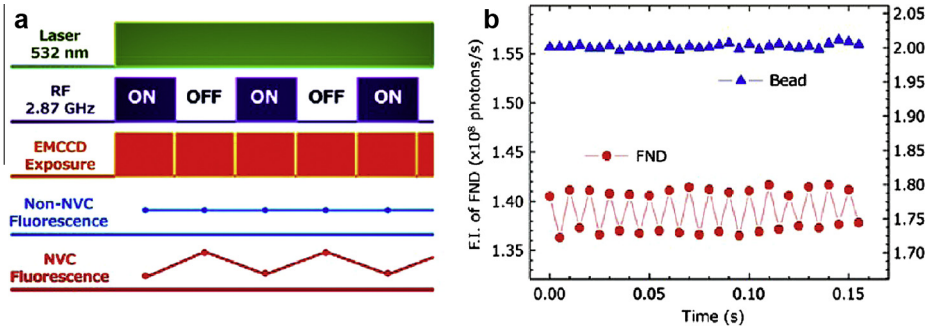

Fig. 2. Background-free detection of FNDs by applying modulated microwave radiation at 2.87 GHz. (a) Time chart of the laser excitation, microwave irradiation, and image acquisition, along with the expected intensity profiles of non-NV and NV fluorescence used for selective imaging of FNDs. (b) Comparison between the time traces of the observed fluorescence intensities of a FND and a fluorescent bead. (From Ref. [16].)

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