

Mass Production and Dynamic Imaging of Fluorescent Nanodiamonds

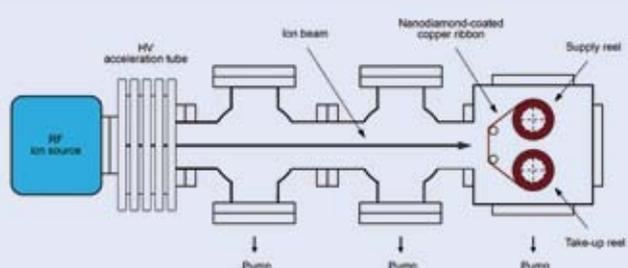
Abstract

Fluorescent nanodiamond (FND) is a new nanomaterial that possesses several unique properties, including good biocompatibility, excellent photostability, and facile surface functionalizability. When excited by a laser, the defect centers within the diamond crystal lattice can emit far-red photons that are capable of penetrating tissue and cells, making them useful for bioimaging applications. Here, we show that bright FNDs can be produced in large quantities by irradiating synthetic type Ib diamond nanocrystallites with medium-energy helium ions. The fluorescence is sufficiently bright and stable to allow three-dimensional tracking of a single FND particle in a live cell by wide-field fluorescence microscopy. The excellent photophysical characteristics are maintained even for particles as small as 25 nm, suggesting that FND is an ideal nanoprobe for long-term tracking and imaging *in vivo*, with good temporal and spatial resolution.

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In many biomedical applications of nanotechnology, diamond has been suggested to be the building block for miniature devices such as nanorobots and nanomachines. These advanced diamond-based nanodevices, while still far in the future, are envisioned to operate in human body, destroy viruses and cancer cells, and repair damaged structures. Several factors contribute to the favorable selection of diamond. First, being an allotrope of carbon, diamond can be synthesized both physically and chemically. Second, the surface of diamond can be readily derivatized with functional groups commonly encountered in organic chemistry. Third, diamond has high rigidity, low chemical reactivity, and good biocompatibility, which earned it the name “Biomaterial of the 21st century”. Very recently, radiation-damaged type Ib diamond nanocrystallites have been demonstrated to be capable of producing bright red fluorescence, when excited by green yellow light. The fluorescence, originating from the photoexcitation of nitrogen-vacancy (N-V) defect centers, is so strong and stable that it has allowed long-term observation of these particles individually in cells. The ongoing technological advancements and impending biomedical needs are making FND one of the most promising optical nanoprobe for *in vivo* imaging and diagnostics.



1 Schematic of the experimental setup for mass production of FNDs with a medium-energy ion beam. Both the radio-frequency ion source and the high-voltage acceleration tube were from a commercial source, while the integrated production system was built in-house.

He⁺ beam. A schematic diagram of the experimental setup is shown in Figure 1. Compared with the previous methods, the setup has boosted the FND yield by nearly two orders of magnitude and, more importantly, it can be installed and operated safely in ordinary laboratories.

The FNDs prepared by the He⁺ irradiation show an emission spectrum peaking at 680 nm (Figure 2). The spectrum, acquired for the particles suspended in water and excited by a 532 nm laser (inset in Figure

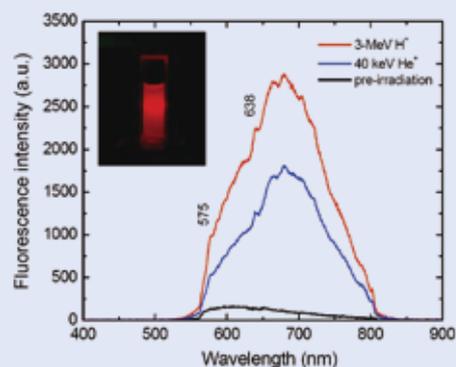
While serving as a promising *in vivo* nanoprobe, FNDs have not yet received widespread exploitation because of the difficulty of mass-producing them. Conventionally, the N-V defect centers in diamond are produced via bombardment of the material with a high-energy (typically 2 MeV) electron beam from a van der Graaff accelerator, followed by annealing at elevated temperatures (typically 800°C). The need for such highly sophisticated and costly equipment hampers the easy availability of FNDs. Herein, we present a practical method to scale up the production of FNDs using a home-built prototype device composed of a high-fluence, medium-energy

2), reveals two types of N-V centers inside the radiation-damaged nanomaterial: $(N-V)^0$ with a zero-phonon line at 575 nm and $(N-V)^-$ with a zero-phonon line at 638 nm. Comparing it with the spectrum of another sample damaged by the 3-MeV protons indicates that the fluorescence spectra of the FNDs prepared under these two drastically different bombardment conditions are essentially the same, except that their intensities differ by $\sim 30\%$. In contrast, the sample without the irradiation treatment yields very weak fluorescence signals.

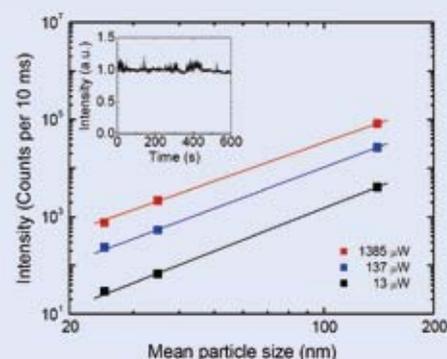
Producing large quantities of FNDs with the He^+ beam irradiation allows us to extract smaller diamond nanoparticles from the 35-nm ensembles by repeated centrifugal fractionation. The particles so extracted have a mean size of 25 nm, as revealed by both transmission electron microscopy and dynamic light scattering measurements. Figure 3 shows the fluorescence intensity of these particles along with those of 35-nm and 140-nm FNDs. The observed signals scale nearly linearly with the volumes of the particles excited at three different laser energies, indicating that the fluorescence intensities of these FNDs are bulk-dependent, little affected by their surface characteristics and, therefore, their environments. Notably, the excellent photostability (i.e. no photobleaching and photoblinking) of the material is preserved even for particles as small as 25 nm (inset in Figure 3).

The excellent photostability and high brightness of the material make it possible to conduct long-term, three-dimensional tracking of a single FND in a live cell. The technique holds great promise to reveal details of intracellular activities such as drug delivery and virus infection. Figure 4a displays the overlap of bright-field and epifluorescence images of a live HeLa cell after uptake of 35-nm FNDs. By using a home-built servo control system and operating the fluorescence microscope in a wide-field mode, we have been able to track a single 35-nm FND (marked with a yellow box in Figure 4a) inside the cell over a time span of more than 200 s. From a mean square displacement analysis of the three-dimensional trajectory (Figure 4b), we determined a diffusion coefficient of $3.1 \times 10^{-3} \mu m^2/s$ for the internalized FND particle. The value is in good agreement with reported diffusion coefficients of quantum dots confined within an endosome.

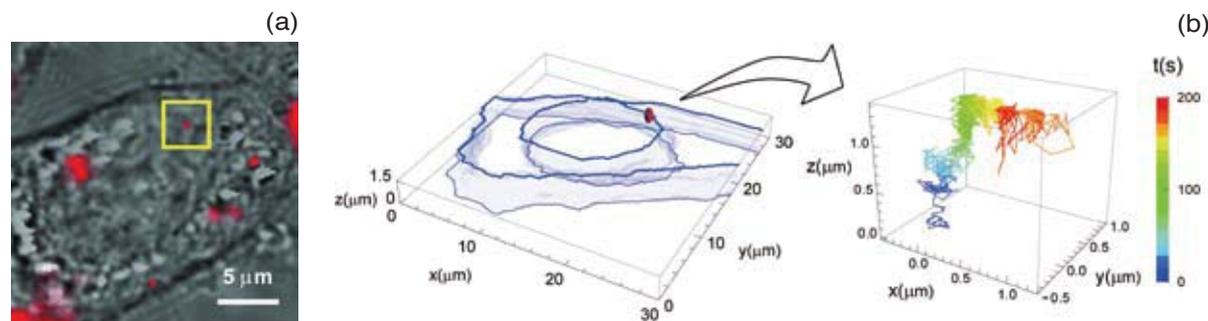
To conclude, we have developed a practical method for FND production in quantity. Compared with organic fluorophores whose time-lapse imaging is limited by photobleaching, the FND as a nanoprobe provides an excellent opportunity to follow both fast (milliseconds) and slow (hours) events in cells, tissues, and even small animals. Integrating the existing and emerging nanodiamond technologies into practice is expected to open up exciting new horizons for applications of this novel material in nanomedicine and nanobiology.



2 Fluorescence spectra of 35-nm FNDs suspended in water, prepared with either 40-keV He^+ or 3-MeV H^+ irradiation. Inset: Fluorescence image of a 35-nm FND suspension excited by 532-nm laser light.



3 Fluorescence intensities of FNDs as a function of particle size at three different laser powers. The slopes of the linear fits vary from 2.65 to 2.95 over the power range used in the measurements. Inset: Fluorescence time trace (intensity normalized) of a single 25-nm FND.



4 Three-dimensional tracking of a single 35-nm FND in a live HeLa cell. a, Overlap of bright-field and epifluorescence (red pseudo-color) images of the cell after the FND uptake. b, (left) Three-dimensional reconstruction, showing the boundaries of the nucleus and the cytoplasm of the cell. (right) Three-dimensional trajectory (shown in pseudo-color) and displacements of a single FND (labeled with a yellow box in a) inside the cell over a time span of 200 s.

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