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## Diamond nanoparticles as photoluminescent nanoprobes

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### Abstract

We present preliminary results showing the potential of diamond nanoparticles with size <50 nm as photoluminescent nanoprobes (i) for biological applications like biomolecule tracking in living cell and (ii) for serving as stable point-like emitters attached at the tip apex of a near-field optical microscope to achieve enhanced spatial resolution.

## 1 INTRODUCTION

There is a growing interest in different fields (biology, nanophotonics, chemical sensing. . .) for bright and stable photoluminescent nanoprobes of smallest possible size for minimal disturbance on the studied systems. We have developed such nanoprobes relying on the photoluminescence of nitrogen-vacancy (NV) colour centres in synthetic diamond nanocrystals (size < 50 nm) [1]. Nitrogen is naturally present in diamond. Vacancies are created by proton irradiation at optimal dose to achieve the highest NV density. NV centers are perfectly photostable emitters in the red and near infra-red, which can be used at single centre level in a various set of quantum optics experiments [2]. They are now envisioned, at a higher concentration, as photoluminescent nanoprobes for biology and nanophysics.

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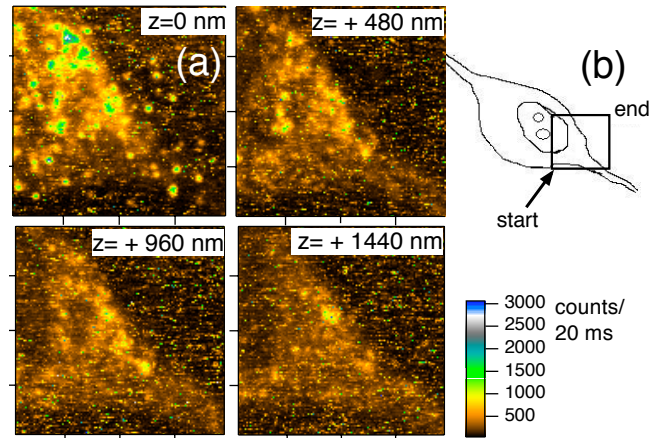
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## 2 PHOTOLUMINESCENT DIAMOND NANOCRYSTALS AS BIOLOGICAL MARKERS

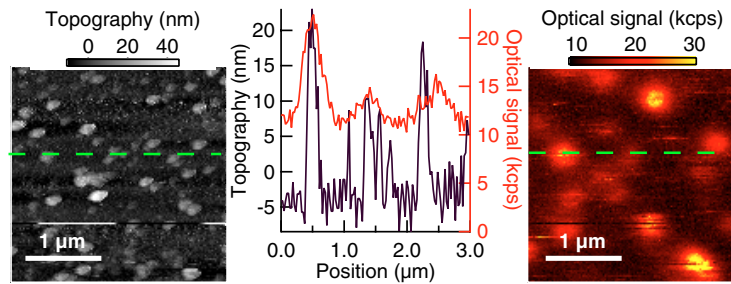
Up-to-date the most used photoluminescent nanoparticles in biology are semiconductor quantum dots (QDs). Although their photostability is much higher than the one of dye molecules, it is limited by blinking and bleaching processes and these particles appear also to be cytotoxic. Diamond material combines many advantages: (i) it is chemically inert, (ii) its surface can be functionalized through chemistry of carbon element, and (iii) it can be strongly photoluminescent thanks to its embedded colour centres. Recent reports show that some photoluminescent nanodiamonds (size  $\approx 100$  nm) can penetrate inside cells, in culture [3]. Commercial powder of synthetic diamond nanoparticles (size  $< 50$  nm) has been first irradiated with protons (2.5 MeV,  $5 \times 10^{16}$  H<sup>+</sup>/cm<sup>2</sup>) after deposition as a 20  $\mu$ m thick layer on silicon wafers. Irradiation step was followed by annealing (2 h, 800 °C), to stabilize the position of the vacancies. Finally nanodiamonds were acid treated (H<sub>2</sub>SO<sub>4</sub>, HNO<sub>3</sub> solution 9:1 vol/vol, for 3 days at 75 °C) to obtain stable aqueous suspension thanks to repulsive electrostatic interactions due to charged surface chemical groups. Further intense ultra-sonication was needed to re-disperse the particles in the solution. The nanoparticles were then deposited on a microscope coverslip. Culture of *HeLa* cells was realised on the same substrate. We expected that nanodiamond would be slowly salted-out and penetrate into the cell individually. Photoluminescence of the coverslips was observed with a home-made confocal microscope (CW 532 nm excitation laser at 200  $\mu$ W mean power). As illustrated in Figure 1, 960 nm above the substrate ( $z = 0$ ) photoluminescent nanoparticles appear that could not be seen on the  $z = 0$  slice, indicating that 50 nm particles have penetrated in the cell cytoplasm. We checked that every bright spot has the spectral signature of NV centers. Note that in plane  $z = 0$  many nanoparticles can still be observed: the majority of them stayed attached to the substrate and was not salted-out into the culture medium as expected.

## 3 PHOTOLUMINESCENT DIAMOND NANOCRYSTALS AS POSSIBLE ACTIVE PROBES FOR NEAR-FIELD OPTICAL MICROSCOPY

In Near-field Scanning Optical Microscopy (NSOM) [4], one approaches a tiny source of light into the near field of the sample, *i.e.* at distances much smaller than the wavelength. Scanning the probe above the sample allows one to build optical images with lateral resolutions better than the usual limitation of far-field optics. This resolution is mainly limited by the size of the light source. That is why fluorescent nanoparticles have received increasing interest among the NSOM community. We have recently reported attempts to use a single CdSe nanocrystal attached to a standard NSOM probe as source of light [5]. In this attempt, the blinking and bleaching behaviour of the nanocrystal has proved harmful to build large-scale images. N-V centres in diamond nanoparticles should avoid these limitations. Such particles have already been used for similar purposes [6], but with particle sizes larger than 100 nm. As shown in Figure 2, we have been able to topographically and optically detect the much smaller nanoparticles presented in



**Figure 1.** (Color online) (a) Photoluminescent confocal raster scans ( $6 \times 6 \mu\text{m}$ ) of part of a cell (depicted in (b); region scanned marked with the square) grown the microscope coverslide initially covered by diamond nanoparticles;  $z$ -serie from  $z = 0$  (coverslide plane) to  $z = 1440$  nm.



**Figure 2.** (Color online) NSOM image of diamond nanoparticles spincoated on a fused silica coverslide. Left: topographic image, obtained in shear force. Right: optical image obtained simultaneously. Middle: line cuts through the two images.

Section 2. The images show that the typical particle size is 15–20 nm, and that not every single diamond nanoparticle exhibits photoluminescence. This opens the possibility of selecting a single active nanoparticle and of attaching it to the tip by a fishing method to form stable active tips.

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