Realization of Nano-Diamond Fluorescent Markers for *In-Vitro* Cell Imaging by Means of MeV Proton Implantation

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INTRODUCTION

Nano-scale materials possess unique optical and chemical properties that allow the creation of innovative biological probes. In particular, the employment of nano-particles has been investigated in different environments, offering promising prospective in biomedical research, diagnostics and therapy. In particular, diamond has received an increasing attention thanks to its non-toxicity and bio-compatibility.

Moreover, diamond lattice can host a large number of defects that can act as single color centers: one of the most appealing ones is the negatively charged Nitrogen-Vacancy (NV^-) defect [1,2]. This defect consists in a complex comprising a substitutional nitrogen atom nearby to a vacancy defect. The NV^- center is optically active, i.e. when optically excited (typically by green light) produces a broad fluorescence emission in the near infrared region around 680 nm. [3]. It is worth stressing that NV^- complex can be found both in bulk material and in micro- and nano-sized crystal; it can also be introduced in the lattice by means of ion-beam-induced damaging [4,5].

Color centers in nanodiamonds (NDs) have many potential biological and medical applications, thanks to their low cytotoxicity. Furthermore, the ultimate cyto-compatibility of the NV-containing NDs and the possibility to optically track them during long-term biological processes is another important issue in this field.

Nanoparticles can be therefore used as biosensors and stable solid supports for synthesis of peptides and as fluorescent markers for cell imaging [6,7].

In this paper we present the realization of nano-diamond fluorescent markers created by means of MeV proton implantation, their morphological and optical characterization and their uptake in cultured cells for *in-vitro* imaging.

ION BEAM IMPLANTATION

The sample under exam is produced by ElementSix™ (UK) and consists in a synthetic diamond powder produced by disaggregation of High Pressure High Temperature (HPHT) single-crystals. Diamond nanoparticles size ranges from ~10 nm to ~250 nm. The crystals are classified as type Ib having a single substitutional nitrogen concentration comprised between 10 ppm and 100 ppm.

Only a few percent of the high content of nitrogen is involved in the formation of NV centers, due to the low amount of vacancies in the pristine powder: ion-induced damaging represents an effective tool to increase vacancy density and therefore the total amount of luminescent centers upon subsequent thermal annealing.

Powders were deposited onto silicon substrates after a chemical cleaning process in HNO_3 for 72 hours, in order to remove superficial graphitic layers (a byproduct of the disaggregation process). The samples were then implanted at room temperature with a 2 MeV H^+ ion broad beam at the AN2000 accelerator facility of the INFN Legnaro National Laboratories (INFN-LNL). An implantation fluence of 5×10^{15} cm^{-2} was achieved. As hilighted in the SRIM [8] Monte Carlo code simulation reported in Fig. 1, the penetration depth of 2 MeV proton is sufficient to pass through all the thin (~20 μm) deposited film of diamond powder, guaranteeing a nearly-uniform vacancy creation over the totality of the nano-particles. A displacement energy of 50 eV and an effective density of 3.52 g cm^{-3} (the same as diamond bulk material, assuming a uniform stacking of the nanoparticles) were set during simulations.

After ion implantation, thermal treatment (800 °C for 1 h in nitrogen environment) was performed to further promote the coupling of nitrogen and vacancy sites into NV^- centers.

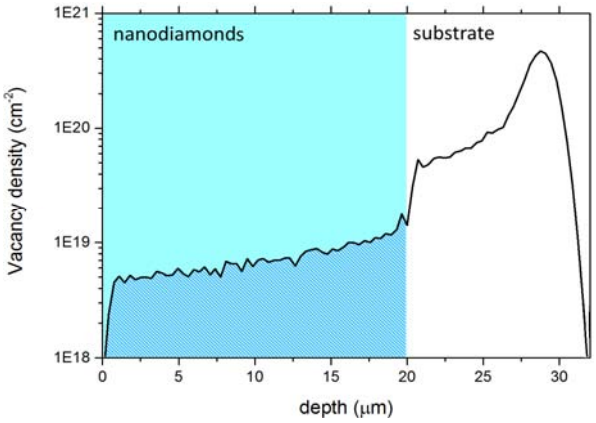

Fig. 1: Damage density profile for a 2 MeV H^+ implantation at a fluence of 5×10^{15} cm^{-2}.

NANODIAMONDS CHARACTERIZATION

After a cleaning procedure, the size of the nanodiamonds was estimated by means of SEM imaging: exemplifying typical SEM micrograph is shown in Fig. 2, where

nanoparticles present the expected dimensions, namely below 250 nm.

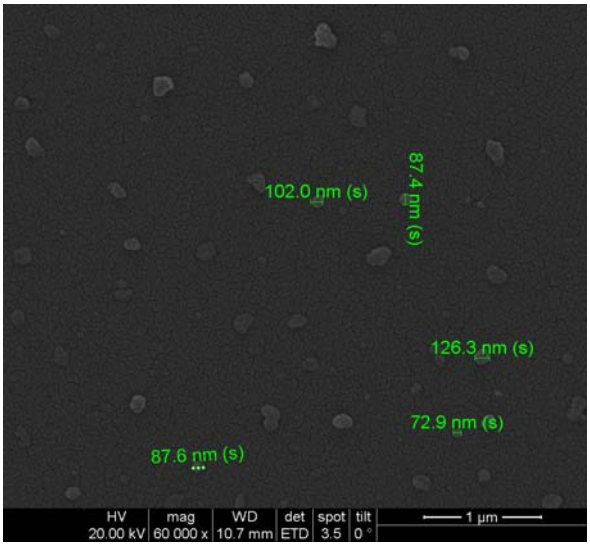

Fig. 2: SEM micrograph of post-processed implanted nanodiamonds. The size of some nanocrystals is highlighted.

Room temperature photoluminescence (PL) spectroscopy was performed with the purpose of assessing the spectral features of the induced NV luminescence in the samples. A Raman micro-spectrometer was employed for this scope, with 532 nm laser excitation and a CCD detector. The typical feature due to the presence of NV centers in NDs is reported in Fig. 3: according to literature [9,10], NV^0 (575 nm) and NV^- (638 nm) zero phonon lines (ZPL) and relative phonon bands are observable.

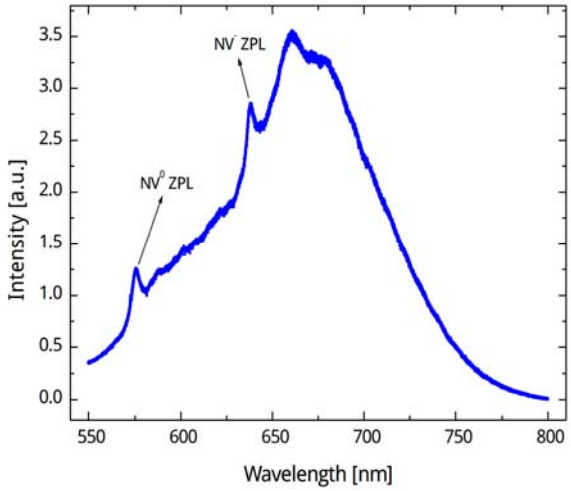

Fig. 3: PL spectrum of ion implanted nanodiamonds, obtained with an excitation wavelength of 532 nm: the spectrum shows the typical zero phonon lines due to the presence of NV centers.

RESULTS

Preliminary results on the uptake of nanodiamonds in cultured cell were subsequently obtained.

Neuroendocrine cells of adrenal gland of mouse were plated on glass substrate and incubated at 37 °C in a water saturated 5% CO_2 atmosphere [11]. Cells reach their optimal adhesion and maturation after an incubation of three days. After this period, an aqueous suspension of NDs with a concentration of ~ 0.3 mg ml^{-1} was added to the culture medium. The nanocrystals uptake process requires approximately 3 hours.

Treated cells were then observed with a confocal microscope in order to obtain *in-vitro* imaging: an excitation wavelength of 543 nm and a photomultiplier detection window of 610-750 nm were employed.

In Fig. 4 we report the comparison between optical transmission and PL micrographs of the same region, where three cells are observable. The efficiency of NDs acting as fluorescent markers in cells is clear.

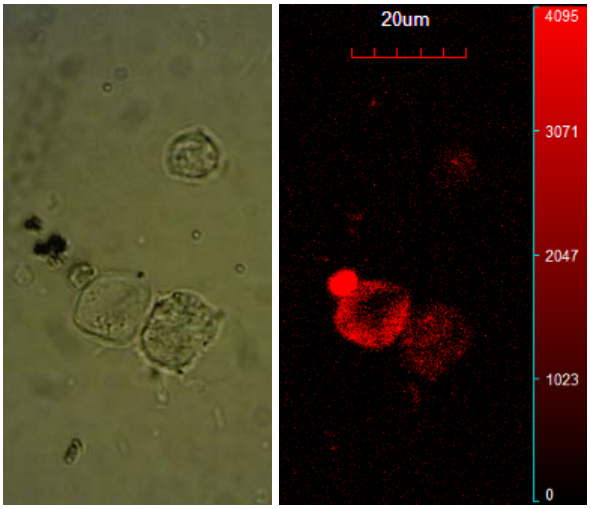

Fig. 4: a) Optical transmission micrograph and b) fluorescence micrograph of nanodiamonds uptaken by neuroendocrinal cells: the presence of luminescent markers inside the cells is clearly observable.

CONCLUSIONS

The preliminary results demonstrate that fluorescent nanodiamonds can be an attractive tool for cell-imaging.

Future activities will be focused on the further characterization and on the surface functionalization of diamonds -particles: it is foreseen to employ these probes in order to monitor neuronal network.

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