NANO MICRO small

Supporting Information

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Fluorescent Nanodiamonds Embedded in Biocompatible Translucent Shells

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Submitted to small NANO MICRO

Supporting Information

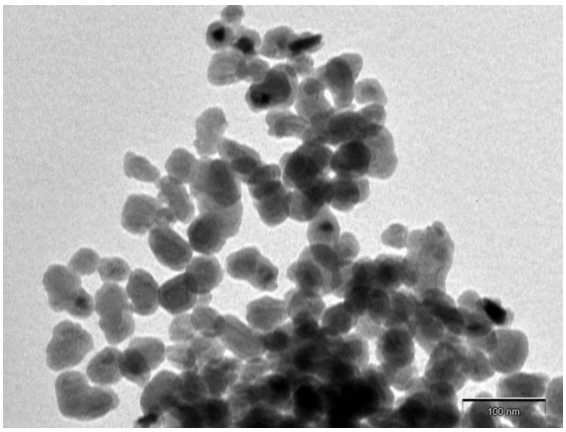

Figure S1: BF-TEM images of aminosilica-coated particles prepared with a half amount of tetraethoxy silane compared to the particles **ND3** (Figure 1B).

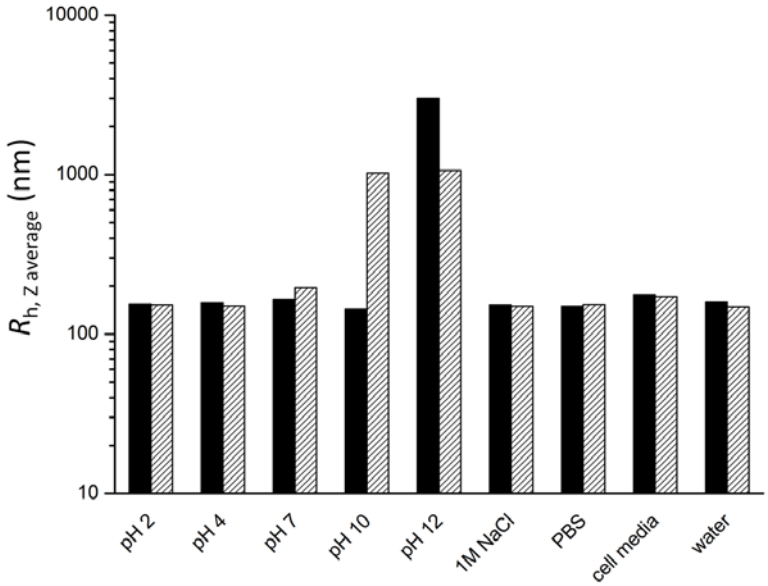

Figure S2: Aggregation states (expressed as zeta averages from DLS measurement) of colloidal aqueous solutions of **ND4** particles at pH 2, 4, 7, 10, and 12 (pH adjusted using HCl and NaOH solutions), in 1M NaCl, PBS buffer and serum-containing cell grow media (Sigma Aldrich RPMI-1640 + serum). Measurements were performed 24 hours (black bars) and 2 weeks (hatched bars) after sample preparation. The sample at pH 12 precipitated during the first two hours, and the sample at pH 10 precipitated after one week. All other samples remained colloidally stable.

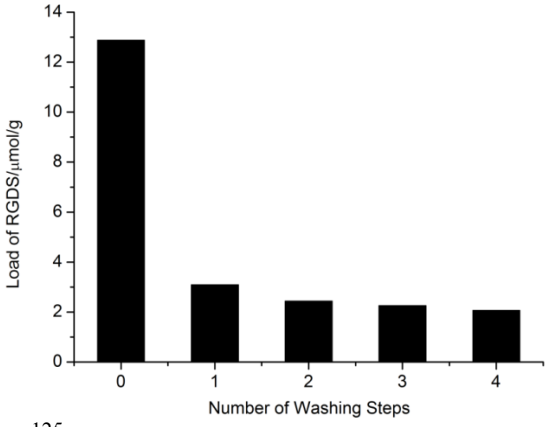

Figure S3: The content of ^{125}I-labeled RGDS peptide in reaction mixture and in the **ND5a** sample after the specified number of washings (1 mg of nanoparticles was washed with 1.2 mL of water and isolated *via* centrifugation. The activity of colloid was measured after each washing step using ionization chamber.

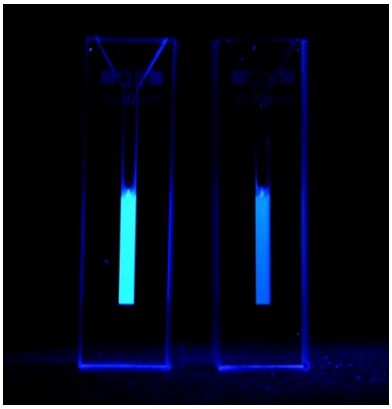

Figure S4: UV-irradiated spectroscopic cuvettes with reaction mixtures after fluorogenic click reaction of **ND4** with coumarin-azide in the presence (left) and absence (right) of Cu(I) catalyst. The fluorescence of product **ND5b** is clearly observable in the left cuvette.

Single particle measurements

Fraction of ND1 displaying NV color center fluorescence

The fraction of fluorescent NDs was determined using a setup combining an atomic force microscope (AFM, MFP- 3D-BIO, Asylum Research, USA) with an inverted confocal microscope sensitive to single NV color center photoluminescence (see ref.[1] for example). Nanodiamonds were first spin-coated on a quartz coverslip, which was then simultaneously raster-scanned by AFM and confocal microscopes. From scans of the mixtures shown in Figure S5 and three additional scans (not shown), we observed that 97 of 134 nanodiamonds, i.e., 74%, were fluorescent. Moreover, the maximal nanodiamond size measured by AFM was about 100 nm (probably an aggregate of two particles), in relatively good agreement with TEM (Figure 1).

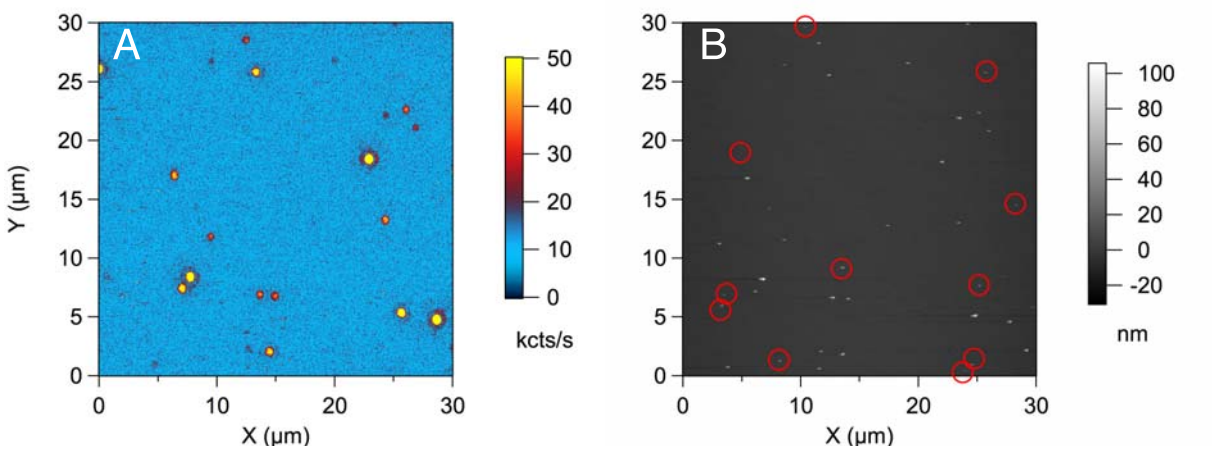

Figure S5: AFM and confocal microscopy simultaneous imaging of non-coated FNDs deposited on a quartz converglass. (A) Photoluminescence image (scale in kilocounts s^{-1} on the single-photon counting module). (B) AFM image of the same field of view. The red circles are centered on NDs that do not exhibit fluorescence. Note that the AFM scan is slightly shifted by about 1 µm towards the negative x-axis, and by about 400 nm towards the positive y-axis.

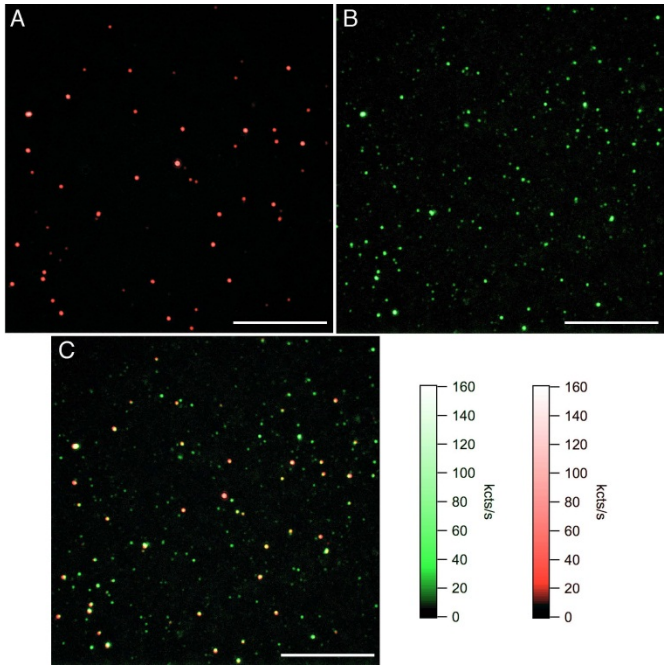

Figure S6: TIRF microscopy images of the fluorescein-labeled particles (**ND5c**), spincoated on a quartz converglass. (A) Red detection channel image showing only NV center fluorescence; (B) Green detection channel, showing fluorescein signal in the same field of view; (C) Merged image in which colocalized spots appear in yellow-white. All red spots of (A) colocalize with green spots of (B). Scale bars: 10 µm, intensity scales in kilo counts per second (kcts s^{-1}) on the detector.

Individual photoluminescence (PL) spectra of ND4

A large number of green spots that do not colocalize with red ones (total of 530), originating from NV centers, were detected in TIRF microscopy images (see Figure S6). To understand this observation, the individual particle spectra were measured using a home-made confocal microscope with an imaging spectrograph, equipped with a cooled CCD array on its detection channel. **ND4** particles were spincoated on a quartz coverslip similarly as for the analysis shown in Figure S5. The PL of the sample was raster-scanned using a continuous wave (cw) excitation laser at a wavelength of 488 nm and a combination of a dichroic beamsplitter (z488rdc Chroma, AHF Analysentechnik, Germany) and a longpass filter with a steep edge at 488 nm (RazorEdge LP02-488RS, Semrock Inc., USA). This allowed us to record the full PL spectra. The excitation laser was then positioned on an individual FND to record the spectrum. Figure S7 shows three different types of spectra obtained for different particles. A large majority of the spectra (about 90%) displays only NV° or NV^- PL characteristics (Figure S7A) with a very low signal in the green bandpass detection channel of the TIRF microscope setup. A rare green signal that could come from a color center emitting in the green like the H3 center[3] or Raman from silica (see Figure S7C) can therefore be ruled out. These observations, along with the fact that only 26% of the **ND1** particles do not display NV center fluorescence (Figure S5), indicate that the green spots in Figure S6 that do not colocalize with NV emission are mostly not related to FND. The most probable cause of the non-colocalized green signal is the presence of fluorescent impurities in the solvent that we can detect considering the single emitter sensitivity of our setup.

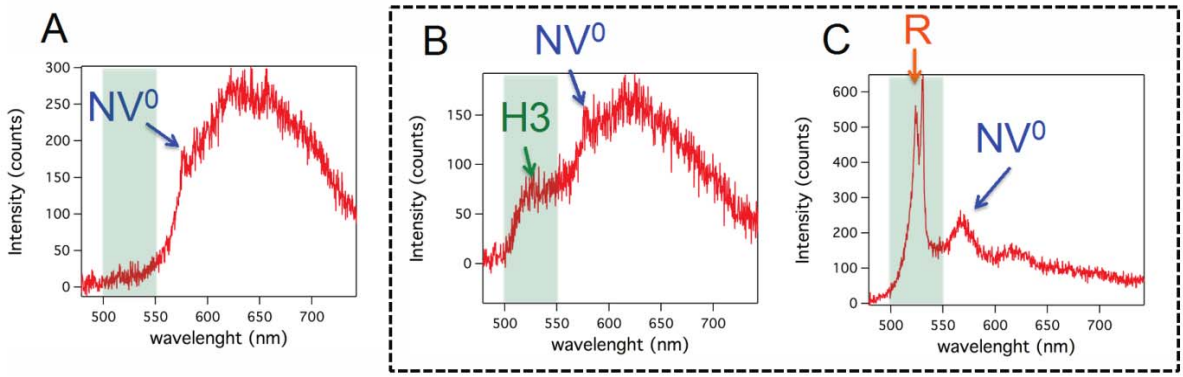

Figure S7: Photoluminescence spectra of individual PEGylated aminosilica-coated (**ND4**) FNDs, excited with a cw laser at 488 nm wavelength (1 mW power). The light green rectangles indicate the detection range of the green channel on the TIRF setup. (A) PL spectrum of an **ND4** particle displaying mostly neutral NV center emission characterized by a zero-phonon line at 575 nm (indicated by the arrow). (B) PL spectrum of another **ND4** particle, showing in addition to NV° emission, the presence of a green emitting center, the H3, corresponding to N-V-N,[2] which would give a significant signal in the TIRF green detection channel. (C) PL spectrum of yet another **ND4** particle displaying narrow peaks in the TIRF green detection channel (labeled with R). We attribute these peaks to the 1873 cm^{-1} Raman peak of amorphous silica,[3] which results in a peak at 537 nm for the 488 nm excitation wavelength used, very close to the 532 nm peak maximum observed.

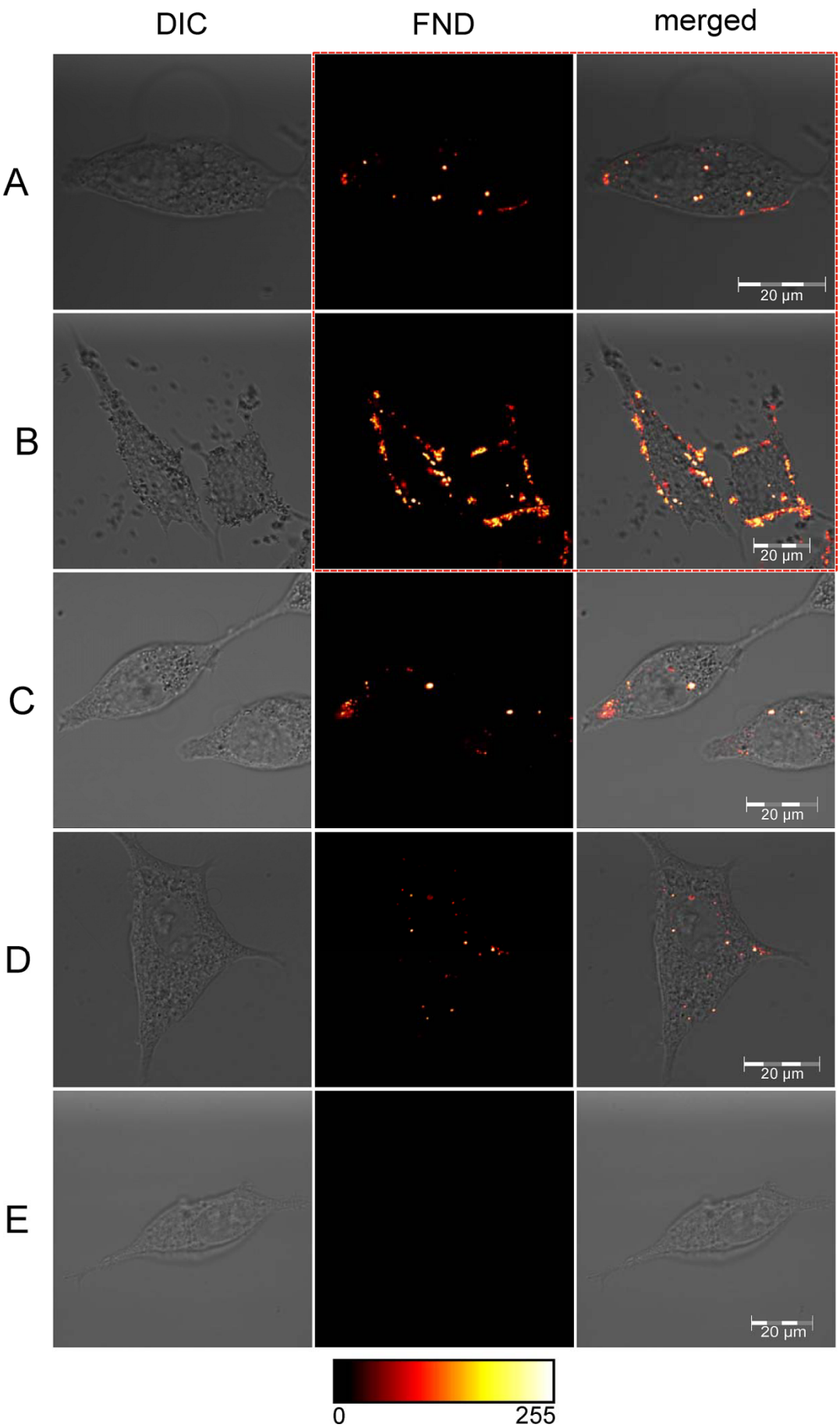
DIC
FND
merged
A
B
C
D
E
20 µm
20 µm
20 µm
20 µm
20 µm
0
255

Figure S8. Images of FNDs internalized by LNCaP cells: (A) PEGylated aminosilica-coated **ND4** and (B) non-coated **ND1**, dispersed in PBS; (C) **ND4** and (D) **ND1** pre-incubated in medium containing serum proteins; (E) cells without nanodiamonds. The displayed images are differential interference contrast (left), fluorescence from NDs (middle), and merged image (right). All the cells were washed by PBS after one hour incubation with NDs (final concentration 200 μg mL^{-1}), incubated for subsequent 23 hours and observed after fixation with solution of 2% formaldehyde. Fluorescence of FND was collected by Zeiss LSM 780 at 639–758 nm upon excitation at 532 nm. The upper right part of the image marked by a red line is identical with Figure 6.

References

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