Diffusion dynamics of fluorescent nano-diamonds in living cells detected by fluorescence-combined iSCAT system

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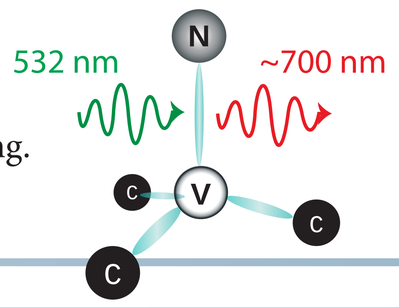
Introduction

Fluorescence nano-diamonds (fND) are promising photoluminescent probes due to embedded, perfectly photostable color centers. An additional benefit of those particles is their high index of refraction, which gives rise to light scattering even for very small particles, and their prominent Raman signal. These properties allow long-term observation in living cells. Here we investigate the diffusion dynamics and uptake mechanism of fNDs of different sizes by interferometric scattering (iSCAT) microscopy combined with fluorescence imaging. With this system, we are able to detect even very small (~10 nm) fNDs and track them in real time. The diffusion of fNDs differs depending on environment, particle size and particle shape.

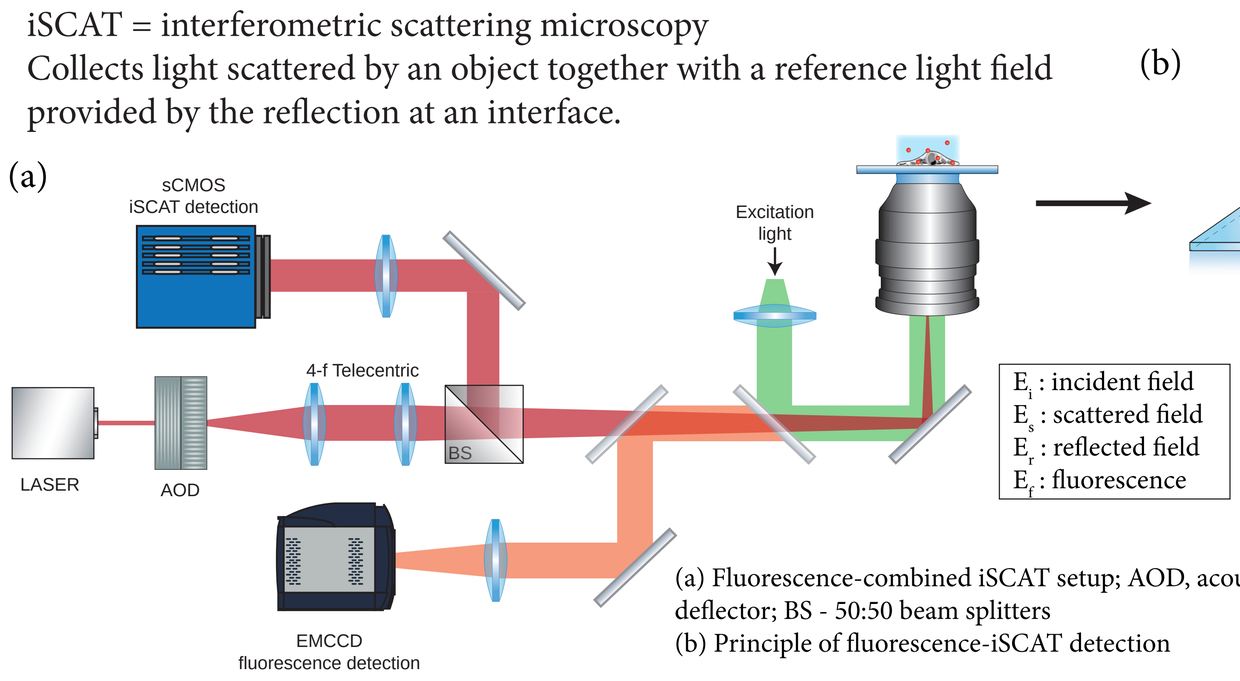
Fluorescent Nano-Diamonds

Carbon-based fluorescence nanoparticles became a suitable option for stable fluorescent probes because of their photostability without photobleaching or blinking, bright fluorescence, long fluorescence lifetime (>10 ns), good biocompatibility and easy surface functionalization.
The nitrogen-vacancy (N-V) defects as fluorescent centers give fNDs the extraordinary photophysical properties. The $(N\text{-}V)^-$ center embedded in the crystal matrix:
◊ absorbs strongly at 560 nm
◊ emits fluorescence at ~700 nm
which is the window for convenient bioimaging.



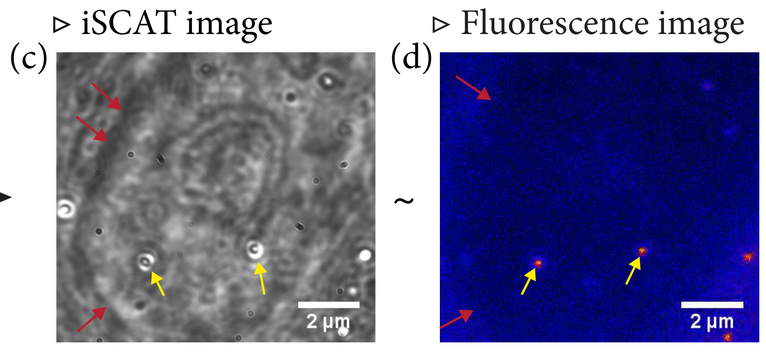
Fluorescence-combined iSCAT System

iSCAT = interferometric scattering microscopy
Collects light scattered by an object together with a reference light field provided by the reflection at an interface.



(a) Fluorescence-combined iSCAT setup; AOD, acousto-optic deflector; BS - 50:50 beam splitters
(b) Principle of fluorescence-iSCAT detection

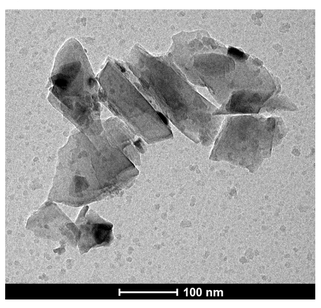
▹ iSCAT image ▹ Fluorescence image



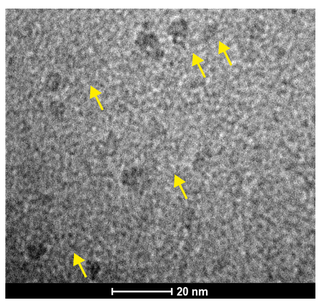
(c) iSCAT image of human osteosarcoma U2OS cell with 100 nm fNDs; scale bar, 2 µm
→ (yellow) scattering signal of fND with its typical fringe pattern
→ (red) cell nucleus boundary
(d) Fluorescence image of U2OS cell with 100 nm fNDs; excitation 532 nm, emission detection range 540 - 600 nm; corresponding image to (c); scale bar, 2 µm
→ (yellow) fluorescence signal of fND
→ (red) cell nucleus boundary

fND TEM

(a) ~100 nm fND

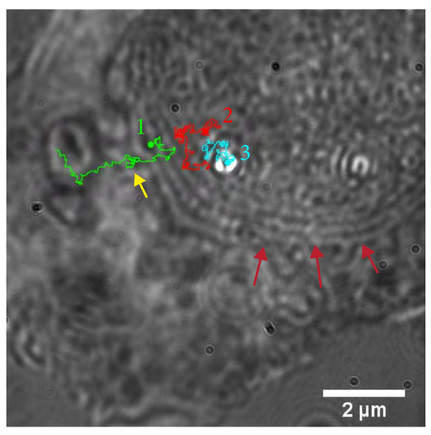


(b) ~10 nm fND

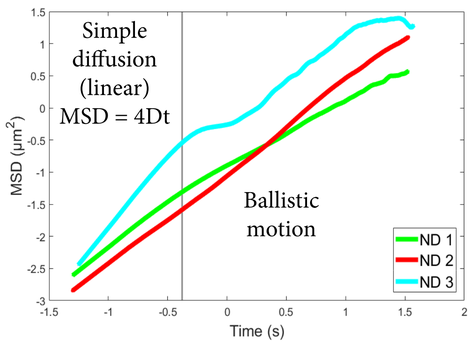


Particle Tracking & MSD Analysis

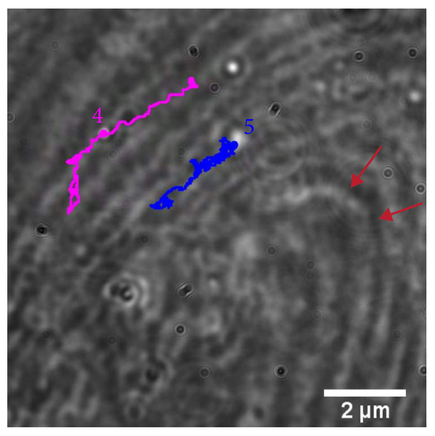
(c) ▹ Diffusion of 10 nm fND



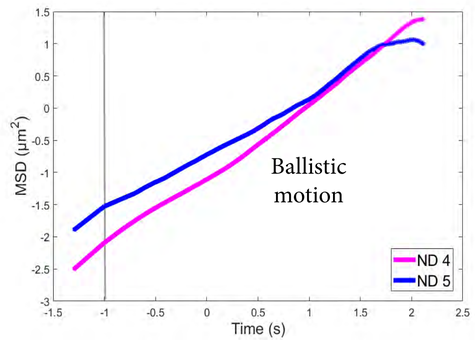
(d) ▹ MSD of 10 nm fND



(e) ▹ Diffusion of 100 nm fND



(f) ▹ MSD of 100 nm fND



(c) (e) iSCAT image of human osteosarcoma U2OS cell with 10 nm (c) and 100 nm (e) fNDs diffusing to perinuclear region; red arrows mark nucleus boundary; yellow arrow moment of endocytosis or transition between cell compartments
1 (green) diffusion along cellular structures
2 (red) corralled diffusion in between cellular structures
3 (turquoise) confined diffusion
4 (pink), 5 (blue) diffusion along cellular structures

(d) (f) Mean square deviation (MSD) as a function of measurement time for fND diffusion inside the cell.
→ linear plot : Brownian motion, simple diffusion
→ curved plot : ballistic motion, (partially) confined diffusion due to cellular structures → longer curve, more structured environment
→ Overall plot should become linear at long timescale

Summary & Future work

◊ We are able to observe and track both 10 nm and 100 nm fNDs in living cells by combining iSCAT and fluorescence detection techniques.
◊ Even scattering from such small NDs (10 nm) can be detected by iSCAT technique.
◊ The diffusion dynamics strongly depends on local environments and particle size.
◊ Both 10 nm and 100 nm fNDs can be internalized by non-phagocytic cells spontaneously and appear to be non-toxic.
◊ We are planning to investigate intrinsic structure of nucleus and target other cellular organelles using fND.

References

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◊ VAIJAYANTHIMALA, Vairakkannu, Yan-Kai TZENG, Huan-Cheng CHANG and Chung-Leung LI. "The biocompatibility of fluorescent nanodiamonds and their mechanism of cellular uptake". Nanotechnology. 2009, 20(42), 425103-

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