doi:10.1017/S1431927616005936
Microsc. Microanal. 22 (Suppl 3), 2016

Fluorescent Nanodiamonds as Fiducial Markers or Nanodiamonds Are Forever.

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Many microscopic techniques rely on the use of fiducial markers or reference points to correlate images or correct for movement during the imaging process. Important techniques including correlated fluorescence and electron microscopy and single molecule localization microscopy such as PALM and STORM, require the use of fiducial markers. Popular choices for fluorescent fiducial markers include quantum dots, fluorescent beads and gold spheres or rods. Nanodiamonds provide another alternative. They are chemically inert and optically transparent. It is well known that defects in the diamond lattice can produce colored diamonds. Other defects, most notably the negatively-charged nitrogen vacancy center $(N\text{-}V)^-$ produce fluorescent diamonds. This defect results in diamonds that are strongly fluorescent at optical wavelengths, with a high quantum efficiency and low photobleaching [1]. These properties make them ideal fiducial markers. In addition, fluorescent nanodiamonds (FNDs) can be functionalized for other biological purposes [2, 3].

Diamonds with $(N\text{-}V)^-$ centers can be produced from diamonds containing substitutional nitrogen of at least 100 ppm nitrogen. Following high energy irradiation, the diamonds are annealed at temperatures above 800°C in vacuum or inert atmosphere. This causes vacancies to migrate to the nitrogen creating both NV^0 and $(N\text{-}V)^-$ centers. $(N\text{-}V)^-$ diamonds are commercially available (Adamas Nanotechnologies, Raleigh, NC). FNDs are available in a variety of sizes.

Confocal scanning images were collected with a Leica SP8 laser scanning confocal microscope, using a 63X, 1.4 NA objective (Leica Microsystems Inc, Buffalo Grove IL). TIRF images were acquired using NIKON Eclipse Ti inverted microscope, 647 nm AOTF modulated LUNB solid state laser (125 mW), 100X, 1.49 NA SR Apochromat TIRF objective lens, Andor iXon Ultra 897 EMCCD camera and 1.5x intermediate magnification lens (Nikon Instruments, Inc., Melville NY). dSTORM localization of TIRF images was performed using Thunderstorm plugin (ver. 1.2) on FIJI software.

Fluorescent nanodiamonds are ideal fiducial markers and biological imaging probes. They have a 10-20 ns excited lifetime, a quantum yield near 0.95 and a 2-photon-cross section. They have biologically compatible fluorescence (Fig.1). FNDs show indefinite photostability and display better temporal stability than nano-gold particles (Fig 1). FNDs can also be used for single molecule localization microscopy to provide fiducials with sufficient longevity and brightness for thousands of images (Fig 2).

In addition, FNDs can be visualized by electron microscopy (Fig 3).

Finally FNDs can be coated in silica and functionalized to produce bright fluorescent markers that can be used in other applications.

Fluorescent nanodiamonds show a number of optical properties that make them ideal fiducial markers. They are bright, long lived and stable. They can be used for confocal fluorescent microscopy, including 2-photon microscopy. They show promise as markers for correlative microscopy as well. In addition, functionalized FNDs could be used as labels for biomolecules. Finally other optical properties not discussed here may allow for background free, improved in-vivo imaging [3].

References:

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[3] SK Sarkar *et al*, Biomed. Opt. Exp. **5** (2015), p.1190.

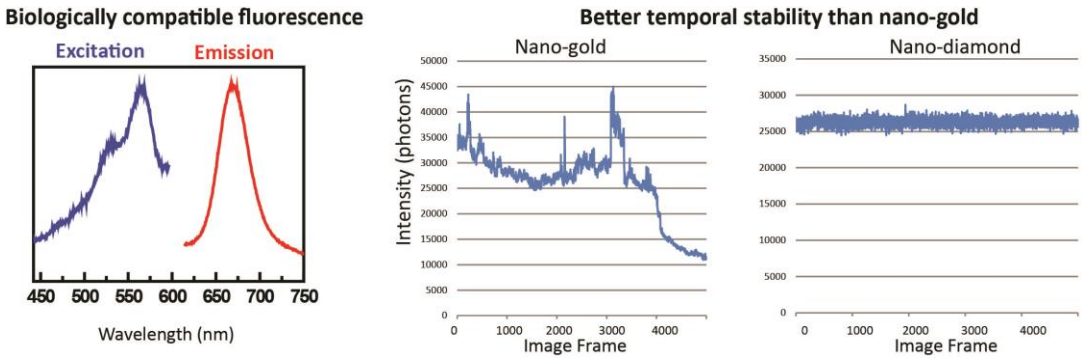

Figure 1. FNDs have excitation and emission properties compatible with biological samples. FNDs show no photobleaching and better stability than nano-gold particles.

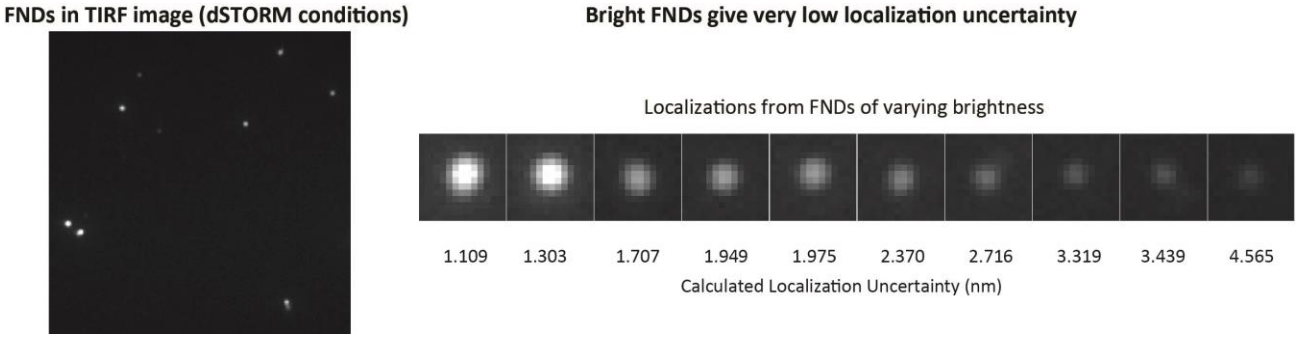

Figure 2. Bright FNDs can give localization errors close to 1 nm. FNDs were visualized in TIRF mode under dSTORM conditions. The single molecule localizations were determined and the error in localization was calculated.

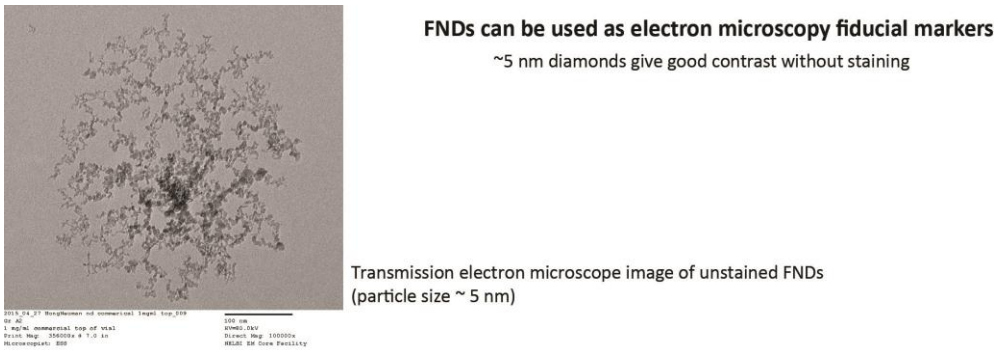

Figure 3. Electron micrograph of unstained FNDs with particle sizes around 5 nm.

https://doi.org/10.1017/S1431927616005936 Published online by Cambridge University Press