

Fluorescent Nanodiamonds: Bio-applications

Fluorescent nanodiamonds (FNDs) offer a unique alternative to currently existing fluorescent biomarkers. With exceptional photo and chemical stability, FNDs are a significantly more robust imaging agent than others currently available. In addition, FNDs may be conjugated with numerous biologically active species and exhibit high biocompatibility, making them particularly well-suited for a number of bio-applications (discussed below).

Content At a Glance:

A brief overview of the use of fluorescent nanodiamonds (FNDs) for various biological applications is discussed, including:

- Basic fluorescence and physical properties of FNDs
- Bio-conjugation chemistry
- *In vitro* and *in vivo* imaging demonstrations
- *In vitro* bio-compatibility studies

Physical and Fluorescence Properties



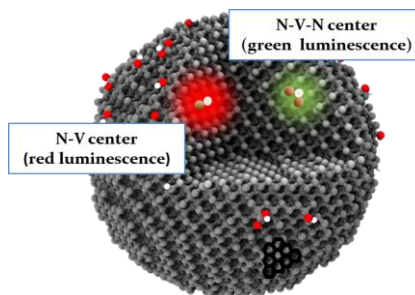
Color Centers

The fluorescence of nanodiamonds originates from atomic substitutions within the diamond lattice called 'color-centers.' Two of these color centers are the nitrogen-vacancy (NV) and nitrogen-vacancy-nitrogen (NVN) centers, which provide red-NIR and green emission, respectively. Being embedded in the diamond core, these color centers are infinitely photostable and do not photobleach, even under high excitation power over long periods of time. Additionally, these centers do not chemically degrade with time. The optimal excitation source for NV centers is between 500-600 nm, with peak emission intensity between 670-710 nm. For NVN centers, the optimal excitation is between 450-500 nm, with peak emission around 510-520 nm.

Particle Size and Shape

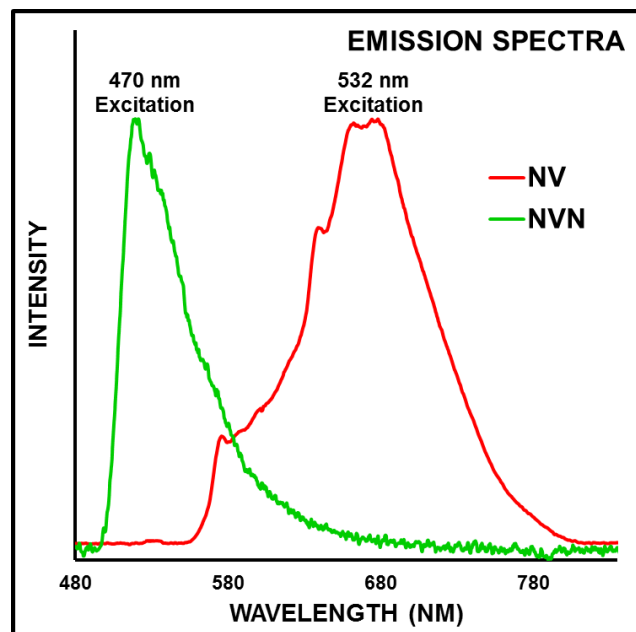
Particles are available in sizes ranging from 10 nm up to 150 μm . Most particles have irregular, rocky shapes, with the exception of the largest micron particles, which can have regular polyhedral shapes.

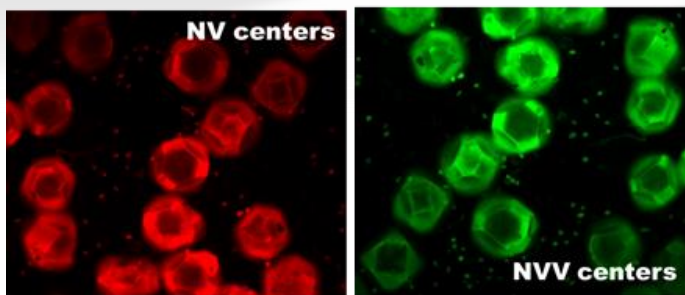
(Right) Fluorescence emission profiles for NV and NVN color centers in diamond.



Color centers are protected by the diamond lattice =
No Photobleaching!

(Above) Schematic model of a nanodiamond particle containing color centers (NV and NVN). Though both the NV and NVN centers can co-exist in the same particle, particles generally contain either one or the other.





(Above): Images of 150 μm diamond particles containing NV centers (left) and NVN centers (right).

Unique Fluorescence Modulation Capability

In addition to fluorescence, NV centers are electron spin active, which allow external fields to modulate fluorescence intensities. Such modulation allows for enhanced contrast capability that helps discriminate against background autofluorescence.

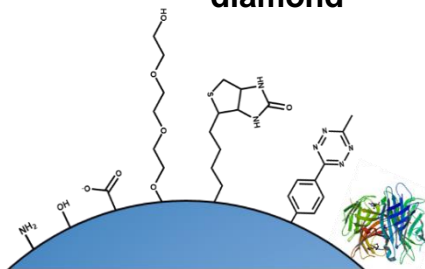
Comparative Brightness

The brightness of FNDs is directly related to the number of color centers that can be fit within the diamond lattice of a single particle. As a result, larger particles tend to be brighter, and smaller particles tend to be dimmer, where a roughly volumetric dependence between particle size and number of color centers is observed. The molar absorption coefficient for a single NV center is reported as $\epsilon_{532\text{nm}} = 8.1 \times 10^3 \text{ M}^{-1} \text{ cm}^{-1}$ ($\sigma = 3.1 \times 10^{-17} \text{ cm}^2$) (*J.Phys.Chem.A 2007 (111), 9379*). For a 100 nm particle containing approximately 300 NV centers, this implies a molar absorptivity of $2.43 \times 10^6 \text{ M}^{-1} \text{ cm}^{-1}$. Comparatively, Alexafluor 647 has a molar absorption coefficient of $2.7 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$. As such, FNDs are suitable for single fluorophore labeling of low concentration targets. On a single particle basis, 100 nm particles containing 3ppm of NV centers are 12x brighter than the fluorophore Atto-532 based on Total Internal Reflection Microscopy (TIRFM) measurements.

Bio-Conjugation: Direct Functionalization or Click Chemistry

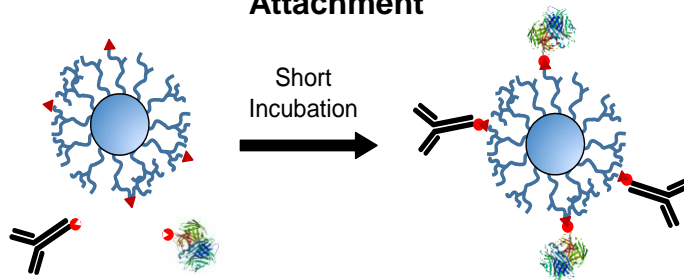
Fluorescent nanodiamonds have a rich surface chemistry which lends itself to a variety of functionalization schemes that are commonplace for the standard bio-lab. Biomolecules such as proteins or antibodies are easily attached to the FND surface by either (1) direct conjugation or (2) click chemistry (highly selective biorthogonal reaction). A range of bio-functionalized FNDs are currently available for use: for example, labeled particles can be directed to a target of interest. In addition, functionalization schemes exist to promote long term buffer colloidal stability.

(1) Direct attachment onto functionalized diamond



FNDs are available with a variety of specific groups for conjugation, including: carboxyl, hydroxyl, amino, PEG, biotin, streptavidin, and click-chemistry ready moieties.

(2) Click Chemistry Mediated Attachment

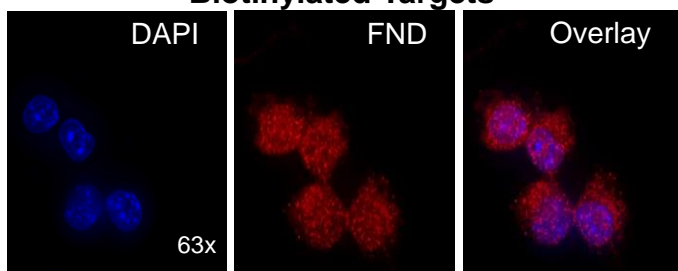


Tetrazine(Tz) functionalized ND can be reacted with transcyclooctene (TCO) at room temperature to label particles with proteins or antibodies with fast reaction kinetics (up to $26,000 \text{ M}^{-1} \text{ s}^{-1}$).

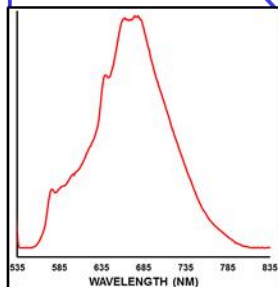
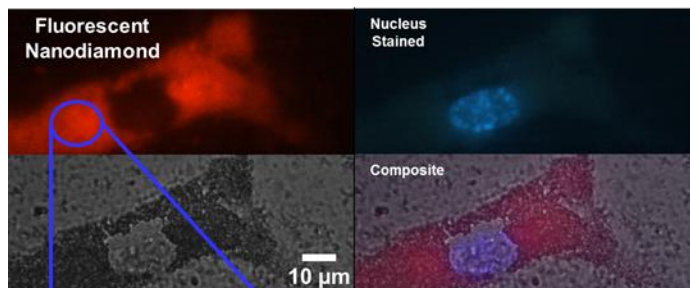
Targeting and *In Vitro* Visualization

Adámas nanodiamonds have been validated in a number of imaging applications. Attachment of biologically active surface groups allows for targeted FND delivery and visualization. The unique fluorescence profile of FNDs also allows for unambiguous verification of localization or conjugation.

Streptavidin Conjugated FND for Biotinylated Targets

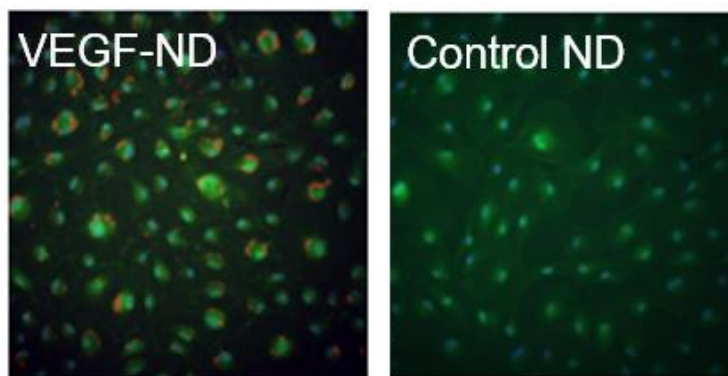


Streptavidin functionalized 40 nm Adámas fluorescent nanodiamond containing NV centers targeting a biotinylated intracellular target, with comparison and overlay to DAPI (4',6-diamidino-2-phenylindole) stained nuclei. Image courtesy of Dr. Lindsay Parker, Macquarie University, Australia.

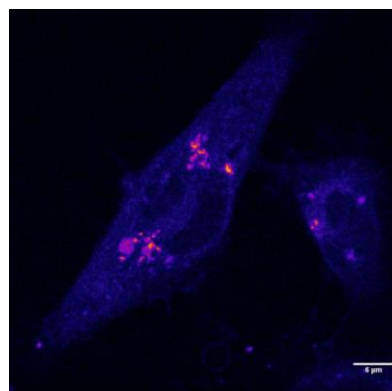


High levels of uptake and facile imaging by colony forming endothelial cells with direct fluorescence visualization and spectroscopic confirmation. Excitation with filtered short-arc mercury lamp.

VEGF Conjugated FND for Cells Expressing VEGF Receptor



Adámas FND click-conjugated to vascular endothelial growth factor (sVEGF, Sibtech) shows targeting affinity to endothelial colony forming cells expressing VEGF receptors, compared to non-functionalized control (merge of Hoechst, ND, and Calcein channels). Creative Scientist, Inc. NC.



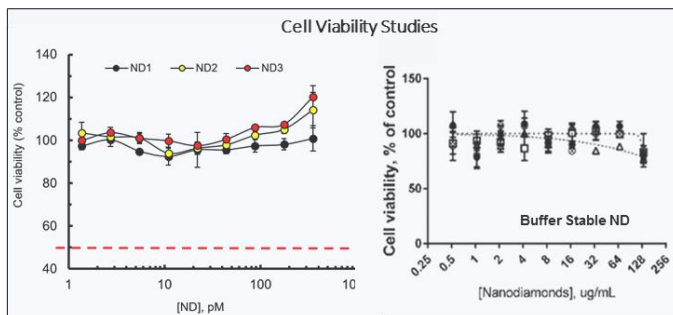
In vitro imaging of 20 nm Adámas FND (20nm-Hj) in MDA-MB-231 Breast Cancer Cells with 488 nm laser excitation following 48 h incubation at 50 µg/mL (650-720 nm detection window). N. Prabhakar, Åbo Akademi, Finland.

FNDs are suitable for both laser confocal imaging as well as traditional filtered excitation of short arc mercury or xenon lamps commonly equipped on epifluorescent microscopes. They can also be functionalized to remain stable in cellular growth media and resist non-specific binding.

In Vivo Visualization

Adámas FNDs are bright enough to be used in whole body imaging applications. Excitation via 605 nm or 640 nm sources provide effective penetration depth with emission compatible with the Cy5.5 window. Intravenous injection into mice showed spleen and liver accumulation which was confirmed via *ex vivo* tissue digestion and analysis. No adverse health effects were observed in the mice over a 24hr period. Because of their high chemical stability, FNDs can be subjected to the harsh acidic conditions of tissue digestion without compromising their fluorescence and used for biodistribution quantification.

Biocompatibility

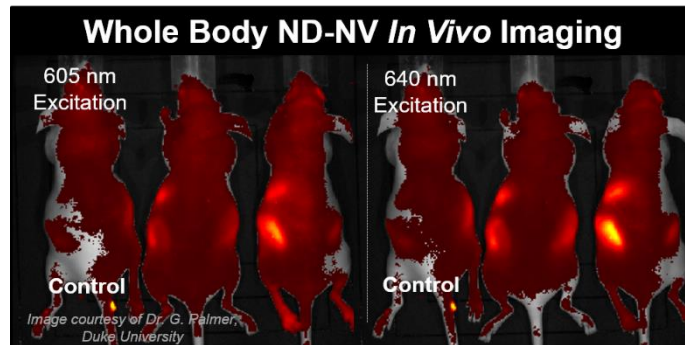


Cell viability of buffer stable ND with 293KDR cells (left) and colony forming endothelial cells (right). No reduction in cell viability was observed in the range tested (up to 0.128 mg/mL)

Adámas FNDs exhibit high *in vitro* biocompatibility, with no significant cellular toxicity observed up to approximately 0.13 mg/mL loading in both 293KDR and colony forming endothelial cells.

Applications

Long-term cell imaging (including stem cells), flow cytometry, bioassays, super-resolution and correlative microscopy imaging (fiducial markers), nanosensors, labeling in tissue engineering, image guided surgery, UV protection, cosmetics, authentication.



Imaging 170 nm FND after IV injection into athymic BALB/c mice. Imaging performed using IVIS system (Duke University School of Medicine).

Takeaways:

Adámas' fluorescent nanodiamonds offer a unique alternative to currently existing fluorescent biomarkers on the market. Key points and advantages are:

- Fluorescence originates from color centers in the diamond lattice.
- Large size range available (10nm-150µm)
- No photo-bleaching and long term fluorescence stability.
- Chemically robust to harsh environments.
- Suitable for *in vitro* and *in vivo* imaging.
- High biocompatibility.
- Unique fluorescence profile for unambiguous verification.
- Range of bio-functional groups can be conjugated to FNDs using multiple reaction schemes, including click chemistry.
- Can be functionalized to promote stability in buffers or cellular growth media with minimal non-specific binding.

Featured Products (High Brightness*)



Functionality	Particle Sizes (nm)	Product	Catalogue No.	Price
Amine (-NH ₂)	100	100nm, -NH ₂	NDNV100nmHiNH210mg	\$650
Hydroxyl (-OH)	100, 40	100nm, -OH	NDNV100nmHiOH10mg	\$550
		40nm, -OH	NDNV40nmHiOH10ml	\$570
Carboxyl (-COOH)	100	100nm, -COOH	NDNV100nmHiCOOH10ml	\$450
			NDNV100nmHiCOOH2ml	\$170
Streptavidin (through PEG)	100, 40	100nm, SA	NDNV100nmHiSA2mg	\$470
		40nm, SA	NDNV40nmHiSA2mg	\$500
Biotin (through PEG)	100, 40	100nm, Biotin 40nm, Biotin	NDNV100nmHibiotin10mg	\$950
			NDNV100nmHibiotin2mg	\$410
			NDNV40nmHibiotin2mg	\$440
PEG	100	100nm, PEG	NDNV100nmHiPEG10mg	\$650
PEG-Tz (click)	100	100nm, PEG-Tz for click chemistry	NDNV100nmHiTz2mg	\$450
PG	100, 40	100nm, polyglycerol (PG) stable in PBS	NDNV100nmHiPG10mg	\$650
			NDNV40nmHiPG10mg	\$690
PG-Tz (click)	100, 40	100nm, polyglycerol (PG) for click chemistry stable in PBS	NDNV100nmHiPGTz2mg	\$550
			NDNV40nmHiPGTz2mg	\$590
PG-Biotin PG-Streptavidin	100, 40	100nm, 40nm, polyglycerol (PG), Biotin or Streptavidin stable in PBS		
PG-scVEGF	100	100nm, PG, scVEGF		
Custom antibody	100, 40	100nm, 40nm, through PEG or PG		

Please inquire at info@adamasnano.com for more information regarding these products.

*High Brightness products contain up to 3ppm of NV⁻ centers

- Products are sterilized; sold as suspensions in DI water or biological buffers

U.S. Patents related to our products: 20160166482 (claims allowed); 8,323,976 and 9,327,980

DISCLAIMER: Product characteristics, specifications, costs, part numbers, and all other details are accurate as of the date of preparation of this document. These values are subject to change. Product characteristics are subject to batch to batch variability and improvements in processing or other developments.

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