Bioconjugated Fluorescent Nanodiamond

Product Sheet

The NV-High Brightness Series features fluorescent nanodiamonds ranging in size from 20 nm up to 150µm containing nitrogen vacancy NV centers with Red-NIR emission. These non-photo-bleaching and non-photo-blinking products are suitable for a number of applications, including but not limited to: 1. *In vivo* and *in vitro* fluorescence imaging, 2. fluorescent tracking of drug delivery 3. fiducial markers for super resolution and correlative microscopy, and 4. Authentication and anti-counterfeiting.

SIZE RANGE : 20-40nm

The 20-40nm size range provides the smallest and brightest currently commercially available fluorescent diamond particles on the market. These sizes are suitable for intracellular imaging and single molecule tracking. The brightness of particles depends on the particle size. The larger the particle, the higher the brightness due to the larger number of color centers that can be accommodated by larger particle volumes. If small size is necessary for your work, then the 20-40nm size range offers the best compromise between brightness and size. For first time users, it Is recommended to start with larger particle sizes to determine if fluorescent nanodiamonds FNDs will provide the necessary contrast in your application.

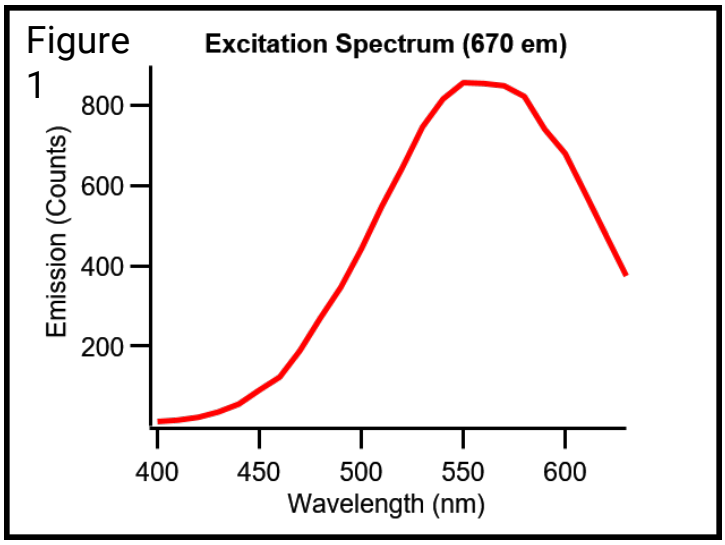

***Figure 1**: General excitation spectra for NV centers in diamond, measured for 670nm emission.*

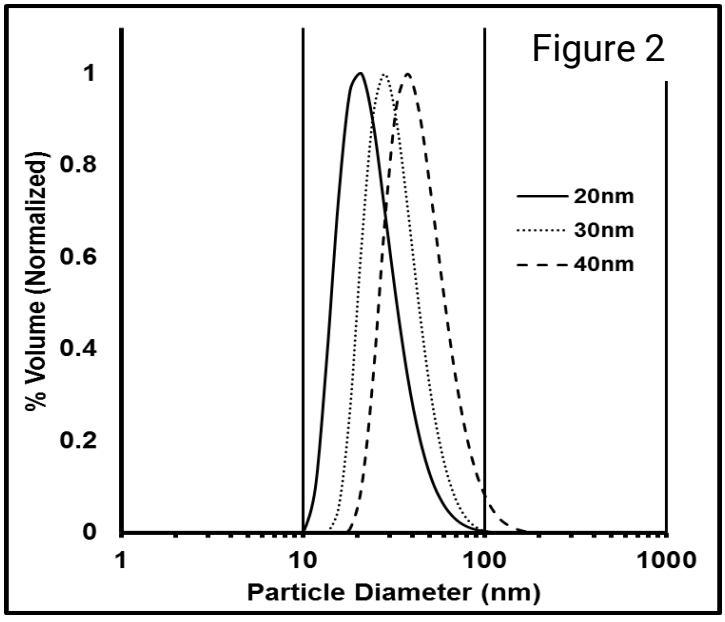

***Figure 2**: Particle size distributions of 20, 30, and 40 nm particles as measured with dynamic light scattering on a Malvern Zetasizer Nano ZS Malvern Instruments, Ltd. UK*

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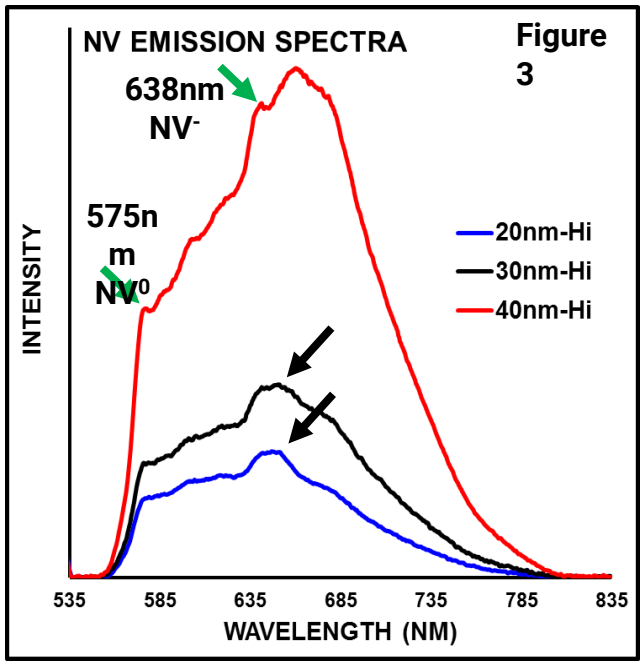

Figure 3 *: Emission spectra of 20, 30, and 40 nm High brightness particle suspensions in deionized water at approximately 1 mg/mL 0.1% w/v concentration. Excitation by 45 mW 532 nm CW laser Coherent – Sapphire . Spectra collected with Ocean Optics HR2000 USB spectrometer with 500msec integration time. Black arrows denote water Raman peak at ~650 nm.*

In addition to the single particle characterization presented above, electron paramagnetic resonance EPR was used to measure the concentration of NV^- in 40nm and larger diamonds. For the 40 nm material, the NV^- concentration was determined to be on the order of 1 ppm.

NV Emission Spectra

Emission spectra for FNDs contain characteristic Zero Phonon Lines ZPLs for the NV^0 and NV^- defect centers located at 575 nm and 638 nm denoted by green arrows in Figure 3 , respectively. As the particle size decreases, the ZPL signature tends to decrease due to the reduced number of emitters per particle, which decreases approximately volumetrically with particle volume.

Because particles are produced by milling/crushing larger particles, induced lattice damage can significantly impact the quality of NV centers and the resultant spectroscopic quality. Generally, speaking, the overall quality of emitting centers tends to decrease with particle size; though much effort is devoted to preserving the quality as much as possible. Adámas was one of the first commercial entities in the world to produce sub-20nm fluorescent nanodiamonds with confirmed NV^- centers.

Size nm	# Color Centers per Particle*	% Fluorescent particles
20	1-2	28%
30	5-6	57%
40	12-14	70%

**Based on weighted average of all particles fluorescent and non-fluorescent .*

Table 1 *: Summary of single particle characterization performed at the University of Stuttgart courtesy of T. Oeckinghaus.*

The average number of NV^- emitters per particle in 20-40nm products Table 1 was estimated from a fit to the measured g2 autocorrelation function using a Hanbury-Brown-Twiss setup Figs 4-6; Table 1 . The crushing process from bulk diamond leaves a population of some particles that do not contain color centers Table 1 . Size height distributions of the samples as measured by atomic force microscopy AFM are smaller than DLS measurements Figure2 , suggesting that the particles might have a plate-like shape. Presence of plate-like particles was observed in high resolution transmission electron microscope HRTEM

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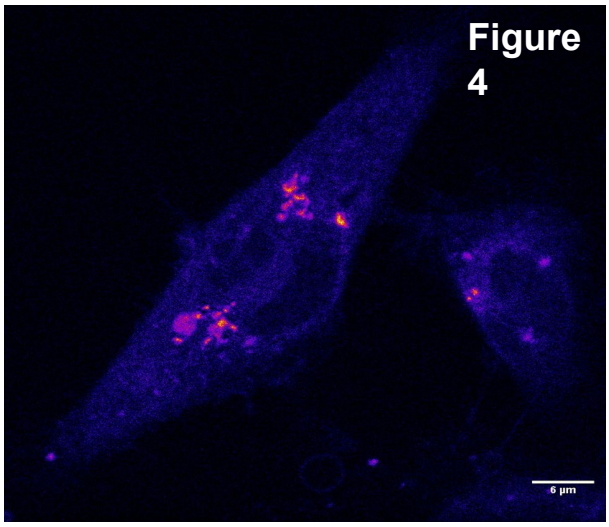

The brightness of aggregates of 20nm particles is high enough to be detected within cells after internalization in a confocal setup *Figure4* .

Figure 4 *: In vitro imaging of 20nm-Hi in MDA-MB-231 Breast Cancer Cells with 488 nm laser excitation following 48 h incubation at 50 µg/mL loading 650-720 nm detection window . N. Prabhakar, Åbo Akademi, Finland.*

SIZE RANGE: 50-200nm

NOTE: High brightness sizes ranging from 50-90 nm are not currently on our website but can be provided upon request.

Adámas large particle size ranges offer significantly higher brightness as compared to the small range. Moreover, these larger particles are typically easier to work with if additional chemical processing at the customer's side is desired. We strongly encourage customers that are performing preliminary experimental work or who do not have prior experience working with fluorescent nanodiamonds to start with these sizes if possible as they provide a good balance between fluorescent intensity and usability for conjugation or targeting schemes.

Total Internal Reflection Microscopy TIRFM measurements allowing side by side comparison with standard organic dyes determined these particles to be ~12x brighter than Atto 532 dyes molecules *Courtesy of K. Neuman, NIH Heart, Lung, and Blood Institute*

Particles in this size range are easier to detect for both *in vivo* and *in vitro* applications. Conjugates of these materials with biologically active groups e.g. biotin, streptavidin are also available upon request.

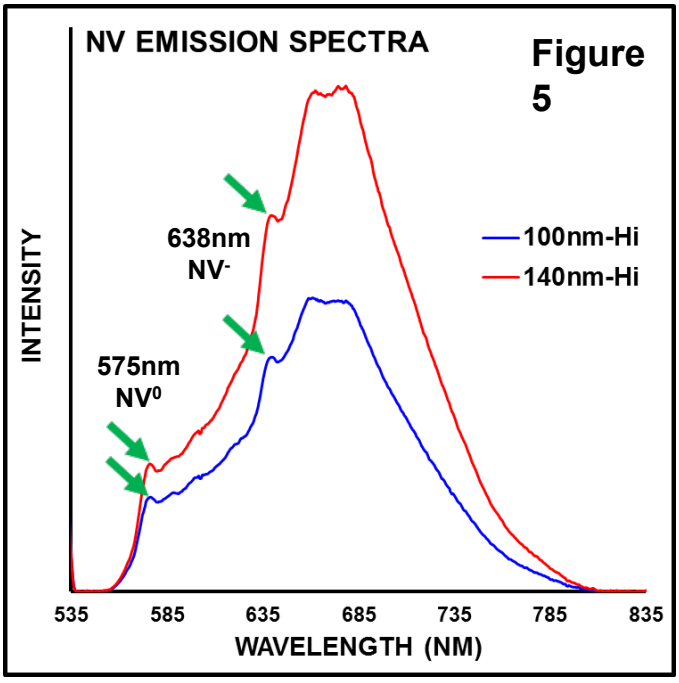

Figure 5 : Photoluminescence spectra of 100 nm and 140 nm high brightness. 15 mW 532 nm CW laser excitation Coherent Sapphire . Ocean Optics HR2000 Spectrometer, 750 msec integration time. ZPL indicated by green arrows.

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Electron paramagnetic resonance EPR studies were performed to evaluate the concentration of NV^- centers in the larger particle size range. These measurements concluded that the NV^- concentration was on the order of 3 parts per million ppm , this equates to approximately ~300 centers per 100 nm particle and ~800 centers per 140 nm particle. In general, a particle volume ratio related dependence on the number of centers per particle is observed with respect to size.

The large size range high brightness particles have been used extensively in *in vivo* and *in vitro* studies. The particles have been successfully conjugated with human vascular endothelial growth factor (VEGf) and preliminary data has suggested effective conjugation and targeting. 200 nm particles have also been used for whole body *in vivo* imaging in mice. Intravenous injection into mice with non-targeting FNDs showed spleen and liver accumulation over time. No observed toxicity was observed over a 24h period. *Ex vivo* digestion analysis of the spleen and liver tissue confirmed the presence of diamond. This is the first commercial demonstration of direct fluorescence whole body imaging using FNDs without external field modulation.

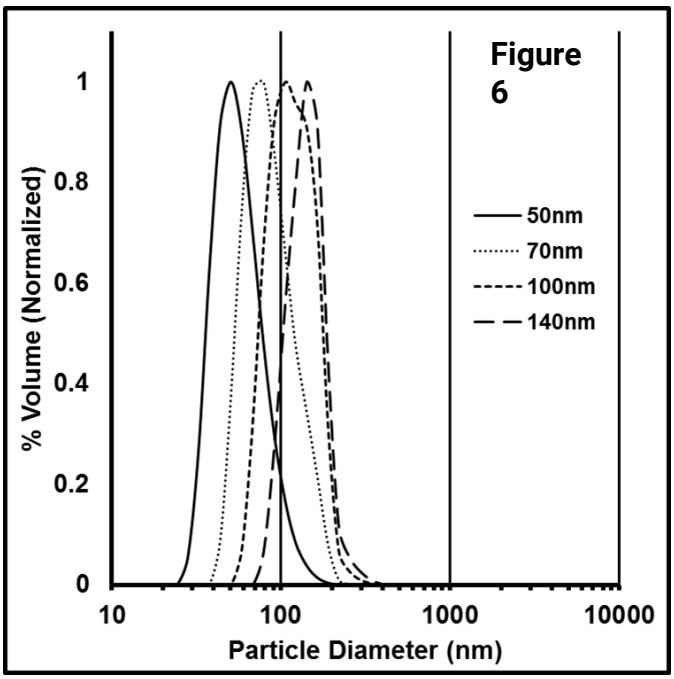

Figure 6 : Particle size distributions of 50, 70, 100, and 140 nm particles as measured with dynamic light scattering on a Malvern Zetasizer Nano ZS Malvern Instruments, Ltd. UK

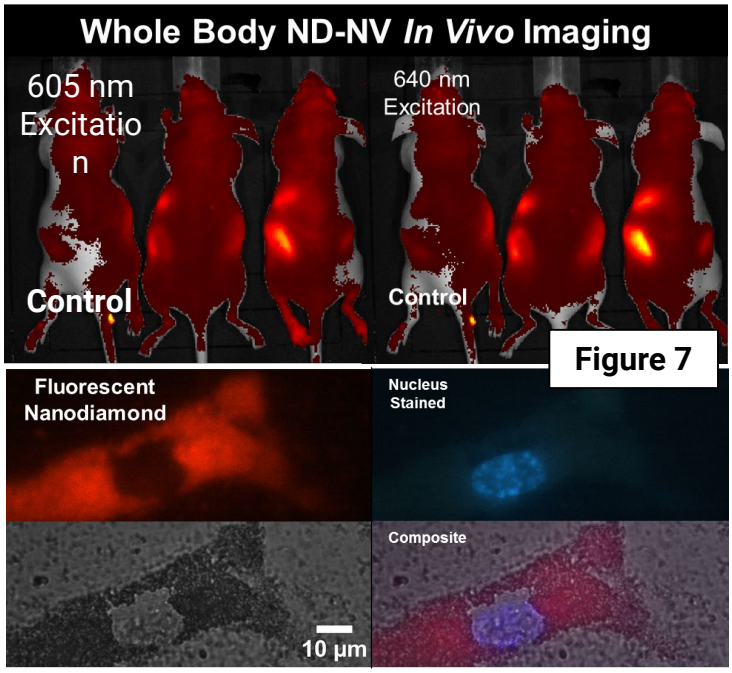

Figure 7: *Top Whole body imaging on IVIS system of live nude mice with two administered, intravenous doses of 200 nm FND particles. Spleen and kidney accumulation observed over 5 hours. Courtesy of G. Palmer, Duke University School of Medicine. Bottom In vitro visualization of 100 nm FNDs in primary endothelial colony forming cells ECFCs .*

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Surface Chemistry:

All products featured in this document exhibit primarily amphoteric surface functional groups carboxylic acids, alcohols, etc. with negative zeta potential. We offer more specifically functionalized products, such as reduced surfaces exhibiting –OH functionalities or bio-functional varieties such as streptavidin and biotin. Reduced diamond tends to exhibit positive zeta potential. Diamond offers a rich surface for a variety of surface functionalization schemes. Contact us if you would like to discuss your specific functionalization schemes at info@adamasnano.com.

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.

ACKNOWLEDGEMENT : Characterization work performed for these products was supported in whole or in part by the National Institutes of Health NIH National Heart, Lung, and Blood Institute NHLBI under contract No. HHSN268201500010C.

Category	Size*	Sold As**	Catalogue No.	Price
Functionalized with Antibodies	100nm	1mg/mL Suspension in PBS	NDNV100nmOPGantiRabbit500ug	$520
	100nm	1mg/mL Suspension in PBS	NDNV100nmOPGantiMouse500ug	$520
Functionalized with Streptavidin	40nm	1mg/mL Suspension in PBS	NDNV40nmHiSA2mg	$500
	100nm	1mg/mL Suspension in PBS	NDNV100nmHiSA2mg	$440
Functionalized with Biotin	40nm	1mg/mL Suspension in PBS	NDNV40nmHibiotin2mg	$440
	100nm	1mg/mL Suspension in PBS	NDNV100nmHibiotin2mg	$410
	100nm	1mg/mL Suspension in PBS	NDNV100nmHibiotin10mg	$950

* Products contain 2.5-3ppm of NV- centers as measured by EPR.
**If you require a specified solvent, or have a specific preference for powder or water, please contact us to discuss your requirements.
Patents related to our products: US 9,889,076

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