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Coherent anti-Stokes Raman scattering microscopy of single nanodiamonds

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S1. SAMPLE PREPARATION

Single particle microscopy. Gridded coverslips (♯ 2 thickness, 520 alphanumeric coded squares of 0.6×0.6 mm each, Electron Microscopy Sciences) were aminated with (3- Aminopropyl)trimethoxysilane. NDs were then diluted with methanol by a factor of more than 1:1000 allowing the carboxyl-to-amine binding to proceed unhindered by the presence of water. Dilutions were calculated based upon spatial requirements of achieving a suitable density of isolated particles. All glass materials were cleaned in sulphuric acid. A drop of MilliQ water was placed on a glass slide to act as immersion medium for the particles. Coverslips were then added to the glass slide (plus immersion medium), and sealed with nail varnish, ready for imaging.

S2. SCATTERING AND EXTINCTION CROSS SECTION MICROSCOPY SET-UP

The experimental set-up consists of an inverted microscope (Nikon Ti-U) equipped with a white-light illumination (halogen lamp 100W with Nikon neutral color balance filter), an oil condenser of 1.4 numerical aperture (NA) with a removable home-built dark-field illumination of 1.1-1.4 NA, a 40x 0.95 NA dry objective, a 1.5x intermediate magnification, and a Canon EOS 40D color camera attached to the left port of the microscope. Images were taken in Canon 14-bit RAW format with 10.1 megapixel resolution. The RAW images were

converted using the DCRAW plugin in ImageJ, providing 16bit RGB images with a linear response to intensity and no color balancing. The color camera enables a coarse spectroscopic detection separating the three wavelength ranges of red (R) 570-650 nm, green (G) 480-580 nm, and blue (B) 420-510 nm. Bright-field microscopy was performed by removing the dark field ring and adjusting the condenser numerical aperture $\mathrm{NA_c}$ to match the objective NA. To quantitatively measure the extinction cross-sections, two bright-field transmission images were taken, one with the NDs in the objective focus and the second one out-of-focus, moving the objective by approximately 15 μm axially, away from the sample. Background images were taken for blocked illumination. To achieve the lowest shot noise, the lowest camera sensitivity was used (100 ISO), for which the full-well capacity of the pixels of about 4×10^4 electrons occurs at 70% of the digitizer range (3.4 electrons/count). The exposure time in the order of 10 ms was chosen to reach the full-well capacity. Averaging over 36 acquisitions was performed for each image set.

The measurement procedure to quantitatively extract the extinction cross-section σ_{ext} from the background-subtracted transmitted intensity of the bright-field image with NDs in focus (I_{f}) and out-of-focus (I_{d}) is described in detail in Ref. 1. Briefly, the extinction cross-section of a ND centered within a circular area A_{i} of radius r_{i} in the image is expressed as $\sigma_{\mathrm{ext}} = \int_{A_{\mathrm{i}}} \Delta dA$ with the relative extinction $\Delta = (I_{\mathrm{d}} - I_{\mathrm{f}})/I_{\mathrm{d}}$. An example of a full color dark-field image and the corresponding Δ image for single NDs is shown in the main paper in Fig. 2. To account for the slight mismatch between I_{d} and I_{f} without ND due to the defocusing, drift of the illumination intensity, and the residual influence of the ND, a local background extinction $\Delta_{\mathrm{b}} = A_{\mathrm{b}}^{-1} \int_{A_{\mathrm{b}}} \Delta dA$ is measured in the area A_{b} between the radius r_{i} and $2r_{\mathrm{i}}$ yielding the background-corrected $\sigma_{\mathrm{ext}} = \int_{A_{\mathrm{i}}} (\Delta - \Delta_{\mathrm{b}}) dA$. The correction area A_{b} is within the defocused image of the NP ensuring a homogeneous influence of the NP over A_{i} and A_{b}. We use $r_{\mathrm{i}} = 3\lambda/(2\mathrm{NA})$ approximately at the second Airy ring of the objective point-spread function. We note that, differently from the gold nanoparticles investigated in Ref. 1, NDs are non absorbing hence their extinction cross-section is a direct measure of their scattering cross-section. We also note that the finite angular range of the 0.95 NA objective implies that it also collects a fraction of the scattered light in the bright-field transmission image, leading to an underestimate of the extinction. This is about 16% for a isotropic scatterer in water.

S3. CARS MICROSCOPY SET-UP

The experimental set-up consists of home-built CARS microscope based on a single-broadband 5 fs laser using spectral focusing. A Ti:sapphire laser source (Venteon, Pulse:One PE) is pumped by a frequency-doubled Nd:vanadate laser (Laser Quantum, Finesse) at 5.5 W and generates 5 fs pulses with a spectral width of 310 nm at 10% of the maximum intensity (660 nm to 970 nm) at a repetition rate of 80 MHz. A long-pass filter (two 6 mm thick Hoya H-66 in Brewster angle) is used to reject the tail of the laser spectrum in overlap with the CARS detection range. The pulse spectrum is then split into three components providing the pump, Stokes and a two-photon excitation beam, the latter not used for the experiments in this work. The resulting pump and Stokes pulses are centred at 682 nm (14,663 cm^{-1}) and 806 nm (12,400 cm^{-1}), with a bandwidth at 10% of 65 nm and 200 nm respectively. We have used SF57 glass blocks of variable length for spectral focussing [2], resulting in a spectral resolution of about 10/cm. Pump, and Stokes beams are recombined using a dichroic mirror prior to entering the scan mirrors (Cambridge Technology, 6210HSM40). A scan lens (from a Nikon A1RMP multiphoton microscope) is used to focus the collimated beam from the scan mirrors into the intermediate image plane at the microscope port, and is imaging the scan mirrors into the back focal plane of the microscope objective. The excitation beams enter a Nikon Ti-U inverted microscope through the left port. For high resolution imaging we use a 60× 1.27 NA water immersion objective (Nikon CFI Plan Apochromat IR λS series) and a 1.4 NA oil condenser. XY sample motion and Z-objective motion is automated by stepper motors (Prior ProScan III). CARS is detected in transmission and epi-geometry by two Hamamatsu H7422-40 PMTs with 38% quantum efficiency (QE) at 560 nm. The epi-detected signal is collected by the microscope objective, travels backwards along the excitation path over the scan mirrors, and is reflected into the PMT by a dichroic mirror. To enable forward detection, the illumination pillar of the Nikon Ti-U microscope was modified to allow computer controlled removal of the 90 degree mirror reflecting the halogen transillumination towards the condenser. The signal collected by the condenser lens is projected upwards into the forward PMT. In both forward / epi detection, the back focal plane of the condenser / objective is imaged onto the PMT cathodes with a matching size by appropriate lenses. Appropriate band-pass filters in front of the PMTs were used. Compared to Ref. 2 the dichroic mirror DM4 was exchanged with Semrock FF538-Di01, and

double stacks of Semrock FF01-609/57 were used in the CARS fwd and epi-detection for the fingerprint region.

S4. QUANTITATIVE DIC

As shown in Ref. 3 the transmitted intensity in a DIC microscope with de Sénarmont compensator can be expressed as

$$I_{\text{out}}(\mathbf{r},\psi) = \frac{I_{\text{ex}}}{2}\left[1-\cos\left(\psi-\delta(\mathbf{r})\right)\right], \tag{1}$$

with I_{ex} being the excitation intensity, $I_{\text{out}}(\mathbf{r},\psi)$ is the output intensity as a function of the position in the sample plain $\mathbf{r}$, ψ is the phase between the two linearly polarized components used in DIC, and $\delta(\mathbf{r})$ is the difference of the optical phase shift φ for the two beams that pass through the sample in two adjacent points separated by the shear vector $\mathbf{s}$

$$\delta(\mathbf{r}) = \phi(\mathbf{r}+\mathbf{s}/2) - \phi(\mathbf{r}-\mathbf{s}/2) \tag{2}$$

To reduce the influence of a possible spatial dependence of I_{ex} we acquire two images at opposite angles θ of the polarizer in the de Sénarmont compensator resulting in the output intensities [3] $I_{\pm} = I_{\text{out}}(\mathbf{r},\pm\psi)$ with $\psi = 2\theta$. The contrast image is then defined as

$$I_{\text{c}}(\mathbf{r}) = \frac{I_{+}(\mathbf{r}) - I_{-}(\mathbf{r})}{I_{+}(\mathbf{r}) + I_{-}(\mathbf{r})}. \tag{3}$$

By combining Eq.(1) and Eq.(3), we obtain an expression that is independent on the local illumination intensity I_{ex}

$$I_{\text{c}}(\mathbf{r}) = -\frac{\sin(\psi)\sin(\delta)}{1-\cos(\psi)\cos(\delta)} \tag{4}$$

In Ref. 3 we used a linear approximation of this expression valid in the limit of $\delta \ll \psi < \pi$. On the other hand, equation Eq.(3) can be solved analytically without the requirement $\delta \ll \psi$. The only requirement is that $0 \leq \psi \pm \delta \leq \pi$, otherwise equation Eq.(3) does not have a unique solution. More specifically, Eq.(1) is monotonic when $0 \leq \delta \leq \psi \leq \pi/2$ and can be solved analytically resulting in:

$$\sin(\delta) = -I_{\text{c}}\frac{1-\cos(\psi)\sqrt{1-I_{\text{c}}^2}}{\sin(\psi)}\frac{1}{1+I_{\text{c}}^2\cot^2\psi} \tag{5}$$

The exact formula in Eq.(5) does reduce to the linear approximation in Ref. 3 in the limit of $\delta \ll \psi < \pi$.

i. Data acquisition and analysis

DIC microscopy was performed with the same Nikon Ti-U inverted microscope used for CARS and a 60× 1.27 NA water immersion objective (Nikon CFI Plan Apochromat IR λS series). Illumination was provided by a halogen tungsten lamp (V2-A LL 100W; Nikon), followed by a blue-green filter (BG40; Schott) to block near-infrared light for which the DIC polarizers do not have sufficient extinction, and a green filter (GIF, transmission band 550 ± 20 nm; Nikon) defining the wavelength range for the quantitative DIC (qDIC) analysis. A de-Sénarmont compensator was used for offset phase adjustment (a rotatable linear polarizer followed by a fixed $\lambda/4$ wave-plate, T-P2 DIC Polarizer HT; Nikon), followed by a Wollaston prism (T-C DIC Module High NA N2 Dry; Nikon) in the condenser unit, a Nomarski prism after the objective (D-C DIC Slider 60X-IV; Nikon), and a linear polarizer (Ti-A-E DIC Analyzer Block; Nikon) in the filter turret.

DIC images were acquired using a charge-coupled device camera (Orca 285; Hamamatsu) with 1344 × 1024 pixels of 6.45 μm size. Considering 60× magnification, the pixel size corresponds to 0.107μm at the sample plane. The camera has a 12-bit A/D converter, and the images were converted to 16-bit grayscale tiff files. An exposure time of 0.1 s was used for each frame in DIC images, limited by the readout speed, and the illumination intensity was adjusted to provide an average of $\sim 75\%$ of digitizer range, corresponding to $\sim 3.8 \times 10^4$ photoelectrons per pixel (at the lowest gain setting of the camera). We averaged over 16 images for each of the two polarizations to improve signal-to-noise. We considered $+30^\circ$ and -30° incident polarizer orientations and calculated the contrast image (the corresponding values of the angle ψ were $+\pi/3$ and $-\pi/3$, respectively). The DIC contrast increases for decreasing $\psi < \pi/2$ however for a polarizer angle approaching zero the DIC image includes a substantial fraction of depolarized scattering from the NDs. In the extreme case of $\theta = 0^\circ$ the DIC image resembled dark-field microscopy, with bright spots at the location of NDs. A setting of $\theta = 30^\circ$ was qualitatively a good choice of the polarizer angle, since the contrast was higher than for $\theta = 45^\circ$ and the image still looked DIC-like, with a darker and brighter region at the loci of the NDs. We did not make a systematic study of the image quality as a function of incident polarization. Note that local inhomogeneity of the incident light as a result of particle extinction and scattering is compensated for by applying the exact formulas Eq.(3) and Eq.(5). The average dark counts (per integration time of 0.1 s) were

subtracted from all images. The dark counts were measured by setting the incident polarizer to $+0°$ (cross-polarization). 5 random pixels were chosen in an area that did not contain any nanoparticle, and the their intensity was averaged and taken as dark counts.

DIC experimental images were analyzed using a Matlab script. The data contained two series of DIC images, with 16 takes of the same sample area. We performed averaging of the DIC images in each series without registration, as the NDs are rigidly bound to the underlying substrate. In order to minimize the computer RAM used, and to decrease the influence of the overall background variation, each ND was analyzed separately by cropping a small square with the center at the ND and a user-defined size. The optimum square size of 32 pixels, which is approximately $3.44\,\mu$m was used. The center of the crops was chosen semi-manually, by mouse clicking at the NDs in the image. For each of the cropped squares, the contrast image I_c was calculated according to Eq.(3). Then, the full analytical formula Eq.(5) was used to produce the differential phase.

To retrieve the integrated phase φ, a Wiener deconvolution procedure was applied with the signal-to-noise parameter [3] $\kappa = 300$. A typical resulting image is shown in the main paper Fig. 4c. The integration artifacts are significant but the high value of κ helps maintaining a more step-like shape of the phase. For each of the integrated phase images $\varphi(\mathbf{r})$ we calculated a two-dimensional integral $A(r_0)$ as follows. A ND area is defined as a circle of radius r_0 centered at the ND such that $A(r_0) = \int_0^{r_0} \phi(\mathbf{r}')\, 2\pi \mathrm{r}'\, \mathrm{d}\mathrm{r}'$ where $\mathbf{r}'$ is the position vector in the sample plane with respect to the ND center. r_0 is taken at approximately the second airy ring of the objective point-spread function for which $A(r_0)$ reaches saturation i.e. does not significantly increase when further increasing r_0. The ND volume V is related to $A(r_0)$ by taking into account that the optical phase introduced by a ND is $\varphi(\mathbf{r}) = 2\pi\Delta n t(\mathbf{r})/\lambda_0$ with λ_0 wavelength in vacuum, Δn refractive index change between the ND and its surrounding medium and $t(\mathbf{r})$ thickness profile of the ND. Hence $A(r_0) = (2\pi\Delta n/\lambda_0) \int_0^{r_0} t(\mathbf{r}')\, 2\pi \mathrm{r}'\, \mathrm{d}\mathrm{r}' = 2\pi\Delta n V/\lambda_0$.

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