1. Introduction

Nanoparticles (NPs) of various chemical compositions, sizes, shapes, and surface-functionalizations are ubiquitous in modern research and industry, with uses ranging from drug-delivery,\(^1\) biomedical imaging\(^2\) and sensing,\(^3\)\(^,\)\(^4\) to catalysis\(^5\) and photovoltaics.\(^6\) For all these applications, knowledge of the NP size and shape is a key requirement. Notably, many NPs used in the aforementioned fields have dimensions well below the diffraction limit (of about 250 nm) for visible light. This presents a significant challenge for the quantification of the size and shape of individual NPs using optical microscopy.

The industry standard for accurate determination of NP sizes and geometry is electron microscopy, particularly transmission electron microscopy (TEM). TEM provides very high resolution, down to atomic detail, owing to the small wavelength of an electron beam, but is complex, expensive, and “low-throughput” in terms of the number of NPs examined in one field of view and corresponding statistics.\(^7\) An alternative, non-optical technique to characterize particle size distributions is tunable resistive pulse sensing\(^8\)\(^,\)\(^9\)\(^,\)\(^10\) (TRPS), which detects the change of the ionic current when a particle passes through a membrane pore. The change in electric resistance is related to the volume, and thus a sizing can be achieved on this premise. The method is high-throughput, accurate, can cover a wide range of particle sizes, and offers the additional benefit of charge and concentration measurement, but is typically limited to NPs larger than about 60 nm, does not provide shape information, and requires calibration before each measurement session.

Centrifugation\(^10\)\(^,\)\(^11\) techniques measure particle size distributions \textit{via} sedimentation rates, allowing good determination even with polydisperse samples, and in some cases are capable...
of resolution down to 1 nm. However, these are inherently ensemble techniques, do not offer shape information, and require or assume a homogeneous particle material density. A related group of methods is field-flow fractionation, which use flow-based elution and separation of particulates. Asymmetric flow field-flow fractionation (AF$_{4}$)$_{10,12}$ uses two flow sources to separate and elute particles, and is often combined with dynamic light scattering (DLS) and multi-angle light scattering (MALS). AF$_{4}$-DLS-MALS$_{10,12,13}$ has been shown to be a robust particle characterization method even capable of providing some indirect shape information by comparing the geometric (MALS) and hydrodynamic (DLS) radii. The setups can however be costly, requiring of significant expertise and sample species-specific setup to make sure samples properly elute.

Recently, a variant of DLS has been developed, called particle tracking analysis$^{14}$ (PTA), which observes Brownian motion of single particles over time to determine their hydrodynamic radius. As with DLS, PTA typically has issues for NP sizes below 20 nm due to their weak scattering. Neither DLS nor PTA can provide information on NP shapes.

Notably, it is possible to relate the magnitude of light absorbed and/or scattered by a NP, with its size and shape. In other words, the optical absorption and/or scattering cross-sections, $\sigma_{\text{abs}}$ and $\sigma_{\text{ sca}}$ respectively, or their sum, known as the extinction cross-section, $\sigma_{\text{ext}}$, carry information on the NP geometry. $\sigma_{\text{abs}}$ can be measured via a technique called photothermal imaging$^{15-17}$ (PTI), achieving detection of gold nanoparticles (GNP) down to about 1.4 nm.$^{18}$ $\sigma_{\text{ext}}$ has been measured by spatial modulation microscopy$^{16,19,20}$ (SMS), with demonstrated detection down to about 2 nm, and GNPs in this size range exhibit $\sigma_{\text{ext}}$ around 1 nm$^2$. However, both techniques require expensive apparatus, careful calibration, and are slow, being laser-scanning based, implying a limitation on throughput. A more direct absorption measurement technique$^{21,22}$ employing laser scanning without modulation has demonstrated detection of absorption of single molecules with $\sigma_{\text{ext}} \approx \sigma_{\text{abs}} \approx 0.1$ nm$^2$. However, this method is also slow and not amenable to high-throughput characterization. Darkfield microscopy is used to measure $\sigma_{\text{ext}}$ and can typically detect particles down to about 20 nm size for gold and silver nanospheres or dielectric particles such as polystyrene beads down to about 70 nm size$^{23-25}$ or nanodiamonds.$^{26}$ In this case, the limit is most significantly a practical one, as scattering by the target NPs is overwhelmed by scattering of their surroundings (typically dielectric debris). Furthermore, $\sigma_{\text{sca}}$ scales as the 6th power of the radius for NPs of dimensions within the dipole approximation, suggesting significant requirements on acquisition time and camera noise properties for particle sizes well below 20 nm.

Ref. 27 presents an on-chip sensing device based on whispering gallery modes (WGM), exhibiting detection and sizing of (non-absorbing) potassium chloride and polystyrene particles from 60 nm to about 200 nm when probing with light close to infrared (wavelength $\lambda = 670$ nm). This technique is notably not limited to the spherical assumption, since the signal is related to the polarizability of the NPs, and thus various shape models could be implemented. However, careful fabrication of the WGM resonator is critical. Ref. 28 uses wide-field reflection microscopy, exploiting the interference between the scattered light of NPs and the substrate reflection, measured as a function of defocus. GNPs and polystyrene beads of 50–70 nm size were separated and their size determined by a machine learning approach. Differential interference contrast wide-field imaging has been used to determine the orientation of gold nanostars,$^{29}$ but not their cross-sections.

We have recently shown that a simple transmission based technique using only widefield illumination is capable of simultaneously detecting hundreds of NPs with $\sigma_{\text{ext}}$ down to 1 nm$^2$, corresponding to a 2 nm GNP.$^{30-32}$ This procedure is rapid, quantitative and can be augmented using excitation light of varying polarisation direction, to gain information on the NP shape, and of varying wavelength, to gather material and shape-specific spectral properties. It is however not a trivial task to solve the inverse problem, i.e. to derive size and shape parameters from the measurement of $\sigma_{\text{ext}}$ especially when treating hundreds of NPs measured simultaneously.

In this paper, we introduce a method solving this inverse problem. We measure $\sigma_{\text{ext}}$ with a simple wide-field technique on hundreds of individual GNPs, both nominally spherical and rod-shaped, and simulate $\sigma_{\text{ext}}$ using a semi-analytical model in the dipole limit. We demonstrate that a retrieval of the NP size, shape, and orientation in three dimensions is possible, by fitting the measurements with the model. The accuracy of this “optical nanosizer” is discussed in relation to the measurement parameters, and bench-marked against state-of-the-art TEM and STEM 2D projection and STEM tomography.

2. Method and model

An object in an incident electromagnetic (EM) field will remove and redistribute power from that field resulting in a “shadow” being cast. In the limit of an object much larger than the wavelength $\lambda$, the shadow can be approximated by a simple projection of the object’s cross-section orthogonal to the propagation direction, hence geometrical optics provides a suitable platform by which to determine the projected physical size and shape of the object. However, diffraction effects dominate for objects whose diameter $D$ is comparable to, or smaller than the wavelength ($D < \lambda$), which in the visible wavelength range is the case for a NP. In this diffraction-limited regime, the “shadow” cast by the NP would be imaged as a shape close to the point spread function (PSF) of the imaging system. The exact form depends on the optics involved, the wavelength and polarisation of the incident light, the NP material, size, shape, and the refractive index of the environment. We also note that the PSF is in principle extended, and one needs to consider the area integral of the intensity of the PSF over the plane of projection, to determine the power removed by the particle from the incident field. In practice, one needs to limit integration to a range close to the diffraction limited size in order to control noise and surrounding structure, as we have discussed previously.$^{33}$ From these measurements, using the inci-
dient intensity $I_{\text{inc}}$ the absorbed power $P_{\text{abs}}$ and the scattered power $P_{\text{sca}}$, the optical absorption and scattering cross-sections, $\sigma_{\text{abs}} = P_{\text{abs}}/I_{\text{inc}}$ and $\sigma_{\text{sca}} = P_{\text{sca}}/I_{\text{inc}}$, respectively, can be determined, whose sum is called the extinction cross-section, $\sigma_{\text{ext}} = \sigma_{\text{abs}} + \sigma_{\text{sca}} = P_{\text{ext}}/I_{\text{inc}}$. Now, measuring these quantities as a function of wavelength and light polarization, and modelling them, the NP properties such as orientation, size, shape, and material can be inferred.

We describe here the model we use to determine the cross-sections $\sigma_{\text{abs}}, \sigma_{\text{sca}}$, and $\sigma_{\text{ext}}$ from the NP properties. We assume the incident field to be a plane wave and a linear optical reference system of unit vectors \( \hat{\mathbf{e}}_{x}, \hat{\mathbf{e}}_{y}, \) and \( \hat{\mathbf{e}}_{z} \), in the directions of the NP semi-axes of lengths \( a, b, \) and \( c \), which are ordered so that \( a \geq b \geq c \). The polarisability of the NP in its reference frame is then given by
\[
\alpha' = \begin{pmatrix}
\alpha_{a} & 0 & 0 \\
0 & \alpha_{b} & 0 \\
0 & 0 & \alpha_{c}
\end{pmatrix},
\]
where \( \alpha_{j} \) with indices \( j = a, b, c \) are the NP polarisabilities for electric fields orientated along the NP’s semi-axes. They are given by
\[
\alpha_{j} = V\varepsilon_{0} \frac{\varepsilon_{\text{NP}} - \varepsilon_{m}}{\varepsilon_{m} + L_{j}(\varepsilon_{\text{NP}} - \varepsilon_{m})},
\]
with the NP volume \( V \), the complex relative permittivity of the NP \( \varepsilon_{\text{NP}} = \varepsilon_{1} + i\varepsilon_{2} \), the positive relative permittivity \( \varepsilon_{m} \) of the surrounding dielectric medium, the permittivity of free space \( \varepsilon_{0} \), and the depolarisation factors \( L_{j} \). Note that the permittivities are frequency-dependent. Further, we note that in non-magnetic materials the relative permittivity is related to the refractive index by \( n_{r} = \sqrt{\varepsilon_{r}} \). The factors \( L_{j} \) are determined by the geometry of the NP, and given by the analytical expression,
\[
L_{j} = \frac{abc}{2} \int_{0}^{\infty} dq \frac{1}{(j^{2} + q)\sqrt{(a^{2} + q)(b^{2} + q)(c^{2} + q)}}.
\]

For the general case these integrals must be solved numerically, but closed-form solutions exist when at least two of the semi-axes are identical, i.e. for prolate and oblate spheroids, as well as spheres.\(^{35}\)

For metallic NPs, \( \varepsilon_{1} \) becomes increasingly negative above the plasma wavelength, resulting in a vanishing real part of the denominator in eqn (2) at a specific wavelength, for which
\[
\text{Re}\varepsilon_{\text{NP}}(\lambda) = \varepsilon_{m}(1 - L_{j}^{-1}).
\]
leading to a large polarizability \( \alpha_{s} \). This effect is called the localised surface plasmon resonance (LSPR) of the NP, and for a sphere, for which \( L_{j} = 1/3 \), it occurs at \( \text{Re}\varepsilon_{\text{NP}}(\lambda) = -2\varepsilon_{m} \). Accordingly, for increasing \( \varepsilon_{m} \), the LSPR wavelength increases, and by way of example, for a spherical gold NP embedded in \( n_{m} = 1.52 \) silicone oil, the LSPR occurs at \( \lambda \approx 530 \) nm. For large \( a/b, L_{a} \) becomes smaller, placing the LSPR at a more negative \( \text{Re}\varepsilon_{\text{NP}} \), which in turn results in a red-shift of the LSPR. We refer to the induced polarisation along the long axis \( a \) as the longitudinal mode. The LSPR of the longitudinal mode is generally sharper and stronger than in the spherical case, since the ratio between the real and imaginary parts of \( \varepsilon_{m} \) increases with increasing wavelengths in this range for gold and other metals. The polarisabilities along the minor axes are instead less affected.

Since a NP can have an arbitrary orientation in the measurement instrument, we need to transform eqn (1) into the measurement frame,\(^{34}\) yielding \( \tilde{\mathbf{a}} \). In the NP’s reference frame, the polarization induced by a field, \( \tilde{\mathbf{E}} \), incident on an NP with polarisability, \( \tilde{\mathbf{a}}' \), is given by
\[
\tilde{\mathbf{p}} = \tilde{\mathbf{a}}' \tilde{\mathbf{E}}.
\]
For the measurement frame, we label the axes of the Cartesian coordinates, \( e_{x}\{x, y, z\} \), with unit vectors \( \tilde{\mathbf{e}}_{x}, \tilde{\mathbf{e}}_{y}, \tilde{\mathbf{e}}_{z} \), where \( \tilde{\mathbf{e}}_{x} \) is along the optical path, and \( \tilde{\mathbf{e}}_{x}, \tilde{\mathbf{e}}_{y} \) are spanning the sample plane, as illustrated in Fig. 1. A vector \( \tilde{\mathbf{v}} \) in the NP frame is transformed into \( \tilde{\mathbf{v}} \) in the measurement frame as,
\[
\tilde{\mathbf{v}} = \tilde{\mathbf{R}} \tilde{\mathbf{v}},
\]
with the rotation matrix, \( \tilde{\mathbf{R}} \), so that for the NP polarization we have
\[
\tilde{\mathbf{R}}^T \tilde{\mathbf{p}} = \tilde{\mathbf{a}}' \tilde{\mathbf{R}}^T \tilde{\mathbf{E}},
\]
and hence the NP polarizability in the measurement frame is given by
\[
\tilde{\mathbf{a}} = \tilde{\mathbf{R}} \tilde{\mathbf{a}}' \tilde{\mathbf{R}}^T,
\]
where we have used that for rotations the inverse matrix, \( \tilde{\mathbf{R}}^{-1} \), is equal to the transposed matrix \( \tilde{\mathbf{R}}^T \). In general, \( \tilde{\mathbf{a}} \) will therefore have non-zero off-diagonal elements. It can be shown\(^{34}\) that \( \sigma_{\text{abs}} \) and \( \sigma_{\text{sca}} \) are given by
\[
\sigma_{\text{abs}}(\tilde{\mathbf{E}}) = \frac{k}{\varepsilon_{0}} \frac{\tilde{\mathbf{E}} \cdot \tilde{\mathbf{p}}}{|\tilde{\mathbf{E}}|^2},
\]
and
\[
\sigma_{\text{sca}}(\tilde{\mathbf{E}}) = \frac{k^4}{6\pi \varepsilon_{0}^2} \frac{|\tilde{\mathbf{p}}|^2}{|\tilde{\mathbf{E}}|^2},
\]
with the wavenumber in the medium \( k = 2\pi n_{m}/\lambda \), the star indicating the complex conjugation, and a time dependence proportional to \( \exp(-i\omega t) \) of the field with \( \omega = 2\pi c/\lambda \) and the light
velocity $c_0$ in vacuum. The square moduli and the dot product in eqn (9) and (10) imply a summation over the different components of the NP polarisation, so that the cross-section depends on the orientation of the NP in the measurement system.

The resulting extinction cross-section spectra are given in Fig. 2 for a gold NP of a volume given by that of a sphere of 30 nm diameter, and different aspect ratios, for fields along the NP semi-axes. Here, and in the remainder of this work, we took $F_{\text{NP}}$ from the tabulated values for gold from ref. 36, interpolated in 2 nm steps over the range $\lambda = 400$ nm to 800 nm. For prolate NPs, the $\sigma_{\text{ext}}$ for fields along the long axis (Fig. 2a top) shows the longitudinal LSPR shifting to longer wavelength and increasing in amplitude, reaching 600 nm and $10^5$ nm$^2$ for aspect ratios $b/a \approx 1/2$. For oblate NPs, similarly, 600 nm and $10^5$ nm$^2$ are reached for $b/a \approx 3$ (Fig. 2b bottom). The geometrical cross-section of the sphere is about 707 nm$^2$, which is similar to the calculated extinction cross-sections at $\lambda = 400$ nm, where gold is strongly absorptive. For NPs with three different semi-axes lengths, shown in Fig. 2c–e, the qualitative behaviour is similar, with the LSPR for a polarization along each semi-axis red-shifting with increasing axis length, but also affecting the LSPRs along the other two axes.

A specific rotational formalism must be chosen in order to calculate $\hat{R}$ in a consistent way. For computational reasons, we wish to remove the angle $\gamma_p$ as an explicit parameter (as described in the ESI section S.II$^\ddagger$), which can be achieved by having the first rotation in $\hat{R}^T$, given by a rotation about the $z$ axis. We therefore define $\hat{R}$ as

$$\hat{R} = R_\phi R_\theta R_\psi, \quad \hat{R}^T = R_\phi^T R_\theta^T R_\psi^T$$

where,

$$R_\phi = \begin{pmatrix} 1 & 0 & 0 \\ 0 & \cos(\phi) & -\sin(\phi) \\ 0 & \sin(\phi) & \cos(\phi) \end{pmatrix},$$

$$R_\theta = \begin{pmatrix} \cos(\theta) & 0 & \sin(\theta) \\ 0 & 1 & 0 \\ -\sin(\theta) & 0 & \cos(\theta) \end{pmatrix},$$

$$R_\psi = \begin{pmatrix} \cos(\psi) & -\sin(\psi) & 0 \\ \sin(\psi) & \cos(\psi) & 0 \\ 0 & 0 & 1 \end{pmatrix},$$

so that in eqn (7), $\hat{R}^T$ is the first multiplier of $\vec{E}$. The rotations $R_\phi$, $R_\theta$, $R_\psi$ when acting in the measurement frame are illustrated in Fig. 1. The unique angular ranges are given by $\phi \in [0, \pi]$, $\theta \in [-\pi/2, \pi/2]$, and $\psi \in [0, \pi]$, as discussed in the ESI section S.II.$^\ddagger$
We can now calculate, in the dipole limit, for a given field \( \vec{E} \), the cross-sections for an elliptical NP of orientation \( \Omega = (\gamma_p, \theta, \phi) \), described by the semi axes \( a \geq b \geq c \), NP permittivity \( \varepsilon_{\text{NP}} \), and medium permittivity \( \varepsilon_m \).

The illumination in the experiment employs a polarizer at an angle \( \gamma_p \) to the \( x \) axis in the back focal plane of the condenser, and a given numerical aperture range. As a result, the field in the sample plane is a superposition of incoherent contributions of a range of incident directions and polarizations. To approximate this illumination, excitation fields \( \vec{E} \) of three orthogonal directions are considered. These are the two fields in the sample plane, one along the polarizer (\( \vec{E}_|| \)), and one orthogonal (\( \vec{E}_\perp \)) to it, given by

\[
\vec{E}_|| \left( \gamma_p \right) = E_{||} \left( \cos(\gamma_p) \sin(\gamma_p) \right), \quad \vec{E}_\perp \left( \gamma_p \right) = E_\perp \left( -\sin(\gamma_p) \right) \cos(\gamma_p), \quad (12)
\]

as well as a field normal to the sample plane,

\[
\vec{E}_z = \begin{pmatrix} 0 \\ 0 \\ 1 \end{pmatrix}. \quad (13)
\]

To treat the illumination approximately, we consider an average of cross-sections for fields in the three directions \( \vec{E}_||, \vec{E}_\perp, \vec{E}_z \), with weights given by the corresponding field intensities created by the illumination (see eqn (3.16) in ref. 24). Briefly, the illumination rays are projected into the respective polarization directions, and their intensity is integrated over the illumination range. For typical illuminations, the highest intensity is along \( \vec{E}_|| \), followed by \( \vec{E}_\perp \), and then \( \vec{E}_z \). Specifically, for illumination with NA = 0.95 in a medium of \( n = 1.52 \), the corresponding relative field strengths are \( E_{||} = 0.999, E_\perp = 0.044, E_z = 0.321 \), while for NA = 1.34, they are \( E_{||} = 0.996, E_\perp = 0.092, E_z = 0.449 \), with the normalization \( E_{\perp}^2 + E_z^2 = 1 \). The normalization does not consider \( E_z \) since this component is propagating orthogonal to \( z \) and is thus not contributing to the measured reference intensity. The corresponding long shadow effect is discussed in ref. 33. As a result, the illumination probes not only the response for in-plane polarization, but also to some extent the response for a polarization along the microscope axis. The expected measured absorption cross-section is then calculated as

\[
\sigma_{\text{abs}}(\gamma_p) = E_{||}^2 \sigma_{\text{abs}}(\vec{E}_||) + E_\perp^2 \sigma_{\text{abs}}(\vec{E}_\perp) + E_z^2 \sigma_{\text{abs}}(\vec{E}_z), \quad (14)
\]

and equivalent for the scattering cross-section \( \sigma_{\text{sca}}(\gamma_p) \). In a measurement, the cross-section will be determined as a power loss for different illumination spectra \( \Lambda \), called “colour channels” in the following, having a normalized spectral intensity \( I_{\Lambda}(\lambda) \), with \( \int I_{\Lambda}(\lambda) d\lambda = 1 \), defining spectrally averaged absorption cross-sections

\[
\sigma_{\text{abs,}\Lambda} = \int \sigma_{\text{abs}}(\lambda) I_{\Lambda}(\lambda) d\lambda, \quad (15)
\]

and equivalently scattering cross-sections \( \sigma_{\text{sca,}\Lambda} \). To model the bandpass filters, we assume a constant value of \( I_{\Lambda} \) over the passband and zero outside, and identify them in \( \Lambda \) by their center wavelength in nm. For the LED illumination, identified by their nominal wavelength in nm, we used the measured LED spectrum, shown in Fig. S1,† to determine \( I_{\Lambda} \).

The resulting extinction cross-sections for various polarizer angles \( \gamma_p \) and colour channels \( \Lambda \) are then compared with the corresponding measured quantities \( \sigma_{\Lambda}(\gamma_p) \), defining the normalized error

\[
S^2 = \frac{1}{N} \sum_{\gamma_p, \Lambda} \left( \frac{\sigma_{\Lambda}(\gamma_p) - \sigma_{\text{abs,}\Lambda}(\gamma_p) - \eta \sigma_{\text{sca,}\Lambda}(\gamma_p)}{\sigma_{\Lambda}(\gamma_p)} \right)^2, \quad (16)
\]

where \( N \) is the number of measurements used in the sum, and \( \hat{\sigma}_{\Lambda}(\gamma_p) \) is the measurement noise of \( \sigma_{\Lambda}(\gamma_p) \). The parameter \( \eta \) accounts for the fact that a fraction of the scattered light is collected by the objective. In the present model, we approximate this fraction by the one for scattering by a spherical NP in a homogeneous medium, for unpolarized illumination, in the dipole limit. Using a NA of 0.95 for both illumination and collection, the fraction of scattered light collected is 13.6%,37 so that we use \( \eta = 0.864 \). We note that this is a small correction, and that accordingly the approximation used is appropriate. Minimising \( S^2 \) over the NP parameters, we determine the most likely parameters describing the NP. We use MatLab R2018b to perform this fitting procedure, which is described in more detail in the ESI section S.II.† Typical computational times are around 1 second per NP fit on a modern CPU (AMD Ryzen Threadripper 2950X).

3. Experiment

3.1. Optical measurements

The optical measurements reported in this work were performed on a Nikon Ti-U inverted microscope, using as illumination source either a 100 W halogen lamp with bandpass filters (center wavelengths 450 nm, 550 nm, 600 nm, 650 nm, or 750 nm) of 40 nm width, or a light emitting diode (LED) source (Thorlabs LED4D106) providing two independent LEDs of center wavelengths 530 nm and 660 nm (for their spectrum see ESI Fig. S1†) coupled via a liquid light guide (Thorlabs AD5LLG). The microscope was setup with a 1.34 numerical aperture (NA) oil-immersion condenser (Nikon MELA41410) limited to NA = 0.95 to match the 0.95NA 40× dry objective (Nikon MRD00405) used with the 1.5× tube-lens. The image data was recorded using a scientific-CMOS (sCMOS) camera (PCO Edge 5.5), capable of acquiring 100 frames per second (FPS) at 2560 × 2160 pixels with 16 bit digitization, 0.54 electrons per count, and a full well capacity of \( N_{\text{FW}} = 30 000 \) electrons.

The illumination intensity and exposure time was chosen to be close to \( N_{\text{FW}} \) without entering the range of non-linear response. A sketch of the setup is given in Fig. 3.

3.2. Structural characterization

Transmission electron microscopy (TEM) was performed on a JEOL-JEM 2100 TEM operating at 200 kV, with samples prepared on 300 Mesh holey carbon. High-angle annular dark-field scanning transmission electron microscope
(HAADF-STEM) images and tomography series were acquired using a FEI Osiris microscope operated at 200 kV. The tomography series were acquired over the tilt range of ±75° with a tilt increment of 3° using a Fischione 2020 single-tilt tomography holder and a pixel dwell time of 6 μs. After alignment of the projection images by using a cross correlation, the stacks of aligned projection images served as inputs for 20 iterations of the expectation maximization reconstruction algorithm implemented in the ASTRA toolbox 1.9.0 using Matlab 2019a.38,39 Amira 5.4.0 was used for the 3D visualization.

3.3. Samples and their preparation for optical measurements

GNPs of nominal spherical shape and mean diameters of 20 nm (GNSS) were obtained from BBI Solutions. Branded ‘ultra-uniform GNPs’ of nominal spherical shape and mean diameter of 30 nm (UGNSS) were obtained from NanoComposix. Finally, gold nanorods (GNRs) of nominal diameter of 30 nm (UGNSs) were obtained from NanoPartz.

Fig. 3 Sketch of the imaging setup used. The transmission of a sample under Köhler illumination of a defined linear polarization and selectable wavelength range is imaged onto a sCMOS camera, using a condenser and an objective of matched high numerical apertures. The sample position is laterally shifted by a piezoelectric stage for referencing.

Three samples and their preparation for optical measurements are described: (i) GNSS-GNs-GNRs, (ii) GNSS-GNs, and (iii) GNSS-GNRs. GNSS-GNs-GNRs were used to validate the optical method. GNSS-GNs were used to measure the optical properties of GNRs. GNSS-GNRs were used to determine the change in the effective refractive index.

3.4. Measurement of extinction

We use a method to quantitatively measure $\sigma_{\text{ext}}$, which has been described in ref. 31, 32 and 37. Briefly, two offset-subtracted brightfield images are obtained; the signal, $I_1$, with NPs in focus at position, $P_1 = (x_1, y_1)$, and the reference, $I_2$, with the sample laterally shifted to position, $P_2 = (x_2, y_2) = (x_1 + \delta x, y_1 + \delta y)$, with the magnitude of the shift, $s = \sqrt{\delta x^2 + \delta y^2}$. Generally, $I_1$ and $I_2$ are averaged over a number, $N_0$, of individual acquisitions, reducing shot noise in the final image. The normalised transmission is obtained by

$$T_\pm = \left( \frac{N_0}{N_0} \sum_{i=1}^{N_0} I_{1,i} \right) / \left( \frac{N_0}{N_0} \sum_{i=1}^{N_0} I_{2,i} \right),$$

with $+$ indicating we have chosen the signal to refer to the sample at $P_1$. Thus, $T_\pm = T_\pm^{-1}$ can be obtained as well by choosing the signal to refer to the sample at $P_2$. To further reduce shot noise, while simultaneously reducing systematic noise due to sensor electronic drift, we repeat this procedure, moving the sample between the two positions at a rate, $\nu_s$. We then determine the extinction images

$$\Delta_\pm = 1 - \frac{1}{N_0} \sum_{i=1}^{N_0} T_\pm,$$

with the number of repetitions $N_0$. The shift rate is limited by the number $N_1$ and the framerate, $\nu_s$, with typical values of $N_1 = 128$, $\nu_s$ about 400 Hz, and $\nu_s$ about 3 Hz. As mentioned above, a single NP appears twice in $\Delta_\pm$, as a bright and dark PSF pair, with flipped contrast between $\Delta_+$ and $\Delta_-$. An example can be seen in Fig. 4, for a selected region of $\Delta_\pm$. The corresponding full field of view of $\Delta_\pm$, seen in the ESI Fig. S2, contains around a hundred simultaneously measured NPs.

3.5. Analysis of $\sigma_{\text{ext}}$

To measure the cross-section of a NP at $P_1$ in $\Delta_\pm$, we integrate $\Delta_\pm$ over an area, $A_\text{int}$, of radius, $r_\text{int}$, centred at $P_1$, and $\Delta_\pm$ over the corresponding area at $P_2$, such that the extinction cross-section is given by

$$\sigma_{\text{ext}} = \frac{1}{2} \int_{\text{int}} (\Delta_+(x, y) + \Delta_-(x + \delta x, y + \delta y)) \, dx \, dy$$

The shot-noise limited noise in the measurement of $\sigma_{\text{ext}}$ is given by

$$\sigma_{\text{ext}} = \frac{r_\text{int} d_{px}}{M} \sqrt{\frac{\pi}{N_0 N_{\text{pix}}}},$$

with the magnification $M$ from sample to detector, the number of acquired frames $N_\text{f} \approx N_0 N_{\text{pix}}$, and $d_{px} = 6.5$ μm the pixel pitch of the sensor. We use $r_1 = 3\lambda/(2NA)$ unless otherwise
4. Results and discussion

Here we discuss the results of the analysis of \( \sigma_{\text{ext}} \) for hundreds of individual NPs of different types. Particular care needs to be taken in understanding the meaning of the statistical distribution of the cross-sections across a set of NPs. Information related to the size and shape distributions is contained within the NP set. Yet, each measured quantity on an individual NP will be affected by the error due to the measurement noise. The evaluation of these effects is discussed below.

4.1. Distributions of \( \sigma_{\lambda} \) and \( \alpha_{\lambda} \)

The distribution of \( \alpha_{\lambda} \) versus \( \sigma_{\lambda} \) is shown in Fig. 5 for three sets of NPs, together with batch-representative TEM images. We call the mean of the polarisation-averaged cross-sections for a given colour channel \( \bar{\sigma}_{\lambda} \), and the associated standard deviation \( \bar{\sigma}_{\lambda}^\prime \). We call the analogous properties of the \( \alpha \) parameter \( \bar{\sigma}_{\alpha} \) and \( \bar{\sigma}_{\alpha}^\prime \). For each NP, we determine the error of the fitted parameters \( \sigma_{\lambda}, \alpha_{\lambda}, \gamma_{\lambda} \) using a Monte Carlo-like simulation. In detail, we add random noise to the measured data, from a Gaussian distribution with a root mean square (RMS) given by the experimentally determined noise \( \delta_{\text{ext}} \) for each \( \gamma_{\lambda} \) and \( \Lambda_{\lambda} \), refit the data, and use the RMS of the resulting parameter distribution as error. Let us in particular denote the standard deviation of the per-NP fitted parameter \( \sigma_{i} \) by \( \delta_{\lambda,i} \), where \( i \) numbers the \( N \) NPs. To remove the measurement noise from \( \delta_{\lambda,i} \), we define \( \delta_{\lambda} = \sqrt{\delta_{\lambda,m}^2 - \frac{1}{N} \sum_{i=1}^{N} \delta_{\lambda,i}^2} \), which represents only the distribution of \( \sigma_{\lambda} \) across the various NPs, due to their different sizes and shapes. Note that for \( \delta_{\lambda,m} \) this correction has not been done, since the corresponding standard deviations \( \delta_{\lambda,m} \) vary strongly depending on \( \sigma_{\lambda,m} \) and \( \sigma_{\lambda} \), and we use \( \delta_{\lambda} = \delta_{\lambda,m} \).

4.1.1 Nominally spherical gold NPs. We have investigated two types of nominally spherical NPs, the UGNSs of nominal 30 nm diameter, and the GNSs of nominal 20 nm diameter (see section 3). Fig. 5a presents the measured \( \sigma_{\lambda} \) and \( \alpha_{\lambda} \) of a set of UGNSs, which exhibit narrow distributions of both \( \sigma_{\lambda} \) and are considerably red-shifted with respect to the resonance of a spherical GNP in the dipole limit. Conversely, the NP is nearly invisible for \( \gamma_{\lambda} \approx 90^\circ \). Additionally, we can see a significant polarisation-dependence for \( \Lambda = 650 \) and \( \Lambda = 750 \), but not for \( \Lambda = 550 \). This is consistent with the redshift of the LSPR polarized along the long axis.

Fig. 4b instead shows a NP with low asymmetry, from the UGNs. For \( \Lambda = (550, 650, 750) \), this NP is described by \( \sigma_{\lambda} = (0.067, 0.043, 0.048) \), \( \alpha_{\lambda} = (614, 1172, 217) \) nm², and \( \gamma_{\lambda} = (75.5, 64.4, 79.1) \). A weak polarisation dependence is observed for all three \( \Lambda \) and the NP is visible in \( \Lambda \) at both polariser angles shown. It exhibits the largest cross-section for \( \Lambda = 550 \), consistent with the expected wavelength dependence discussed in section 2.
and $\alpha_{\Lambda}$. We find $\alpha_{600}$ to be below 0.2 (apart from some outliers accounting for 4.7% of the population) with a mean and standard deviation $\bar{\alpha}_{600} \pm \hat{\sigma}_{600} = 0.058 \pm 0.031$, indicating NPs of very low ellipticity. $\hat{\sigma}_{450}/\bar{\alpha}_{450}$ and $\hat{\sigma}_{550}/\bar{\alpha}_{550}$ are both about 18%. At these wavelengths the cross-section is roughly proportional to the NP volume, hence 18% relative volume distribution corresponds to 6% radius distribution. $\hat{\sigma}_{600}/\bar{\sigma}_{600}$ instead is larger, about 41%, consistent with the fact that the variability of $\sigma_{\text{ext}}$ at a wavelength above the LSPR of a spherical NP is sensitive to shape deviations, in fact elliptical shapes result in a red-shifted $\sigma_{\text{ext}}$ as shown in Fig. 2. This can also be seen in the correlation of increasing $\sigma_{600}$ with $\alpha_{600}$ for the outliers having larger deviations from the spherical shape in Fig. 5a. Notably, correlating $\sigma_{600}$ with $\sigma_{550}$ (see inset), two groups can be identified, with different ratios $\sigma_{600}/\sigma_{550}$, identifying two distinct shapes. Qualitatively, these observations are consistent with TEM, where most of these ultra-uniform NPs are defect-free and have a faceted shape, while a few NPs contain defects and hence vary from these shapes, as shown in the ESI section S. III.† Additionally, we see that $\hat{\sigma}_{550} > \hat{\sigma}_{450} > \hat{\sigma}_{600}$ as expected for spherical gold NPs with a LSPR at $\lambda \approx 530$ nm in a homogeneous environment of $n_r = 1.52$.

Fig. 5b shows the results for a set of GNSs, which we measured using LED illuminations centred at 530 nm and 660 nm. For this sample, the TEM indicates a large variability in shape, dominated by multicrystalline roundish NPs, and monocristalline triangular plates with rounded edges. The plates are seen also side-on, where they appear rod-shaped.
Indeed, we find that the measured $\sigma_\lambda$ is highly distributed for both available $\Lambda$, with $\sigma_{530} \pm \delta_{530} = [645 \pm 469]$ nm$^2$, and $\sigma_{660} \pm \delta_{660} = [298 \pm 482]$ nm$^2$. For many NPs in this sample, $\sigma_{660}$ is comparable to the noise level, with $\delta_{660} = 43$ nm$^2$, resulting in a large error of $\sigma_{660}$, as estimated by the dashed line. Nonetheless, for NP with larger $\sigma_{660}$, also a large $\sigma_{660}$ is observed, consistent with the presence of plate-like shapes in TEM. Likewise, $\sigma_{330}$ ranges from about 0 to 0.4, with most values below 0.25 ($\sigma_{330} \pm \delta_{330} = 0.12 \pm 0.18$), indicating that on average the NPs have some distinct non-sphericity. The large value of $\sigma_{530}/\sigma_{330} \approx 57\%$ suggests a wide distribution in average size as well. However, $\sigma_{530} > \sigma_{660}$, showing that most NPs do not have an aspect ratio above 1.5 (see Fig. 2), consistent with TEM analysis (see also Table 1 and ESI Table S1†).

4.1.2. Rod-shaped gold NPs. Results for the GNRs are shown in Fig. 5c. Considering the large nominal aspect ratio, we performed measurements with an additional channel in the red part of the spectrum, at 750 nm. Looking at Fig. 2, we find the longitudinal resonance for channel in the red part of the spectrum, at 750 nm. 

### Table 1

<table>
<thead>
<tr>
<th>NP type</th>
<th>$p_0 \pm \delta p_0$ (nm)</th>
<th>$p_{660} \pm \delta p_{660}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>30 nm UGNS</td>
<td>28.59 ± 1.77</td>
<td>0.973 ± 0.024</td>
</tr>
<tr>
<td>20 nm GNS</td>
<td>17.84 ± 1.25</td>
<td>0.865 ± 0.091</td>
</tr>
<tr>
<td>30 × 10 × 10 nm$^3$ GNR</td>
<td>15.36 ± 2.86</td>
<td>0.394 ± 0.135</td>
</tr>
</tbody>
</table>

We therefore expect $\tau_1 = 0.25$ to $\tau_2 = 1.43$ for the UGNs, and $t_1 = 0.1$ to $t_2 = 1.75$ for $\Lambda$ and $\gamma_\rho$, of the UGNs in Fig. 5a. The cross-section data to be fitted were calculated for a NP of a volume characterized by the sphere-equivalent diameter $D_\gamma = 25.6$ nm (taken from the average of the fitted $D_\gamma = \sqrt{6V/\pi}$ in Fig. 7), and of an orientation $y = \theta = \phi = 0$, so that $a$ and $b$ are in the measurement $x$, y plane. The distribution of fitted parameters results from the set of noise realizations, with $\delta_{\text{ext}} = (57.5, 53.1, 30.9)$ nm$^2$ for $\Lambda = (450, 550, 600)$, respectively. These values of $\delta_{\text{ext}}$ correspond to the measurements in Fig. 5a, and amount to 2–20% of the respective $\sigma_{\text{ext}}$. The different colors in the plots show different simulated aspect ratios $b/a = c/a$ as indicated. The fit parameter space is 6-dimensional (three orientation angles and three semi-axis lengths), and we have 18 simulated measurements ($6 \gamma_\rho$ at 3 $\Lambda$). Notably, measuring a spherical NP ($b/a = c/a = 1$), the noise results in fitted values of aspect ratios between 1 and 0.8. For a strongly prolate NP instead (e.g. $b/a = c/a = 0.6$), the average aspect ratio $\sqrt{bc/a}$ is rather well defined (see right inset, e.g. magenta histogram), while $b/a$ and $c/a$ still vary significantly. The retrieved volume (see histogram in left inset) shows a smaller dependence on the aspect ratio, while still being of decreasing variation with decreasing aspect ratio. Reducing the measurement noise, the distributions narrow, as shown in the ESI Fig. S3.† Equivalent simulations for the oblate and general cases are discussed in the ESI section S. IIC.† Generally, the interplay between NP orientation, aspect ratios, and probed colour channels goes beyond an intuitive
4.2.1. Geometrical parameters from measured nominally spherical gold NPs. The fit results are presented below for the investigated nominally spherical NPs. Let us start discussing the UGNs, using the colour channels \{\Lambda\} = \{450, 550\}. The resulting values of \(c/a\) and \(b/a\) are shown in Fig. 7a, with the colour indicating the ellipsoid average diameter \(D_v = 2\sqrt{abc}\), and the symbol size indicating the fit error \(S\). We find that \(c/a\) and \(b/a\) are mostly above 0.9, and the \(D_v\) ranges between 24 and 27 nm. Fig. 7b shows the average aspect ratio \(\sqrt{bc/a}\) versus \(D_v\) and a histogram of \(D_v\). Notably, the values of \(D_v\) are about 10% smaller than measured in TEM, the latter shown as black points in Fig. 7b (see also ESI Fig. S14† for a comparison with 2D projection HAADF-STEM). A possible explanation of this small discrepancy is the fact that in TEM we measure only the NP size in plane, and the corresponding values in Fig. 7b are calculated using \(D_v = 2\sqrt{ab}\).

To appraise the measured parameter distributions, it is again important to understand the effect of the measurement noise characterized by \(\hat{\sigma}_{\text{ext}}\). To this end, four measured NPs were selected, indicated by (black, red, blue, magenta) stars in Fig. 7a, which have \(\sigma_{550} = (0.092, 0.026, 0.023, 0.203)\), and \(\sigma_{550} = (1150, 1117, 2566, 1473)\) nm\(^2\), respectively. Random Gaussian noise of standard deviation \(\hat{\sigma}_{\text{ext}}\) was added to the experimental data, and the NP parameters were retrieved from the resulting data. This was repeated for 10,000 noise realizations, and the results are shown in Fig. 7c, each NP in a separate panel. The contours indicate the regions of highest probability density containing 68% and 95% of the data, which for a Gaussian distribution are the \(\sigma\) and \(2\sigma\) confidence intervals, respectively. The results show that, depending on the NP size and shape, the measurement noise results in about 1 nm full-width-at-half maximum (FWHM) in \(D_v\) and 10% in \(b/a\) and \(c/a\). These values account for about half the parameter spread in the set of investigated UGNs, confirming that the size and shape distribution of these NPs is indeed very uniform.

The NPs indicated by the black and red stars show a confinement of the data to \(b \approx c\), which is consistent with the simulated result in Fig. 6 for a spherical NP. The blue star NP shows a tighter distribution due to its high cross-section, reducing the effect of noise. The measured in-plane anisotropy is very small (2.3%) for this NP, and the fit shows a slightly oblate particle with \(b/a > 0.95, c/a \approx 0.92\), and the \(c\) semi-axis along \(z\) in the measurement system, as in Fig. S4.† The magenta star NP has a sizeable anisotropy (20.3%) and three different semi-axes, with \(c/a \approx 0.71\) and \(b/a \approx 0.82\). Overall, we see that the measurements give detailed information about these nominally 30 nm diameter NPs, with diameter precision around 1 nm and anisotropy precision in the 10% range for a measurement noise around 50 nm\(^2\). Decreasing the measurement noise by about an order of magnitude would increase the precision, as seen in Fig. S3,† and is possible with the experimental technique. Notably, the precision of the diameter would increase by the same factor, while for the asymmetry, the situation is more complex. For an isotropic case, the degeneracy of all semi-axes allows for a larger parameter space resulting in similar cross-sections, while for a significantly
Fig. 7 Fitted parameters for UGNSs using the channels \( \Lambda = 450, 550 \) from the measurements shown in Fig. 5a. (a) \( c/a \) versus \( b/a \), with the colour scale indicating the volume equivalent diameter \( D_v \). (b) magenta points: geometric average aspect ratio, \( \sqrt{bc}/a \), versus \( D_v \). Black points: \( b/a \) versus \( D_v = 2\sqrt[3]{abc^2} \), for 30 NPs examined using TEM. The magenta bars give the histogram of the magenta points. In both (a) and (b), the size of the points is given by \( S = 1/(1 + S) \), so larger points indicated lower error, as shown in (a). Inset in (b) shows the fitted NP orientation angles \( \theta \) versus \( \phi \), with the colour indicating \( \sqrt{bc}/a \). (c) For the measured NPs indicated by stars in (a and b), fit results for 10 000 realizations (1000 shown by filled circles) of added random measurement noise. Contours are the boundaries of regions of highest density containing 68% (black) and 95% (grey) of the data. Insets show the distributions of \( D_v \) and provide the symbol colour scale. The inset in the red starred plot is a TEM tomographic reconstruction of an exemplary NP (see also ESI section S.III†) from the same sample stock with similar \( D_v \) as the fitted NP.

asymmetric NP, this degeneracy is at least partially lifted, reducing the resulting parameter uncertainty. This is exemplified in the simulated cases seen in Fig. S4 and S5† comparing \( c/a = 1.0 \) with \( c/a = 0.6 \), and for three distinct semi-axes in the ESI Fig. S6." 

The fitted angles are shown in the inset of Fig. 7b. In particular we plot \( \theta \) and \( \phi \), the two angles which rotate the NP out of the substrate plane. The in-plane rotation \( \psi \) creates only a shift in the measured dependence of \( \sigma_\text{L}(\gamma) \) on \( \gamma \), and thus does not change the measured cross-section set. The observed angular distribution is spread over the full ranges of \( \phi \) and \( \theta \), consistent with the near spherical shape of the NPs. Some reduction around \( |\theta - \pi/2| = 0 \), and around \( |\phi| = \pi/2 \) is visible, which we can understand considering that as soon as there is some asymmetry, the NP is more likely to lie down on the substrate plane, so that \( a \) and \( b \) are in the substrate plane, corresponding to \( \theta = \phi = 0 \), as opposed to standing on end, which is either \( \theta \approx \pi/2 \) (a normal to substrate), or \( \theta \approx 0 \) and \( |\phi| \approx \pi/2 \) (b normal to substrate).

To determine the full three-dimensional NP shape with EM, going beyond 2D projection TEM as shown in Fig. 5, we have performed high-angle annular dark-field scanning transmission electron microscope (HAADF-STEM) tomography for a few of the UGNSs, as described in the ESI section S.III.† We found that most NPs are mono-crystalline and nearly spherical, consistent with our optical measurements and discussed fitting results. The aspect ratios of two NPs obtained from tomography are 0.99 and 0.96. For illustration one 3D rendering is shown in the inset of Fig. 7c.

We note that the selection of color channels \( \Lambda \) influences the results to some extent, as different channels are sensitive to different NP parameters and shape ranges, as discussed in detail in the ESI section S.II E.† Using \( \{\Lambda\} = \{450, 600\} \) is found to produce oblate NPs in the fit, which shows that the model underestimates the cross-section in the \( \Lambda = 600 \) channel. This can be attributed to the neglected surface damping20,25 in the gold permittivity used in our model, and the absence of surface faceting in the ellipsoidal shape assumed.

We now move to the more conventional GNSs, which, as we have seen in Fig. 5b, have a more variable shape. This is visible also in the fit results shown in Fig. 8, where there are rod-shaped NPs with \( b/a \approx c/a < 0.6 \), plates with \( b/a > 0.8 \) and \( c/a < 0.5 \), and reasonably spherical NPs with \( c/a > 0.7 \), as indicated in Fig. 8a. The distribution of the diameter results in a mean \( \bar{D} = 21.8 \pm 5.8 \) nm. We see a narrow mode around 18 nm, in good agreement with the observations from TEM (black
The comparison with results of 2D projection HAADF-STEM are given in the ESI Fig. S13. A tail of outliers to higher volume with a small aspect ratio is also found. Such NPs are not obvious in the TEM data, which mainly show roundish NPs with defects, and triangular plates in either side or top view. They could be thin plates of larger size, which are sporadically seen in TEM, and possibly NP dimers formed by aggregation. We also note that by dropping the \( \Lambda = 450 \) channel compared to the UGNS data shown in Fig. 7, the NP volume is less constrained. The orientation of the NPs (inset in Fig. 8b) shows again a broad distribution, but with a stronger tendency to lie flat (\( \theta \approx 0 \), \( |\phi - \pi/2| \approx \pi/2 \)), consistent with the larger deviation from spherical shape. To assess the effect of the measurement error, also in this case four measured NPs were selected, indicated by (black, red, blue, magenta) stars. Random Gaussian noise of standard deviation \( \hat{\sigma}_{\text{ext}} \) was added to the experimental data, and the NP parameters were retrieved. The corresponding distributions are shown in Fig. 8c. We find an uncertainty in the diameter of about 2 nm full width at half maximum in \( D_v \), while quite well defined in the average aspect ratio \( \sqrt{2c/a} \). The volume has been calculated assuming a cylindrical shape. These findings are in line with observations from TEM, and the optical results in Fig. 5d. We do see a large proportion of cases with \( b/a \) and \( c/a \) around 1/3, as expected from the nominal specifications, and TEM (see also ESI Fig. S14 for a comparison with 2D projection HAADF-STEM). Importantly, the individual NP fits shown in the lower four panels exhibit narrow bands at small values of \( c/a \) and \( b/a \), similarly to the synthetic cases seen in Fig. 6. The NP orientations (see inset Fig. 9b) are distributed in \( \phi \) but concentrated around \( \theta = 0 \), as expected for rods, where \( \phi \) is rotating around the rod axis, having little effect, and \( \theta \) is tilting the rod axis out of plane. Additionally, a fraction of NPs, such as the black star one, are plate-like, with \( c/a < 0.25 \) and \( b/a > 0.5 \). Again we have performed tomographic TEM for a few of the GNRs, as described in the ESI section S.III. We found, consistent with the above fitting results, that almost all NPs are defect free, and rod-shaped NPs are dominating. The aspect ratios obtained from tomography for two NPs are 0.36 and 0.42. For illustration, one 3D rendering is shown in the inset of Fig. 9c.

4.2.2. Rod-shaped gold NPs. The fit results for the GNRs are shown in Fig. 9, and are highly distributed in \( D_v \), \( b/a \), and \( c/a \), while quite well defined in the average aspect ratio \( \sqrt{2c/a} \). The volume has been calculated assuming a cylindrical shape. These findings are in line with observations from TEM, and the optical results in Fig. 5d. We do see a large proportion of cases with \( b/a \) and \( c/a \) around 1/3, as expected from the nominal specifications, and TEM (see also ESI Fig. S14 for a comparison with 2D projection HAADF-STEM). Importantly, the individual NP fits shown in the lower four panels exhibit narrow bands at small values of \( c/a \) and \( b/a \), similarly to the synthetic cases seen in Fig. 6. The NP orientations (see inset Fig. 9b) are distributed in \( \phi \) but concentrated around \( \theta = 0 \), as expected for rods, where \( \phi \) is rotating around the rod axis, having little effect, and \( \theta \) is tilting the rod axis out of plane. Additionally, a fraction of NPs, such as the black star one, are plate-like, with \( c/a < 0.25 \) and \( b/a > 0.5 \). Again we have performed tomographic TEM for a few of the GNRs, as described in the ESI section S.III. We found, consistent with the above fitting results, that almost all NPs are defect free, and rod-shaped NPs are dominating. The aspect ratios obtained from tomography for two NPs are 0.36 and 0.42. For illustration, one 3D rendering is shown in the inset of Fig. 9c.
5. Conclusions and outlook

To summarise, we have demonstrated a rapid, quantitative and accurate method to “optically size” hundreds of individual nanoparticles, by measuring their optical cross-sections in widefield extinction microscopy for different light wavelengths and excitation polarizations, and solving the inverse problem of determining the particle size, shape and orientation from the measurements.

We have shown examples for three different gold nanoparticle samples, nominally spherical and rod-like, in practice exhibiting various sizes and shapes, which were fitted using an ellipsoid model in the dipole limit. The results are, in general, in good agreement with non-correlative 2D projection TEM and HAADF-STEM, and HAADF-STEM tomography of the same batches. Depending on the experimental noise and the choice of colour channels in the measurements, we have quantified the sensitivity of the method. We show an uncertainty down to about 1 nm in mean diameter, and 10% in aspect ratios when using two or three colour channels and an experimental noise of about $\hat{\sigma}_{\text{ext}} = 50 \text{ nm}^2$. Notably, the precision can be increased by decreasing the measurement noise.

As an outlook, further advances in the method on the experimental side could include the direct probing of the nanoparticle axial direction, through the addition of a radial polarizer and a high numerical aperture illumination lens to generate an axial polarization component. This would improve the capability of the technique to measure particle geometries in 3D, presently originating from the interplay between particle shapes and plasmon resonance shifts. On the modelling side, calculating the response using a fully numerical solver, e.g. via COMSOL as presented in ref. 25 and 40, and optimizing the material permittivity used, would enable a more accurate and general description of essentially arbitrary shapes and material types. Combined, these future developments are poised to provide a powerful nanoparticle sizing method by optical means, presenting an alternative to complex and expensive TEM tomography analysis, merging accuracy with simplicity of use, easy access to statistics over large particle sets, and applicability to any nanoparticle size, shape, and composition.

Author contributions

L.P., W.L. and P.B. conceived the work. L.P. prepared the samples for the optical measurements and performed the related measurements and data analysis. W.A. prepared the samples for the STEM tomography, and performed the related measurements and data analysis. L.P, P.B. and W.L. developed the numerical model and fitting methods. L.P. performed the numerical simulations and data fitting. All authors contributed to the data interpretation and writing of the manuscript.

Data availability

Information on the data underpinning the results presented here, including how to access them, can be found in the

Conflicts of interest

There are no conflicts to declare.

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