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On the sensitivity of Interferometric Cross-Polarisation Microscopy for nanoparticle detection in the near-infrared.

Benjamin T. Miles,¹ Elizabeth C. Robinson,¹ Erik M. H. P. van Dijk,² Ian D. Lindsay,¹ Niek F. van Hulst,^{3,4} and Henkjan Gersen^{1,*}

¹*Nanophysics and Soft Matter Group, H.H. Wills Physics Laboratory, University of Bristol, Bristol, BS8 1TL, United Kingdom*

²*Faculty of Science and Technology, University of Twente, 7500 AE Enschede, The Netherlands*

³*ICFO - Institut de Ciències Fotoniques, 08860 Castelldefels (Barcelona), Spain*

⁴*ICREA - Institució Catalana de Recerca i Estudis Avançats, 08010 Barcelona, Spain*

We address the sensitivity of Interferometric Cross-Polarisation Microscopy by comparing scattering and absorption by spherical 10 nm nanoparticles through a combination of modelling and experiment. We show that orthogonality of light in the two polarisation branches of Cross-Polarisation Microscopy ensures that only light that has interacted with a nanoparticle is interferometrically enhanced. As a result background-free shot noise-limited detection is achieved for sub- μ W optical powers at the sample. Our modelling in particular shows that in the near-infrared regime, above the plasmon resonance frequency of spherical nanoparticles, the cross-polarisation approach is several orders of magnitude more sensitive than conventional extinction based detection. This enhanced near-infrared sensitivity for spherical nanoparticles is promising for applications requiring low absorption and low power imaging of nanoparticles in cells.

Keywords: Confocal Microscopy, Nanoparticle detection, Mie Scattering, Cross-Polarisation Imaging, Gold nanoparticles, Background free detection, Detection Sensitivity

Inside the living cell a multitude of dynamic processes occur, which are routinely studied by a large variety of biological and physical methods based on detection of single fluorescent molecules [1]. For example, the detection of fluorescent labels, or auto-fluorescence from proteins, is used to track the conformation, position and movement of proteins, filaments and DNA, both in-vitro and in-vivo [2–4]. Moreover, the photo-physical dynamics of the fluorophore, such as fluorescence lifetime and energy transfer, directly reports on the local nano-environment [5]. Clearly the detection of single fluorophores for nanoscale bioimaging has found a wide range of applications, as confirmed by the 2014 Nobel prize [6]. Yet the reliance on fluorescence is accompanied by a number of limitations. Firstly, organic molecules do convert to a non-fluorescent state after a limited number of photo-cycles, which limits the observation time of the experiment and simultaneously induces phototoxicity [7]. Secondly, the finite lifetime of the excited state results in saturation, which limits the maximum emitted intensity, and thereby the precision and/or time resolution with which individual fluorophores can be tracked [8].

An attractive alternative to fluorescent labels that does not suffer from the above limitations is provided by metallic nanoparticles, which are already widely used in biology for single particle tracking and localization [9]. The optical signal from these particles is strong, very stable and does not suffer from photobleaching. Moreover, as the scattered signal from these nanoparticles is proportional to the incident intensity, the limitation on achievable time resolution is removed as the arrival rate of scat-

tered photons can be enhanced by increasing the incident intensity [8]. To operate at this higher intensity it is advantageous to work in the near-infrared regime (NIR) where absorption by water and biomolecules is minimal which, combined with lower photon energies, substantially reduces phototoxicity [1, 10]. However, to follow biological processes it is paramount that the label used is small compared to the molecular machinery inside the cell, typically a few tens of nanometers in size [1]. Detecting nanoscale objects in the 10 nm size regime is challenging as their cross-section is small typically limiting the detection to particles with a diameter larger than ~ 30 nm in conventional microscopy [1, 11].

The clear potential for biological applications has triggered the development of a variety of optical approaches to detect individual nanoparticles smaller than ~ 30 nm, as recently reviewed in depth by Yurt *et al.* [12] as well as Zijlstra and Orrit [11]. Several of these approaches have now even demonstrated the detection of a single molecule in absorption; convincingly demonstrating the sensitivity that can be achieved [13–16]. However a drawback to nearly all of these approaches is that they are resonant and require high optical powers incident on the sample at the wavelengths where absorption by biomolecules is large, which is prone to induce photodamage. To overcome such limits, we have recently demonstrated an Interferometric Cross-Polarisation approach [17] which enables detection of single gold nanoparticles down to 5 nm diameter at excitation powers below 1 μ W incident on the sample.

In this paper we provide a theoretical estimate and understanding of the sensitivity of the interferometric cross-polarised detection scheme by a quantitative comparison to the simplest case of direct detection of the trans-

* h.gersen@bristol.ac.uk

mitted light absorbed by an individual nanoparticle [15]. Against the commonly held notion that absorption-based detection schemes are more sensitive [18] our analysis shows that, for the same signal-to-noise ratio, a scattering based interferometric detection scheme allows spherical 10 nm gold nanoparticles to be detected at near-infrared wavelengths with two orders of magnitude less incident optical power when operating above the plasmon resonance of the nanoparticle. We confirm this concept through the first experimental demonstration of detecting 10 nm gold nanoparticles at both visible and near-infrared wavelengths at sub-1 μW powers incident on the sample. This ability to achieve detection using extremely low power levels at near infrared wavelengths holds considerable promise as an approach to exploit the potential of such small nanoparticles as biomarkers in living cells while causing minimal photodamage.

First let us consider the photon-limited sensitivity. Due to the discrete nature of photons, the precision with which one can detect an optical signal is fundamentally limited by shot noise [19]. A photon counter detecting, on average, N_s photons per time interval will, for many such intervals, yield a distribution of counts with a standard deviation $\sqrt{N_s}$, this uncertainty being the shot noise. The resulting signal-to-noise ratio(SNR) in the background-free, shot noise-limited case is then given by:

$$\text{SNR}_{\text{shot noise}} = \frac{N_s}{\sqrt{N_s}} = \sqrt{N_s}, \quad (1)$$

which sets the classical limit for detecting changes in N_s [20].

A molecule or nanoparticle with extinction cross-section σ_{ext} passing through a beam of linearly x -polarised light with area A results in a reduction of the incident photon flux N_{in} (in photons/sec) after the nanoparticle by $N_{\text{ext}} = \frac{\sigma_{\text{ext}}}{A} N_{\text{in}}$. Reducing the area A by focusing the incident light obviously increases the signal N_{ext} , however one still has to detect this signal against the shot noise induced by the background of photons that have not interacted with the molecule or nanoparticle. For detection of a nanoparticle passing through the focus in transmission, as depicted in figure 1(a), the photon flux at the output of the collection lens and incident on the detector is given by:

$$N_{\text{out}} = N_{\text{in}} - N_{\text{ext}} = \left(1 - \frac{\sigma_{\text{ext}}}{A}\right) N_{\text{in}}. \quad (2)$$

This expression implicitly assumes that a plane polarised wave is incident on the scattering object, which is not strictly true for focussed light [21, 22]. However, instead of considering the extinction, absorption and scattering from first principle as done by Mohammadi and Agio [22], we choose to follow this classical treatment as it enables a clearer discussion of SNRs.

To be detectable, the signal, N_{ext} , must exceed the uncertainty in the total photon count due to shot noise, $\sqrt{N_{\text{out}}}$. The SNR in this case is therefore given by:

FIG. 1. Focusing linear x -polarised light will create field components in all directions that are scattered or absorbed by a molecule or nanoparticle passing through the focus as shown schematically in the inset. (a) Direct detection in which both excitation light and light scattered by the nanoparticle are collected, with the signal originating from the extinction of light. (b) Cross-polarised detection scheme in which a polariser with its transmission axis perpendicular to the incident linear polarisation direction only transmits the scattered field components with a polarisation perpendicular to the incident polarisation in principle enabling background-free detection. (c) Interferometric Cross-Polarisation detection scheme in which linear x -polarised light is split in a signal and reference branch. Interfering light from the signal-branch with a y -polarised reference that is frequency shifted by an Acousto-Optic Modulator(AOM) enables background-free detection of only y -polarised scattered photons by eliminating objective-induced depolarisation that in practise limits the scheme in (b).

$$\text{SNR}_{\text{direct}} = \frac{N_{\text{ext}}}{\sqrt{N_{\text{out}}}} = \frac{\frac{\sigma_{\text{ext}}}{A} N_{\text{in}}}{\sqrt{(1 - \frac{\sigma_{\text{ext}}}{A}) N_{\text{in}}}} \approx \frac{\sigma_{\text{ext}}}{A} \sqrt{N_{\text{in}}}, \quad (3)$$

in which the assumption is made that $\frac{\sigma_{\text{ext}}}{A} \ll 1$. Equation 3 enables us to estimate the photon flux needed for direct detection of the absorption by a single molecule in transmission. A single terrylene diimide(TDI) molecule, as used by Celebrano *et al.* [16] to demonstrate that it is possible to measure the absorption of a single molecule, is stated to have a cross section of $9 \times 10^{-16} \text{ cm}^{-2}$ at 632.8 nm when its electronic dipole matches the polarisation of the incident light. For light focused by an objective with a Numerical Aperture(NA) of 1.45 this

FIG. 2. Shows the absolute value of the electric field in the x -, y -, and z -direction (from left to right) in the focus of a 1.45 NA objective when linear x -polarised light ($\lambda = 632.8$ nm) is focused to a diffraction limited spot. The incident polarisation direction is indicated by the arrow in (a). The spatial extent of these images is $3 \mu\text{m} \times 3 \mu\text{m}$ and they are scaled to their maximum value using the multiplication factors indicated. The relative magnitude of this maximum field strength is used to provide a measure for the relative fraction γ of the incident linear x -polarised photons that are converted to x -, y -, and z -polarised photons, respectively.

corresponds to $\frac{\sigma_{\text{ext}}}{A} \approx 4.0 \times 10^{-7}$, which shows that at least 6.1×10^{12} photons/s are needed to reach an SNR equal to one. At the wavelength used this corresponds to a power of at least $1.9 \mu\text{W}$ impinging on a molecule with its absorption dipole moment aligned with the incident polarisation if we assume a perfect detector that is able to count each photon. This number is consistent with the optical power of $\sim 100 \mu\text{W}$ used by Celebrano *et al.* [16] once optical losses (60 %) and quantum efficiency (50 %) of their detector are accounted for, assuming an SNR of 4.

From the above discussion, it can be seen that noise at the detector is dominated by the shot noise contribution from the background of photons that do not interact with the nanoparticle, represented by the unity term in equation 2 and 3. As both the cross-section and A are fixed by the physical properties of the molecule and objective, respectively, the only available approach to increase SNR, thus enabling detection at lower incident power levels, is the reduction of this background term. Here we consider background reduction through the use of a cross-polarised detection scheme which exploits the change in polarisation direction of the light scattered from a nanoparticle. This effect arises because a tightly focused x -polarised beam yields a field in the focal plane that is no longer purely x -polarised but also contains y - and z -polarised components [23]. In the case of unperturbed focusing, these components in principle propagate to reconstruct an x -polarised far field. However, perturbation of the focal region due to a nanoparticle results in these components appearing in the far field, allowing a polarisation-based detection scheme to be sensitive to only those photons that have interacted with the nanoparticle, as schematically depicted in Fig 1(b).

The x -, y - and z -component of the electrical field for a high NA oil-immersion objective with $\text{NA}=1.45$ can in fact be calculated [23] and are displayed in figure 2 for a filling factor of 2. Fig. 2(b) and 2(c) are multiplied by 12.6 and 2.43, respectively, to enable comparison

of the different field components using the same colour scale. From this we see that the different field components have both a different strength as well as a different spatial distribution. To discuss SNRs one is most interested in the ability to distinguish maxima, hence we use the relative magnitude of the maximum field strength as a measure for the relative fraction γ of the incident linear x -polarised photons that are converted to x -, y -, and z -polarised photons, respectively. For an objective with $\text{NA} = 1.45$ we find $\gamma_{x \rightarrow x} = 0.8513$, $\gamma_{x \rightarrow y} = 0.0054$, and $\gamma_{x \rightarrow z} = 0.1433$ showing that the field in the focal plane is dominated by the x -component as would be expected. Note that as the incident and scattered field are collected by an objective a similar conversion between polarisation states will be induced upon collection. However, a lower NA collection objective with $\text{NA} = 0.90$ yields $\gamma_{x \rightarrow x} = 0.8973$ showing that ignoring the polarisation conversions for a lower NA air objective is a reasonable simplification.

To analyze the sensitivity of cross-polarised detection, we consider that a perfect polariser is used to make the incoming light linearly x -polarised with a photon flux N_{in} passing this first polariser as depicted in figure 1(b). Focusing this light will convert a fraction γ of the incident photon flux in x -, y - and z -polarised photons, respectively. This light, when scattered by a nanoparticle, will be collected by the collection objective leading to a scattered photon flux in specific polarisation states of:

$$N_{\text{scat},o} = \gamma_{i \rightarrow o} \frac{\sigma_{\text{scat}}}{A} N_{\text{in}}, \quad (4)$$

in which i and o represent the input and output polarisation direction of interest, respectively and σ_{scat} is the scattering cross-section. This change to the scattering cross-section reflects that we detect scattered photons in this detection scheme. The second perfect polariser in Fig 1(b) will transmit light polarised in y , but block all x -polarised light. As a result, behind an ideal polariser there will in principle be a photon flux of only $N_{\text{out}} = N_{\text{scat},y}$. In this case the shot noise on a perfect detector behind the polariser will be $\sqrt{N_{\text{scat},y}}$ yielding a SNR of:

$$\text{SNR}_{\text{crossed}} = \frac{N_{\text{scat},y}}{\sqrt{N_{\text{scat},y}}} = \sqrt{\gamma_{x \rightarrow y} \frac{\sigma_{\text{scat}}}{A} N_{\text{in}}}. \quad (5)$$

From this equation we see that the required photon flux N_{in} to achieve the desired SNR becomes linear in $\frac{A}{\sigma_{\text{scat}}}$ and no longer quadratic in $\frac{A}{\sigma_{\text{ext}}}$, as was the case for direct detection (see Eq. 3), albeit with different cross-sections. Despite this clear theoretical advantage, in practice the SNR as given in Eq. 5 is not achievable using the detection scheme depicted in figure 1(b) due to objective induced depolarisation as we will explain below.

The extinction coefficient is an important figure of merit to consider in analyzing polarisation microscopy [24]. The extinction coefficient is defined as

the ratio of the intensity of light transmitted between parallel polarisers to that transmitted when polarisers are crossed. Ideally this extinction coefficient would be infinite, however the image formation in wide-field microscopes prevent this even if the polarisers are perfect and in fact drops rapidly as the NA of the objectives is raised and typically a value of 1×10^{-3} is obtained [24]. This loss of extinction originates from the different transmission-coefficients and phase-shift experienced by s- and p-polarised rays as they pass through the optical interfaces under the large angles used in high-NA objectives [25]. In practice one sees this phenomena manifest itself in the form of a Maltese cross at the output of a system [17, 25] as drawn in figure 1(b). For an illumination objective of NA=1.45 and a collection objective with NA=0.90 this Maltese cross is clearly visible and one finds an extinction ratio of only 2.2×10^2 when this combination of objectives is placed between Glan Thompson polarisers that have an extinction ratio of 5×10^5 . As a result of this objective-induced depolarisation effect, the SNR as given in equation 5 is therefore not achievable in wide-field microscopes even in the case of perfect polarisers. However this changes for single-point scanning confocal imaging systems as the polarisation aberrations that occur at the exit pupil of the system have opposite phase in different quadrants in the exit pupil [25]. As a result these aberrations cancel when overlapped with a reference beam to enable detection of the amplitude of the transmitted field instead of the intensity [24, 26]. In fact, detecting the light's amplitude in principle allows infinite extinction ratios to be obtained as shown theoretically by Wilson and Tan [27]. In this measuring amplitude rather than intensity can be done by either using infinitely small pinholes or through interferometric detection of the light transmitted [24].

Here we focus on the use of interferometric detection of the light scattered by the nanoparticle as schematically depicted in figure 1(c). In this scheme the polariser in the frequency shifted reference branch is set to transmit E_y so that from the field exiting the collection objective, only E_y components that do not have anti-phased equivalents (as occur for polarisation aberrations in the imaging system) will interfere with the reference field to generate a signal at the modulation frequency, $\Delta\omega$. In the geometry displayed in Fig. 1(c), linear x -polarised light with a photon flux N_1 incident on the illumination objective is transformed by the scattering from a nanoparticle in the focus to:

$$\mathbf{E}'_1 = \begin{bmatrix} E_1 - E_{\text{ext},x} \\ E_{\text{scat},y} \end{bmatrix} \approx \begin{bmatrix} \sqrt{N_1} - \sqrt{N_{\text{ext},x}} \\ \sqrt{N_{\text{scat},y}} \end{bmatrix} \quad (6)$$

at the output of the collection objective in which $E_{\text{ext},x}$ corresponds to the reduction of the x -polarised field as a result of both absorption and scattering. Note that in the schematic diagram drawn in Fig. 1(c) the photons in both x - and y -polarisation are incident on the detectors at the outputs of the interferometer as there

is no polariser present after the collection objective. We write the frequency shifted field in the reference branch as $\mathbf{E}_2(\omega + \Delta\omega)$, which is linearly y -polarised with a photon flux N_2 . Assuming a 50/50 beam splitter the resulting irradiance(I) on one of the perfect detectors at the output of the interferometer becomes:

$$I_{\text{det}} = \frac{\langle |\mathbf{E}'_1 + \mathbf{E}_2|^2 \rangle}{2} = \frac{1}{2} \langle \mathbf{E}'_1{}^2 + \mathbf{E}_2{}^2 + 2\mathbf{E}'_1 \cdot \mathbf{E}_2 \rangle \quad (7)$$

where $\langle \rangle$ denotes a time average over the response time of the detector. If we assume perfect overlap this reduces for the case of orthogonal polarisations to:

$$I_{\text{det}} \approx \frac{1}{2} \langle E_1^2 + E_2^2 - 2E_1 E_{\text{ext},x} \cos(\phi_1) + 2E_2 E_{\text{scat},y} \cos(\Delta\omega + \phi_2) \rangle, \quad (8)$$

in which we assume that $E_{\text{ext},x}, E_{\text{scat},y} \ll E_1, E_2$ and where ϕ_1, ϕ_2 corresponds to the phase difference between the interfering fields. From equation 8 we see that the small signal $E_{\text{scat},y}$ corresponding to the y -component of the field scattered by the nanoparticle is interferometrically enhanced by the light in the reference branch, E_2 . This enables us to select the optical flux in the reference branch sufficiently large so that the limiting factor is the shot noise of all the light incident on the detector and not the background electronic noise. Moreover, it shows that we can distinguish between scattered photons interfering with the incident light (E_1) and those interfering with the reference (E_2) as a result of the applied frequency modulation in the reference branch. If we maximize the signal by ensuring that $\phi_1 = \phi_2 = 0$ (constructive interference) and rewrite the measured irradiance in terms of photon flux we obtain:

$$I_{\text{det}} \approx \frac{N_1 + N_2}{2} - \sqrt{N_1} \sqrt{N_{\text{ext},x}} + \sqrt{N_2} \sqrt{N_{\text{scat},y}} \cos(\Delta\omega). \quad (9)$$

As the amount of light that interacts with a nanoparticle is small compared to N_1, N_2 the noise is given by $\sqrt{N_1 + N_2}$. With this approximation the SNR becomes:

$$\text{SNR}_{\text{inter}} \approx \frac{\sqrt{2N_2} \sqrt{N_{\text{scat},y}}}{\sqrt{N_1 + N_2}} = \frac{\sqrt{2N_2}}{\sqrt{N_1 + N_2}} \sqrt{\gamma_{x \rightarrow y} \frac{\sigma_{\text{scat}}}{A} N_1}. \quad (10)$$

For the case where $N_2 \gg N_1$ as is the case here due to losses arising from overfilling the objective and total internal reflection occurring at the air-glass interface, this reduces to Eq. 5, albeit with an additional factor of $\sqrt{2}$. This factor results from our earlier assumption that we operate at constructive interference. The above equation demonstrates that the use of an interferometric cross-polarisation scheme allows an SNR to be achieved equal to that of ideal cross-polarised detection, with perfect extinction and no objective-induced depolarisation. Comparing the given SNR expressions for direct and cross-polarised detection highlights the key advantage that in

the cross-polarised interference case the required photon flux, N_{in} , becomes linear in $\frac{A}{\sigma_{\text{scat}}}$ rather than quadratic in $\frac{A}{\sigma_{\text{ext}}}$.

Direct detection and interferometric cross-polarised detection depend on distinct cross-sections, thus one expects different wavelength dependence. Specifically relevant is the question which of these methods is more sensitive in the NIR. Fortunately the general theory for scattering by spherical nanoparticles is well established and these cross-sections can be calculated using Mie theory [21]. In this theory the interaction of a nanoparticle with light is characterized by the efficiencies of absorption and scattering resulting from their respective cross-sections, σ_{abs} and σ_{scatt} . Both of these processes reduce the number of photons incident on the detector leading to $\sigma_{\text{ext}} = \sigma_{\text{abs}} + \sigma_{\text{scatt}}$ under the assumption that a plane wave is incident on the particle. In the limit of particles that are small compared to the wavelength, the absorption and scattering cross-sections are given by [21]:

$$\sigma_{\text{abs}} = \frac{\pi^2 n_s}{\lambda} D^3 \text{Im} \left(\frac{m^2 - 1}{m^2 + 2} \right) \quad (11)$$

and

$$\sigma_{\text{scatt}} = \frac{2\pi^5 n_s^4}{3\lambda^4} D^6 \left| \frac{m^2 - 1}{m^2 + 2} \right|^2 \quad (12)$$

where n_s is the refractive index of the medium, m the ratio of complex refractive indices of the nanoparticle and the medium, and D the diameter of the nanoparticle. These expressions allow analytical expressions to be derived for the required photon flux in the limit for spherical particles that are small compared to the wavelength showing that both approaches scale with D^{-6} .

To appreciate the wavelength dependence one has to introduce the dispersion of the complex refractive index of the particle. The extinction and scattering cross-sections needed for this are calculated using Mie code published by Bohren and Huffman [21] (dashed lines) as well as in the small particle limit using Eq. 11 and Eq. 12 (solid lines). The green and blue curve in Figure 3(a) show the calculated extinction and scattering cross-section, respectively, for a 10 nm gold nanoparticle where the published Mie code overlaps with the curves obtained from Eq. 11 and Eq. 12. The refractive index values for gold have been taken from Johnson and Christy [28] and we assume that the particles are embedded in a homogeneous material with refractive index $n_s = 1.5$. The wavelength and particle-size dependence of the required optical power to achieve a $\text{SNR} = 1$ for both approaches are displayed in Fig. 3(b–d), in which the green and blue curves show the required optical power using direct and cross-polarised interferometric detection, respectively. These calculated powers follow from Eq. 3 and Eq. 10, by multiplication with the photon energy. The spotsize, A , is treated as wavelength dependent with

$\gamma_{x \rightarrow y}$ a constant with a value of 0.0054 as used previously. Fig. 3(b) shows the required incident power to detect a single gold nanoparticle with a diameter of 10 nm and $\text{SNR}=1$ as a function of wavelength, while (c, d) show the diameter dependence for selected wavelengths in the visible and near-infrared. Powers needed to achieve other SNRs can be found from (b–d) by multiplying the displayed values with SNR^2 .

From these figures it is evident that for wavelengths in the NIR that are well above the plasmon resonance frequency of the spherical nanoparticle two orders less optical power is required in the case of cross-polarised interferometric detection (blue lines). This observation is counter-intuitive as the commonly held notion is that for small nanoparticles absorption-based techniques are more sensitive than scattering based approaches, which is clearly not the case when operating above the plasmon resonance frequency. This increased sensitivity in the NIR for detecting spherical nanoparticles when using cross-polarised detection as opposed to direct detection is a clear consequence of the fact that for cross-polarised detection the required power to obtain a specific SNR scales linearly with $\frac{A}{\sigma_{\text{scat}}}$ rather than quadratically in $\frac{A}{\sigma_{\text{ext}}}$ as is the case for direct detection. So despite the fact that at the plasmon resonance the scattering cross-section is 3 orders of magnitude lower than the extinction cross-section as can be seen in Fig. 3(a) both show a similar reduction with wavelength and are 2 to 3 orders of magnitude lower in the NIR. As a result of the linear vs. quadratic dependence of the required optical power this hence leads to the observed enhanced sensitivity of roughly 2 orders of magnitude for a cross-polarised scattering based approach at $\lambda = 780$ nm compared to direct detection. Interestingly enough this advantage for cross-polarized interferometric detection only applies to particles that are well within the small particle approximation as we can see from the cross-over visible in Fig. 3(d) at a diameter of 80 nm. A similar trend is found for silver nanoparticles that have a lower plasmon resonance frequency, although for those it is beneficial to use interferometric cross-polarised detection at all displayed wavelengths and in fact 4 orders less optical power is needed in the NIR. It is important to realize that the sensitivity for interferometric cross-polarised in the NIR is below that of direct detection when working at the plasmon resonance as can be seen directly from 3(b) by comparing the minimum powers needed at 532 nm and 780 nm, respectively.

To provide experimental evidence that it is indeed possible to detect 10 nm spherical gold nanoparticles at ultra-low excitation powers by a scattering based approach we imaged single gold nanoparticles with a diameter of 10 ± 1 nm at both visible ($\lambda = 532$ nm) and NIR ($\lambda = 780$ nm) wavelengths using an excitation power of less than 1 μW incident on the sample. For the NIR measurements we used a laser-diode emitting at 780 nm (Aixiz, A-780-10-3.2) and further details on sample preparation and the experiment can be found elsewhere [29]. The cross-polarised amplitude image for

FIG. 3. (a) Green and blue curves depict the extinction and scattering cross-section, respectively, for a 10 nm gold nanoparticle embedded in a medium with $n = 1.5$. (b–d) Green and blue curves display the optical power required to detect a gold nanoparticles with an SNR=1 using direct detection and cross-polarised interferometric detection, respectively. From these curves the required power to achieve other SNRs can be calculated through multiplication by SNR^2 . Dashed lines use Mie code by Bohren and Huffman [21] to calculate cross-sections for gold spheres embedded in a medium with $n = 1.5$, while solid lines use cross-sections in the small particle approximation. (b) Power required as function of wavelength for a gold nanoparticle with $D = 10$ nm highlighting that for wavelengths above 600 nm two orders less optical power is required for interferometric cross-polarised detection. (c,d) Power required as function of particle diameter for two typical wavelengths. Black and red dotted lines correspond to the wavelength and particle diameter used for the measurements presented in Fig. 4.

$\lambda = 532$ nm and $\lambda = 780$ nm is shown in Fig. 4(a) and (b), respectively. In this the observed amplitude distribution of each individual nanoparticle in first approximation resembles the expected E_y field component displayed in Fig. 2(b) as discussed by Hong *et al.* [17]. The exact image formation is, however, slightly more complex as it results from interference with the reference. For these images collected at the focal plane the interference primarily reduces the amplitude of the sidelobes visible in Fig. 2(b) [29].

To highlight the SNR obtained Fig 4(c) and 4(d) show line-traces across the centre of the two left-hand lobes of the patterns visible for five selected nanoparticles at $\lambda = 532$ nm and $\lambda = 780$ nm, respectively. For clarity high features arising from other particles on the same line have been removed where needed. These results were obtained using a power of $1.2 \mu\text{W}$ in the signal branch in both cases. With a filling factor of 2 this corresponds to a power of less than $1 \mu\text{W}$ incident on the sample demonstrating that it is possible to detect single nanoparticles with a good SNR for ultra-low excitation powers for both visible and NIR wavelengths. Note that at these excitation powers it would not be possible to image these particles with the obtained SNR using the direct detec-

tion scheme depicted in Fig. 1(b) as can be determined from the required optical powers depicted in figure 3(b). To achieve the SNR of 4 seen in Fig. 4(d) would require $2 \mu\text{W}$ incident on the sample, which exceeds the power used here. Unfortunately, it is difficult to directly compare the SNRs obtained for $\lambda = 532$ nm and $\lambda = 780$ nm against our modelling due to practical limitations. The sample preparation for the experiments shown has been identical, yet different illumination fibers had to be used and in the NIR the SNR is severely affected by mode-hopping noise of the laser used [30], which completely dominates the noise visible in Fig. 4(d). It is worth noting that this type of noise is not a fundamental limitation for the technique as amplitude noise of this type can be suppressed by using a more stable laser diode or through balanced detection of the interferometric signal as we recently demonstrated [31].

To conclude: we have shown that the orthogonality of the light in the two polarisation branches of the interferometer ensures that only light that has interacted with a nanoparticle passing through the focus will be interferometrically enhanced. This results in a SNR corresponding to background-free shot noise-limited detection of spherical nanoparticles at optical powers signifi-

FIG. 4. Interferometric cross-polarization imaging of 10 ± 1 nm diameter gold nanoparticles at visible and near-infrared excitation. The detected amplitude of the scattered light is shown for particles excited by (a) $\lambda = 532$ nm, with a power of $1.2 \mu\text{W}$ and $9.5 \mu\text{W}$ in the signal and reference branch, respectively. (b) $\lambda = 780$ nm, with a power of $1.2 \mu\text{W}$ and $3.2 \mu\text{W}$ in the signal and reference branch, respectively. With a filling factor of 2 this in both cases corresponds to a power of less than $1 \mu\text{W}$ incident on the sample. The spatial extent of the images is $18.4 \mu\text{m} \times 18.4 \mu\text{m}$, for the fast and slow axis, respectively, with a 1.5 ms pixel dwell time and a lock-in integration time of $366 \mu\text{s}$. Vertical line-traces across the centre of the two left-hand lobes for five selected particles to highlight the SNR obtained for (c) $\lambda = 532$ nm and (d) $\lambda = 780$ nm.

cantly lower than would be possible in direct detection of transmitted light when operating above the plasmon resonance frequency, enabling the use of low optical powers incident on the sample reducing associated photodamage and phototoxicity. We have shown this experimentally by demonstrating that single gold nanoparticles with a diameter of 10 ± 1 nm can be detected using cross-polarised detection with a good SNR using an illumination power of less than $1 \mu\text{W}$ incident on the sample at both visible and NIR wavelengths. The use of an interferometer combined with single-point imaging, moreover, enables the removal of polarisation aberrations resulting from the imaging system enabling polarisation measurements with high spatial resolution and low illumination power in living cells. Our preliminary work towards cell imaging indicates that the signal levels resulting from the cell interior in interferometric cross-polarisation imaging are below those obtained for 10 nm gold nanoparticles provided that sufficient care is taken during sample prepa-

ration to reduce edge birefringence of the cell surface. This highlights the potential of this approach to localize single gold nanoparticles in living cells with high spatial resolution in the wavelength regime where absorption by biomolecules and water is low.

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