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Wavelength-Dependent Differential Interference Contrast Inversion of Anisotropic Gold Nanoparticles

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Abstract

Gold nanorods are promising nanoparticle-orientation sensors because they exhibit wavelength and angle-dependent optical patterns in their differential interference contrast (DIC) microscopy images. In this paper, we report a finite-difference time-domain method to simulate DIC images using nanorods as model probes. First, we created a DIC image library of nanorods as a function of imaging wavelength and rotation angle that showed good agreement with experimental results. Second, we used this simulation tool to explain why the patterns inverted from bright to dark when the imaging wavelength increased from below to above the plasmon resonance of the nanorod. We found that this intensity inversion resulted from reversal in electric field direction depending on wavelength relative to the nanorod plasmon resonance. Finally, we showed that this DIC contrast inversion is a general phenomenon by measuring and simulating DIC images from gold nanorods of different sizes and gold nanostars.

TOC Graphic

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ASSOCIATED CONTENT

Supporting Information

Post-processing script for DIC image generation; simulation conditions for DIC image simulation; single-particle scattering spectra; DIC contrast inversion in wide-field image; charge distribution inversion; full rotational study for three different gold nanorods; gold nanorod going through out-of-plane rotation; simulation of a gold nanostar.

Introduction

Noble metal nanoparticles (NPs) have emerged as attractive nanoscale imaging probes because of their unique optical and spectral properties.^{1–3} Anisotropic nanoparticles such as gold nanorods are of particular interest because they exhibit orientation-dependent optical responses.⁴ Polarization-based imaging, including dark-field (DF) polarization microscopy,⁵ photothermal heterodyne imaging (PHI),⁴ and differential interference contrast (DIC) microscopy^{6–7} have been used to resolve the orientation of single gold nanorods. Scattering-based DF microscopy is challenging for distinguishing nanorods from biological backgrounds, however, and particles smaller than 20 nm in diameter are difficult to detect since they have low scattering cross-sections.⁸ PHI requires two separate laser excitations to probe the longitudinal and transverse modes of nanorods in addition to raster scanning for image collection, which limits applications in dynamic *in vitro* systems.⁴

Although a diffraction-limited technique, DIC microscopy can be used to probe the orientation and rotational motion of gold nanorods by interference and polarization anisotropy.^{9–11} Moreover, gold nanorods show wavelength and orientation-dependent bright and dark intensities in their DIC images.¹² Previous work has indicated that DIC image contrast was strongest near the localized surface plasmon (LSP) resonance wavelength (λ_{LSP}) of the NP, with the longitudinal and transverse modes showing different optical responses.¹⁰ How near-field interactions between the plasmonic NP and the incident light translate to the final far-field DIC image at different imaging wavelengths, however, is less clear.

To understand how the particle LSP wavelength affects DIC image patterns, simulations that consider the fundamental details of how light interacts with the metal probes in a DIC optical set-up are required. Experimental images are inherently noisy and limited in resolution, and simulations can predict full image patterns for improved pattern recognition instead of relying on single-intensity levels alone.¹³ Calculations based on the point-spread function of the DIC microscope require arbitrary assumptions of phase-offsets of orthogonal illumination beams due to light-NP interactions.¹⁴ Also, analytical solutions that can determine the scattering of isolated plasmonic NPs, such as Mie theory, are typically limited to simple shapes (e.g. spheres)¹⁵ and do not provide the near-field information needed to understand the resulting image patterns. Finite-difference time-domain (FDTD) numerical simulations can visualize both the electric near-field distribution around NPs and far-field responses.¹⁶ FDTD methods determine the electromagnetic field around structures by discretizing the differential form of Maxwell's equations in both time and space, which enables modeling of nanostructures with more complex geometries.¹⁷ By setting of appropriate boundary conditions within the simulation region, arbitrary particle sizes and aspect ratios can be systematically modeled. FDTD models have been developed for imaging methods including DF microscopy¹⁸ and phase-contrast microscopy,^{19–20} but not for DIC imaging.

Here, we describe a three-step FDTD simulation procedure for DIC microscopy of anisotropic metal NPs. We focus primarily on gold nanorods as a model optical probe. This calculation tool could reproduce experimental DIC image patterns as a function of particle

orientation and imaging wavelength. Different from the literature,^{6–7} we found that gold nanorods showed DIC contrast inversion depending on the wavelength used for DIC imaging relative to the LSP resonance of the rod. By examining how the nanorod interacted with each polarization of the Nomarski prism within the DIC microscope, we discovered that phase shifts occurred only to the beam aligned with longitudinal axis of the rod. Near-field calculations revealed a contrast inversion when the direction of the electric fields reversed with wavelength change. Finally, we demonstrated that this DIC contrast inversion around the particle LSP wavelength is a general phenomenon, and other anisotropic nanoparticles NPs such gold nanostars also showed this effect.

METHODS

FDTD simulations of DIC microscopy

Calculations were performed with commercial software (FDTD Solutions, Lumerical Inc.). Periodic boundary conditions with a width of 12 μm in both x and y dimensions were used to avoid interactions between particles in neighboring simulation zones (Supporting Information, Figure S1). Perfectly matched layer conditions were used with 2 μm in z . A plane wave source (broadband or single frequency) was incident along the z -axis 50 nm below the gold nanorod. The rod was designed as the union of two spheres and a cylinder having dimensions from SEM images and material properties defined by a built-in model based on data from Johnson and Christy.²¹ A mesh override region around the particle forced a 2-nm mesh in all dimensions. A 2D monitor recorded the electric and magnetic fields 50 nm above the sample. Two orthogonal polarizations were simulated separately by plane-wave sources in two setups, where the beam was polarized along the x -axis in setup 1 and along the y -axis in setup 2. The nanorod was placed at $x = +60$ nm (-60 nm) in setup 1 (setup 2) to simulate the 120-nm shear distance between the two polarizations by the Nomarski prism. To simulate DIC images of the rod at different orientation angles, a sweep function was used to rotate the rods in 10° increments. Results were post-processed with a Lumerical script (Supporting Information, S2).²²

Gold nanorod sample preparation

Reference markers were created on #1.5 coverslips by shadow deposition of 5-nm Cr through an indexed TEM reference grid (Ted Pella). A 10x diluted solution of bare gold nanorods (40×92 , 25×75 , Nanopartz Inc.) was drop-cast on the coverslips for one minute and then rinsed with deionized water and dried with nitrogen gas. The coverslip was taped to a glass slide either with an air gap or a drop of $n = 1.52$ immersion oil in the gap.

Gold nanostar sample preparation

Reference markers were created on #1.5 coverslips by shadow deposition of 10-nm Cr through an indexed TEM reference grid (Ted Pella). Anisotropic gold nanostars were synthesized by mixing 100 μL of chloroauric acid (40 mM) and 19.9 mL of 3-(N-morpholino)propanesulfonic acid (MOPS, 150 mM, Sigma-Aldrich). The solution was left in dark overnight for AuNS to grow and their final shapes to be stabilized.²³ The solution then was washed with water two times through centrifugation (8000 rpm, 8 minutes) and diluted by 200x. Diluted solution was drop cast on the coverslip for 30 s and rinsed with

deionized water and dried with nitrogen gas. The coverslip was taped to a glass slide with a drop of deionized water in the gap.

DF imaging

DF imaging was carried out on an inverted Nikon TE2000-U with an oil immersion 100× objective (variable NA = 0.7–1.3) and either an air darkfield condenser (NA = 0.8–0.95) or an oil darkfield condenser (NA = 1.2–1.5). The light source was an unfiltered, unpolarized 100 W tungsten-halogen lamp. Transmitted light was sent to a spectrometer (Acton SP2300, Princeton Instruments) with a liquid nitrogen cooled CCD (Princeton Instruments).

DIC imaging

DIC imaging was performed on an inverted Nikon TE2000-E with an oil immersion, NA = 1.4 condenser, and an oil immersion NA = 1.49, APO, TIRF, 100× objective. The light source was a 100-W unfiltered, unpolarized tungsten-halogen lamp. The light passed through a band pass filter (hard-coated OD4, center wavelength = 600, 640, 680, 700, 730, 750, 780, 830, 940 nm with 10-nm bandwidth, Edmund Optics), a dé Sénarmont compensator, Nomarski prism, condenser, sample, objective, second Nomarski prism, analyzer, and hit the detector (Andor Zyla 4.2 sCMOS). Metamorph software was used to collect the image files, and they were processed with ImageJ or custom MATLAB scripts.

SEM imaging

Scanning electron microscopy (SEM) images were obtained on gold nanoparticles-on-glass samples with a FEI Quanta environmental SEM. The low-vacuum mode (1.2 torr) enabled imaging of the insulating substrates.

Calculation of Normalized Contrast

To quantify the intensity contrast from the DIC images, we calculated the normalized contrast of the images in each rotational series. First, the particle centroid within each frame was identified and assigned (x , y) coordinates at each angle. Particle intensity (I) was averaged among all pixels within a circular region of interest defined as the particle ROI, either 10- or 15-pixel diameter depending on the size of the particle) centered on the particle coordinates. To correct for the intrinsic variable background intensity across the field of view, we defined the local background ROI as either 41×41 or 61×61 pixel square (depending on the spot size of the particle) that was centered on the particle coordinates. The local background intensity (I_{bkg}) was taken as the average pixel intensity in this background ROI excluding the particle ROI. Contrast in each frame was defined as $C = (I - I_{bkg})/I_{bkg}$, and was normalized from 0 to 1. Higher contrast indicated brighter DIC signals, and lower contrast corresponded to darker DIC signals. Normalized contrast values were fit to $\sin^2(\theta)$ function to compare trends for different wavelengths.

RESULTS AND DISCUSSION

DIC microscopy simulation scheme

Unlike phase-contrast microscopy,²⁴ a method that visualizes refractive index differences against a reference, DIC microscopy is a phase-contrast imaging technique that represents *gradients* in the optical path length (i.e. refractive index) as intensity contrast.²⁵ Nomarski prisms separate elliptically polarized light into two orthogonally polarized and spatially separated beams (labeled ordinary and extraordinary), direct them through a sample, and recombine the beams into a new elliptical polarization state. The separation distance between the two intermediate beams is referred to as the shear distance and is typically on the order of 100 nm, slightly below the resolving power of the objective lens. A linear polarizer before the image detector—the analyzer—with orientation parallel to the minor axis of the polarization ellipse, maps the final polarization state to an intensity distribution.

Figure 1a summarizes our three-stage FDTD simulation process for DIC microscope imaging of plasmonic NPs. The initial polarization state of light was determined by experimental settings of the first polarizer and the quarter-wave plate. The effect of the Nomarski prisms was represented by preparing two separate simulation environments with orthogonal polarization and the sample objects within them shifted by the experimental shear distance of 120 nm. Of the two spatially separated polarization states, the polarization that was aligned with the longitudinal axis of the nanorod experienced major phase delay. The FDTD region only exists between the condenser and objective lenses and probes the local interactions between the incident light and the NP; all other optics of the DIC microscope do not need to be represented in the FDTD simulation. To represent the optics between the sample and the CCD detector, a 2D monitor (in x - y plane and normal to z axis) was placed above the NP to record the transmitted and scattered light. The results were analyzed by a post-processing script (Supporting Information, S2) modified from a Lumerical Inc. script for Phase Contrast Microscopy.²² Once the light beams were recombined through the simulated prism and analyzer, the DIC image of the particle was created by Fourier transforming the far-field propagating light waves.

DIC FDTD simulations of single gold nanorod and gold-nanorod dimers

Figure 1b illustrates the mechanism for a gold nanorod to generate bright and dark contrast as a function of orientation. The background intensity was defined by the initial elliptical polarization state determined by the angle of the first polarizer with respect to the quarter-wave plate. Bright and dark intensities of gold nanorod images were optimized when the longitudinal axis of the particle was aligned to the polarization direction of one of the two beams. In both cases, the particle induced a phase shift in the transmitted light. The bright intensity resulted from the NP increasing the phase difference between the two light beams (ϕ) and creating a more circular polarization. In this case, the component of the ellipse aligned to the analyzer was larger than that of the background. Similarly, the dark intensity occurred when the phase difference between the two beams was reduced ($\phi = 0$, no phase difference), and the final polarization state was more linear, resulting in the component of the ellipse aligned to the analyzer to be less than that of the background. If the sample did

not induce any phase delay to either light beam, the final polarization state remained identical to the initial elliptical polarization state, and the background was gray.

With the two beams treated in separate simulations, we could examine the interaction of the gold nanorod with each beam separately, which is not possible to deconvolute experimentally. Figure 2 summarizes how the DIC phase image is represented by the phase difference between the two orthogonal beams and is calculated as $\phi \sim \phi(Ey) - \phi(Ex)$. $\phi(Ex)$ is defined as the phase difference between the extraordinary beam with respect to the background, and $\phi(Ey)$ as the phase difference between the ordinary beam with respect to the background. When the imaging DIC wavelength (λ_{DIC}) was at the λ_{LSP} of the particle, and the long axis of the particle was aligned along the extraordinary axis, the final DIC image showed bright contrast (Figure 2a). Since the particle axis only caused a phase delay in the beam aligned with particle orientation, a positive phase difference resulted when the beams were recombined. Conversely, when the particle was rotated by 90° , the ordinary beam experienced a phase delay, resulting in an opposite phase difference when the beams were recombined (Figure 2b). For gold nanorods with dimensions of 40×90 nm (typical experimental dimensions), the maximum phase difference was approximately 0.06π when aligned to one of the two beams. This value showed good agreement with the experimentally determined phase-offset between the intermediate beams of the DIC microscope and was the highest DIC contrast for this gold nanorod.

We validated the FDTD simulation results with an in-plane rotational correlation study of gold nanorods by combining scanning electron microscopy (SEM) images, DF scattering spectra, and DIC images. DIC images of a single rod were acquired over 180° in 10° increments (Figure 3a). The nanorod size and shape were measured by SEM to setup the FDTD simulations. The same series of rotational angles was simulated, and the resulting DIC images were in good agreement with experimental images. Orientation-dependent contrast of the gold nanorod was observed in both simulation and experiment, with bright and dark maxima occurring at the same orientation: aligned parallel (0°) and perpendicular (90°) to the shear axis. For intermediate angles in which the rod orientation was in between bright and dark axes, the relative intensities of the bright and dark regions decreased as expected. At angles near 40° and 130° , nearly equal intensities of bright and dark regions were observed. Additionally, rods aligned at 40° and at 130° showed DIC image patterns different from each other, with the images at 40° showing 3–4 isolated bright and dark regions with 4-fold-like geometry, and the images at 130° showing a more evenly split bright/dark pattern. At intermediate angles, both ordinary and extraordinary beams experienced phase delay, and the resulting DIC pattern was determined by the difference in phase change of the two beams. Similar to experimental measurements, our simulation set-up could distinguish the different DIC patterns at intermediate angles (40° and 130°). DIC image patterns of nanorods going through out-of-plane rotation could be generated that could be used as part of the training data to predict the 3D orientation of the particles in dynamic environments (Supporting Information, Figure S9).

In general, the simulated images are different from experimental images in two ways: (1) high-contrast rings around the central feature in the simulated images but not in experiment; and (2) larger simulated spot sizes. These discrepancies can be explained by noting that

FDTD images contain Airy diffraction patterns because the light source is a plane wave with a constant intensity over the aperture area.²⁶ In experiment, imperfect optics, an incoherent light source, and baseline noise in the detector can obscure the faint rings. Also, the different spot sizes of DIC patterns can be attributed to different numerical apertures (NA) used in simulation and experiment. Since a single plane wave was used as a light source in simulation, the effective NA was 0, while the NA of the experimental oil-immersion condenser was 1.4/1.55.

As a numerical method, our simulated DIC process allows for any arbitrary NP geometry to be tested. To examine more complex geometries than a gold nanorod, we selected a rod dimer was imaged by DIC and simulated in FDTD (Figure 3b). In this dimer, one of the particles (left) was aligned 45° counterclockwise from the ordinary axis while the other nanorod aligned 45° clockwise from the ordinary axis. With the left rod orientated the same as the single rod (Figure 3a), comparison between the two structures allowed us to determine the influence of the second rod on the overall image. FDTD images showed scattering rings with higher intensity around the dimer than with a single particle because of the combined scattering effects from each particle. In both experiment and simulation, DIC images revealed a shift and offset of approximately 40° in rotation angle corresponding to the maximum bright and dark images compared to the single rod. We attribute this shift in maximum intensity to two nanorods being closer together than the shear distance of the microscope and optically behaving as a *single* larger NP with its long axis shifted ~45° from that of a single rod. By probing the changes in DIC images with respect to the rotation angles of the particles, relative angle between the rods can be predicted by probing the angle shift and offset in maximum intensity.

Notably, our simulations make no assumptions about the interaction between incident light and the nanorod. While previous analytical simulations based on a DIC point-spread-function assumed a $\pi/2$ phase shift between the two intermediate beams (*i.e.* circular polarization) and a $\pi/6$ phase shift from the rod,¹⁴ we relied on experimental parameters to determine phase differences. The phase offset between the two intermediate beams that gave highest experimental DIC contrast was $\sim\pi/18$, consistent with the calculated phase shift induced by the NP in simulation. The nanorod-induced phase shift is a consequence of the dielectric function of gold²¹ and the dimensions of the specific particles.

Wavelength-dependent image contrast of gold nanorods

Although previous studies suggested that DIC intensity is strongest when imaged at wavelengths close to the LSP resonance of NPs,^{7, 10} heterogeneity in particle shapes and corresponding LSP wavelengths produced by solution-based synthesis techniques poses challenges in choosing a single imaging wavelength. We found that within a single field of view of the DIC microscope (250 μm^2), although particle orientations were fixed, many NPs showed inverted DIC contrast when imaged at 640 nm and at 750 nm (Supporting Information, Figure S3). We also examined the angle-dependent contrast for one selected nanorod through a systematic in-plane rotational study (Figure 4). DF spectroscopy revealed that the LSP wavelength at the maximum scattering intensity was 678 nm (Figure 4a); particle dimensions were 43 $\times$ 78 nm in SEM. The simulated and measured scattering

spectra matched well, with LSP resonances close to each other. DIC images of the nanorod were acquired for angles between 0° and 180° at 10° increments and compared with simulation results at 640 nm (Figure 4b) and 750 nm (Figure 4c). At 640 nm ($\lambda_{\text{DIC}} < \lambda_{\text{LSP}}$), for both experiment and simulation, alignment of the particle to 0° resulted in a fully bright contrast image, while 90° alignment produced a fully dark contrast image. At 750 nm ($\lambda_{\text{DIC}} > \lambda_{\text{LSP}}$), an inversion in the contrast was observed; 0° resulted in a dark-contrast image, while 90° alignment produced a fully bright contrast. At an intermediate angle of 50° , the positions of dark and bright lobes within the DIC patterns were reversed for $\lambda_{\text{DIC}} = 640$ nm and $\lambda_{\text{DIC}} = 750$ nm. At $\lambda_{\text{DIC}} = 640$ nm, dark lobes were in the lower left and upper right quadrants of the DIC pattern, and in the upper left and lower right quadrants for $\lambda_{\text{DIC}} = 750$ nm. Similarly, at 130° , both wavelengths showed a half bright image pattern where the dark lobe was on the left (right) for $\lambda_{\text{DIC}} = 640$ nm ($\lambda_{\text{DIC}} = 750$ nm). These results suggested that both rotation angle and imaging wavelength contributed to the final DIC image pattern. To quantify angle and wavelength-dependent changes in DIC patterns, we also calculated the normalized contrast values for simulated and experimental DIC images at both wavelengths (Figure 4d). Clear negative correlation between 640 nm and 750 nm images for both experiment and simulation further supports that contrast inversion occurs for all examined angles. Compared to polarization anisotropy,⁹ our metric normalized contrast calculation corrects for inherent variability in background DIC intensity by taking the intensity of the local background into account.

We examined the near-field data recorded by FDTD simulation of this nanorod to identify the origin of the contrast inversion at different wavelengths. Figure 5a shows the scattering cross section of the longitudinal mode of the rod with LSP resonance at 690 nm. The NP was illuminated with a broadband plane wave propagating in the $+z$ -direction, polarized along the x -axis. The total scattering cross section was calculated based on all the scattered light collected from the particle. Although only the x -axis polarized scattered light was collected, the normalized scattered intensities between experimental DF and this FDTD setup were comparable since scattering is dominated from the longitudinal mode. To probe the near-field profile at different wavelengths, the electric field amplitude was recorded with a 1D line monitor along the longitudinal axis of the rod from 500 nm to 900 nm (Figure 5b). With increasing wavelength, electric fields on the rod showed maximum intensity at 660/720 nm and reversed direction abruptly at the LSP. A 2D plane monitor was placed at $z = 0$ to observe 2D cross-sections and visualize the real part of the electric near field $\text{Re}(E_x)$ at these two wavelengths (Figure 5c). Comparison between $\text{Re}(E_x)$ maps at $\lambda = 660$ nm and at $\lambda = 720$ nm showed that the direction of the electric field was reversed as the imaging wavelength crossed the LSP wavelength. Surface charge distribution of the longitudinal dipole mode in the nanorod was also inverted (Supporting Information, Figure S4). When light was polarized along the y -axis, the field intensity of $\text{Re}(E_y)$ was over an order of magnitude weaker and did not show sign change (Supporting Information, Figure S5), indicating that the transverse plasmon mode did not contribute to the DIC contrast inversion.

To investigate further the relationship between imaging wavelength (λ_{DIC}) and LSP wavelength (λ_{LSP}), we examined three additional nanorods that have different LSP resonance wavelengths. These three rods were selected because they represent three possible relationships between λ_{LSP} and λ_{DIC} with the two imaging wavelengths ($\lambda_{\text{DIC-1}} = 640$ nm;

$\lambda_{\text{DIC-2}} = 750 \text{ nm}$) (Table 1). As the ensemble LSP resonance of the nanorod solution was 700 nm, two imaging wavelengths were chosen to probe the DIC response at both the longer and shorter wavelengths than the rod LSP. In-plane rotational correlation study of gold nanorods was carried out by combining their DF spectra, SEM images and DIC images (Figure 6). Nanorod **I** showed weak DIC intensity at $\lambda_{\text{DIC}} = 750 \text{ nm}$, and nanorod **III** showed weak DIC intensities at $\lambda_{\text{DIC}} = 640 \text{ nm}$, suggesting that DIC intensity decreases at λ_{DIC} far from λ_{LSP} . Our DIC rotational study at representative angles (Figure 6c; **angles from 0°–180° in Supporting information, Figure S6-S8**) confirmed that there was no contrast inversion for nanorod **I** and nanorod **III**, and that there was a clear contrast inversion for nanorod **II** at 0° and 90°. Regardless of nanorod size, when the long axis of the rod was aligned to the shear axis of the Nomarski prism, $\lambda_{\text{LSP}} < \lambda_{\text{DIC}}$ resulted in a fully dark DIC image, while $\lambda_{\text{LSP}} > \lambda_{\text{DIC}}$ produced a fully bright DIC image. This phenomenon suggests that DIC contrast inversion is only present when λ_{DIC} is tuned across the λ_{LSP} .

We further examined DIC contrast for gold nanostars to demonstrate that our contrast inversion principles applied to NPs with 3D anisotropy (Figure 7). Gold nanostars were synthesized by a seedless mechanism using Good's buffer with a morpholine ring (MOPS) and exhibited multiple LSP resonances in the near-infrared (NIR) and second NIR window.²³ The specific nanostar of interest had four different branches protruding from the core (Figure 7a, inset) and showed two LSP resonance peaks: $\lambda_{\text{LSP-1}} = 760 \text{ nm}$ and $\lambda_{\text{LSP-2}} = 870 \text{ nm}$.

We conducted a similar DIC rotational study at four different wavelengths ($\lambda_{\text{DIC-1}} = 730 \text{ nm}$; $\lambda_{\text{DIC-2}} = 780 \text{ nm}$, $\lambda_{\text{DIC-3}} = 830 \text{ nm}$; $\lambda_{\text{DIC-4}} = 940 \text{ nm}$) so that $\lambda_{\text{DIC-1}} < \lambda_{\text{LSP-1}} < \lambda_{\text{DIC-2}}$ and $\lambda_{\text{DIC-3}} < \lambda_{\text{LSP-2}} < \lambda_{\text{DIC-4}}$. As expected from diffraction effects, the spot sizes of the DIC images increased with increasing imaging wavelength. Consistent with the rods, contrast inversion was observed as the DIC image was fully bright at 0° for $\lambda_{\text{DIC-1}}$ and fully dark for $\lambda_{\text{DIC-2}}$. DIC patterns at $\lambda_{\text{DIC-3}}$ were similar to those of $\lambda_{\text{DIC-2}}$ as λ_{DIC} did not cross the λ_{LSP} peak, and only a minor difference in phase delay was observed. Another DIC contrast inversion was observed from $\lambda_{\text{DIC-3}}$ to $\lambda_{\text{DIC-4}}$, as the DIC image pattern changed from dark back to bright at 0°. Simulated scattering spectra and DIC images of the nanostar agreed with experimental results (Supporting Information, Figure S10). Hence, wavelength-dependent DIC contrast inversion can be observed in plasmonic particles with more complex geometries.

CONCLUSION

We developed an FDTD simulation set up for DIC imaging of anisotropic gold NPs. The NPs showed DIC contrast inversion when the imaging wavelength was tuned across the LSP resonance. By calculating the local near-field distribution, we discovered that this contrast inversion occurred because the electric field reversed sign after this wavelength change. The simulation we developed can be extended to predict DIC images of NPs of arbitrary shape, composition and dielectric environment, which holds promise for work related to probing single-particle motion in solution or cellular environments. We anticipate that image patterns generated by our calculations can be used as training data for machine learning algorithms to predict particle shape and orientation in dynamic systems.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. FDTD Simulation of DIC microscopy process with a gold nanorod probe.

(a) Side-view of the optical path for a DIC microscope. There are three components to the FDTD-DIC simulation environment: Setup, FDTD simulation, and Processing. Each section produces optical polarization states corresponding to specific components of the DIC microscope. (b) Top-down views of polarization states at different locations in the microscope. The phase shift in the intermediate beams induced by the gold nanorod causes the bright and dark contrast depending on particle orientation.

Figure 2. Deconvolution of final DIC phase image into two orthogonal beams of Nomarski prism. Images displaying the phase difference between the two beams induced by (a) nanorod that produces bright contrast or (b) nanorod that produces dark contrast. The dimensions of the rod are 40 nm (width) and 90 nm (length). The images on the right show phase difference of each beam with respect to the background. 0 on the color scale bar refers to no phase delay and represents the background.

Figure 3. Rotational study of gold nanorods.

DIC images were measured and simulated for **(a)** a single nanorod and **(b)** a V-shaped dimer with rotation angles from $\theta = 0^\circ$ to $\theta = 180^\circ$ in 10° increments. Dimensions of each rod were 40 nm (width) and 103 nm (length). The DIC images were correlated with SEM images and the SEM images shown represent the particle orientation for $\theta = 0^\circ$. Measurement and simulations were done at $\lambda_{\text{DIC}} = 700$ nm.

Figure 4. Wavelength dependence of DIC images for a single gold nanorod.

(a) SEM and scattering spectrum for a single gold rod. Solid line represents experimental spectrum, dotted line represents simulated spectrum. (b) Comparison of experimental and simulated DIC images at $\lambda_{\text{DIC}} = 640 \text{ nm}$ (c) Comparison of experimental and simulated DIC images at $\lambda_{\text{DIC}} = 750 \text{ nm}$. (d) Normalized contrast calculated from the DIC images showing the contrast inversion between the two wavelengths. Solid lines with markers are from experimental data; dotted lines are calculated from simulated DIC images.

Figure 5. FDTD near-field analysis of DIC contrast inversion.

(a) Scattering simulation for the longitudinal plasmon mode of the gold nanorod in Figure 4 with a scheme of the 1D (dotted line) and 2D (blue plane) monitor locations (inset). (b) E-field amplitude (1D monitor) as a function of wavelength for the rod. Minimum and maximum values for E_x are labeled with square (660 nm) and circle (720 nm) respectively. (c) 2D cross-sections of E-field amplitude on the blue (square, $\lambda_{\text{DIC}} = 660 \text{ nm}$) and red (circle, $\lambda_{\text{DIC}} = 720 \text{ nm}$) sides of the LSP wavelength.

Figure 6. DIC response of gold nanorods with three different resonance locations.

(a) The measured scattering spectra (normalized) of individual rods. (b) SEM images with long axis of rods aligned to $\theta = 0^\circ$. (c) DIC image sets for $\lambda_{\text{DIC}} = 640 \text{ nm}$ (blue outline) and $\lambda_{\text{DIC}} = 750 \text{ nm}$ (red outline) for $\theta = 0^\circ$ to 150° .

Figure 7. DIC response of a single gold nanostar at four different imaging wavelengths. (a) Measured scattering spectrum (normalized) of a single gold star. LSP resonances were around 760 nm and 870 nm. Inset shows SEM image of the particle. (b) DIC images of gold nanostar at $\lambda_{\text{DIC}} = 730 \text{ nm}$, 780 nm , 830 nm , and 940 nm . Blue outline corresponds to wavelengths less than LSP resonances, and red outline corresponds to wavelengths longer than LSP resonances.

Table 1.Relationship Between λ_{LSP} and λ_{DIC} of Three Different Nanorods

	λ_{LSP}	$\lambda_{\text{DIC-1}} = 640 \text{ nm}$	$\lambda_{\text{DIC-2}} = 750 \text{ nm}$
Nanorod I	609 nm	$\lambda_{\text{LSP}} < \lambda_{\text{DIC-1}}$	$\lambda_{\text{LSP}} < \lambda_{\text{DIC-2}}$
Nanorod II	694 nm	$\lambda_{\text{LS}} > \lambda_{\text{DIC-1}}$	$\lambda_{\text{LSP}} < \lambda_{\text{DIC-2}}$
Nanorod III	798 nm	$\lambda_{\text{LSP}} > \lambda_{\text{DIC-1}}$	$\lambda_{\text{LSP}} > \lambda_{\text{DIC-2}}$