Physical insight into light scattering by photoreceptor cell nuclei

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A recent study showed that the rod photoreceptor cell nuclei in the retina of nocturnal and diurnal mammals differ considerably in architecture: the location of euchromatin and heterochromatin in the nucleus is interchanged. This inversion has significant implications for the refractive index distribution and the light scattering properties of the nucleus. Here, we extend previous two-dimensional analysis to three dimensions (3D) by using both a numerical finite-difference time-domain and an analytic Mie theory approach. We find that the specific arrangement of the chromatin phases in the nuclear core-shell models employed have little impact on the far-field scattering cross section. However, scattering in the near field, which is the relevant regime inside the retina, shows a significant difference between the two architectures. The "inverted" photoreceptor cell nuclei of nocturnal mammals act as collection lenses, with the lensing effect being much more pronounced in 3D than in two dimensions. This lensing helps to deliver light efficiently to the light-sensing outer segments of the rod photoreceptor cells and thereby improve night vision.
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The vertebrate retina is an optics puzzle: for light to reach the sensory portions of the photoreceptor cells (PRCs), it has to traverse the entire thickness of the retina [1].

While light propagation through a considerable part of the retina is facilitated by the light-guiding Müller cells [2], potential for scattering arises in the outer nuclear layer. The outer nuclear layer contains the nuclei of the PRCs and is located in the optical path before the light sensitive outer segments. The latter have been described extensively in the literature for their optical properties (c.f. [3]), while the nuclei have only recently been discovered to serve an optical function [1]. A recent study showed that the rod PRC nuclei in nocturnal mammals possess a unique "inverted" structure [1]. Transcriptionally active euchromatin is situated at the periphery of the nucleus, while inactive and denser heterochromatin is located at its center. This highly unusual nuclear architecture is not seen in other vertebrate cells [4]. Most cells possess a "conventional" architecture in which euchromatin is situated at the center of the nucleus. While the exact reason for this predominance is unclear, it seems to present advantages related to gene expression regulation [5], which is supported by the fact that the conventional architecture has been largely conserved throughout evolution. Therefore, the discovery that nocturnal mammals had clearly diverted from the conventional nuclear architecture was all the more surprising.

Given the low-light conditions in which nocturnal mammals thrive, this nuclear inversion may have its genesis in optics. Quantitative phase microscopy indicates that inverted (nocturnal) PRC nuclei have the maximum refractive index in the middle of the nucleus, while their conventional (diurnal) counterparts have the maximum at the periphery [1]. This is consistent with the assumption that condensed heterochromatin has a higher refractive index than less dense euchromatin. Assuming a core-shell model for the nucleus, the authors of the aforementioned study used a finite-difference time-domain (FDTD) technique [1,6] in two dimensions (2D) to show that the inverted nuclear architecture results in a lensing effect not present with the conventional nuclear architecture. This focusing effect is believed to optimize light transmission through the outer nuclear layer of the retina.

The purpose of the present study is to extend the results of the previous work on the nuclear inversion into three dimensions (3D) while providing further physical insight into the optical implications of the inverted architecture of the PRC nucleus. We first use a far-field Mie theory approach to compare both the total scattering cross sections over the entire visible spectrum and the angular scattering distribution at 500 nm. Second, we show that our Mie code can be extended to efficiently calculate near-field distributions. This region is of particular interest as the dense packing of cells and their nuclei in the retina leads to multiple scattering events preceding extension into the far field. We use an FDTD code to validate these results and to allow for comparison with previously employed two-dimensional models.

For Mie theory calculations in the far and near fields, the electromagnetic fields are expanded in terms of the standard vector spherical harmonics, $\vec{M}$ and $\vec{N}$, following Bohren and Huffman [7]. The coefficients for the incident and scattered fields in these expansions are obtained by adapting the code for light scattering from a coated sphere in Appendix X therein. The methods used in this study are applicable for arbitrary incident illumination.

To simulate illumination with a Gaussian beam, we use the electromagnetic field components given by Barton [8]. They are based on a power-series expansion in the (small) parameter, $s = 1/(kw_0)$, where k is the wave vector and w_0 is the beam waist. The radial fields, E_r and H_r, serve as a basis for an expansion in terms of vector spherical harmonics:

$$E_r(r,\theta,\varphi) = \sum_{n=1}^{\infty} \sum_{m=-n}^{m=n+1} P_{mn} N_{r,mn}(r,\theta,\varphi). \qquad (1)$$

If the particle lies on the optical axis, the expansion coefficients P_{mn} of the incident fields can be determined

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using the orthogonality of the vector spherical harmonics. The procedure used to achieve this is similar to that suggested by Gouesbet *et al.* to find the g_n-coefficients for a Gaussian beam in the framework of generalized Mie theory [9]. However, we use a formulation in terms of vector spherical harmonics rather than scalar potentials.

FDTD simulations were carried out on a message passing interface (MPI)-coordinated computer cluster using libraries from the open-source FDTD package Meep [10]. Computational cells were implemented with periodic boundary conditions in lateral dimensions and limiting perfectly matched layers [11] in the longitudinal direction to allow for plane wave illumination. A single wavelength was supported by at least 20 points on a subpixel smoothing Yee lattice.

For both Mie theory and FDTD simulations, nuclei were approximated as coated, dielectric spheres with 4.0 and 5.0 μm core and shell diameter and relative refractive indices of $m = 1.02$ and $m = 1.04$ for the euchromatin and heterochromatin phases, respectively. Diameters and refractive index values are consistent with measurements previously published [1]. Simulations with averaged polarization were run at various wavelengths with particular emphasis on 500 nm, as this corresponds to the wavelength of highest rod sensitivity [12].

We find that scattering cross sections for the conventional and inverted architectures do not differ substantially throughout the visible spectrum. Reduced perturbations of plane waves by inverted nuclei are only observed for short wavelengths [Figs. 1(a)]. Also, the inversion of the nuclear architecture manifests a suppression of high spatial frequencies only at larger angles, which constitute a negligible contribution to the total scattering [Fig. 1(b) and 1(c)]. As a comparison, scattering amplitudes of spheres with a uniform refractive index contrast ($m = 1.03$) lie mostly between those of the two core-shell models. These results change only insignificantly when broadening the incident angular spectrum by choosing a Gaussian beam illumination (beam waist $w_0 = 2.5$ μm) that is symmetrically incident on the nucleus [Fig. 1(d)]. The results reveal that the presence of a (higher refractive index) heterochromatin phase in the center of the nucleus only moderately suppresses the generation of higher angular frequencies in the far field.

While the inversion of nuclear models has little impact on scattering intensities in the far field, near-field distributions—which are physiologically more relevant—can still be drastically different. This can be understood intuitively by noticing that, within the limit of ray optics, collimated light scattered by lenses with focal lengths f and $-f$ results in the same far-field intensity distributions while showing substantial differences in the proximity of the lenses. To obtain intensity distributions immediately behind the nuclei from Mie theory, we superimpose the incident plane waves on top of the scattered fields. In the near field, the scattering patterns of the conventional and inverted nuclei differ significantly [Figs. 2(a) and 2(b)]. While one observes a diffraction pattern characterized by pronounced spatial oscillations behind the nucleus of the conventional type, the same incident plane wave is strongly focused after passage through an inverted nucleus. These results were confirmed using FDTD [Figs. 2(c) and 2(d)]. Moreover, the focusing effect is robust against changing from plane wave to Gaussian illumination (data not shown).

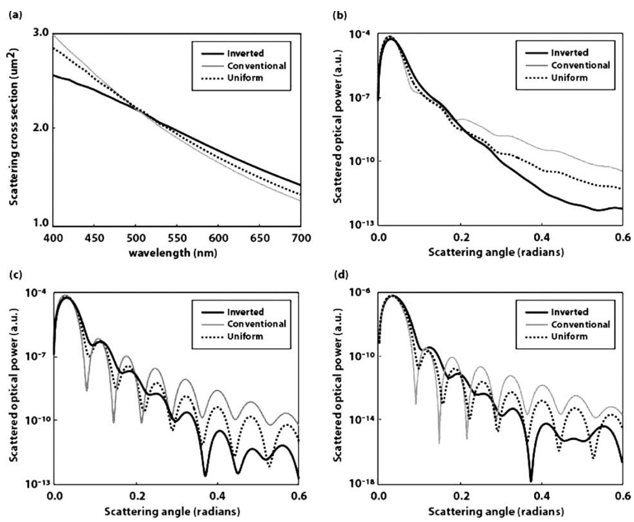

Fig. 1. Far-field scattering properties of photoreceptor cell nuclei obtained with the coated-sphere Mie theory model. (a) Scattering cross sections of conventional and inverted nuclei (and a homogeneous sphere for comparison) as a function of wavelength for plane wave incidence. (b) Scattered power (i.e., intensity multiplied with the area element of the scattering arc) averaged over the visible spectrum (400 to 700 nm) for illumination with a plane wave. (c) Scattered power for incidence of a single plane wave ($\lambda = 500$ nm) and (d) for a symmetrically incident Gaussian beam ($\lambda = 500$ nm, $w_0 = 2.5$ μm).

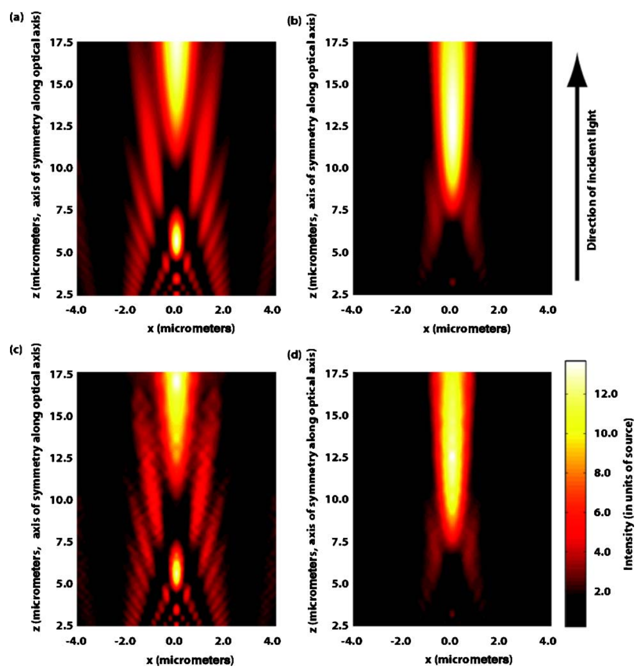

Fig. 2. (Color online) Scattering of an incident plane wave by conventional and inverted nuclei in the optical near field. Nuclei (not shown; for parameters see text) are centered on the coordinate origin. Light propagates along the positive z axis (see arrow). Mie theory results for the scattering from the (a) conventional (b) and inverted nuclear architecture. FDTD simulations for the scattering from a conventional (c) and (d) inverted nucleus.

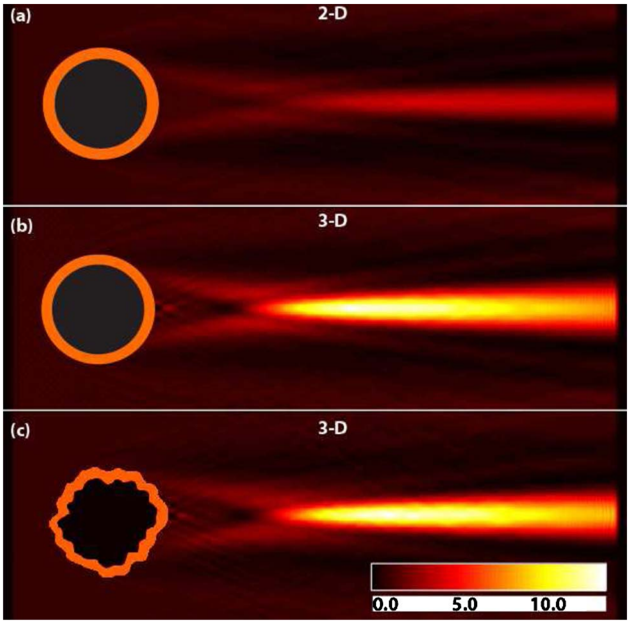

Fig. 3. (Color online) Comparison of the FDTD results for the near-field scattering intensities in 2D and 3D for incidence of a plane wave ($\lambda = 500$ nm; propagation from left to right) on a nucleus of inverted architecture with 5 μm outer diameter. The focusing effect in (b) 3D is much more pronounced than in (a) 2D and is also insensitive to relatively large deviations from (c) a spherical shape (cross section through the center of the nucleus is shown on the left). The two-dimensional results were generated using the code from [1].

Comparing these findings to the previous study identifies the focusing effect, which was shown to lead to an effective channelling of light through the outer nuclear layer in 2D, to be at least three times as strong in 3D [Fig. 3(a) and 3(b)]. This is not surprising because, for problems involving only thin optical elements, light propagation is separable by dimensions, i.e., a phase shift acquired in one dimension does not affect spatial modulations in another [13]. Consequently, the focusing by a lens in 3D must be quadratically stronger than in 2D. This increased focusing effect is still prominent if the shape of the nucleus deviates from a perfectly spherical geometry [Fig. 3(c)].

We conclude that, within our models, the induction of higher-order angular frequencies is not significantly suppressed by the inversion of the nuclei. However, we can confirm the focusing of light by inverted nuclei previously reported in 2D with even greater strength in 3D. It will be the objective of further studies to investigate the channelling of light through three-dimensional stacks of these nuclear microlenses forming the outer nuclear layer. We believe that, considering the spatial dimensions of the problem, an extension of the presented Mie theory model to match boundary conditions between multiple scatterers is superior to the more commonly employed FDTD simulations. This technique will also enable us to account for heterochromatic chromocenters as potential scatterers that are found in some conventional nuclei. The results presented in this paper further support the surprising fact that, as a response to evolutionary pressures, the packaging of DNA—heavily implicated in gene expression and biological function—is subverted to confer an optical advantage to nocturnal mammals.

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