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SPECTRAL SIMULATION APPROACH OF LIGHT SCATTERING BY BIOLOGICAL MICROSTRUCTURES

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ABSTRACT. A light scattering simulation method for characterization of spectrally resolved microscopic images is presented. Based on the discrete dipole approximation, this method can be applied to analyze complex biological microstructures. In this contribution, spectral scattering features are compared to Fraunhofer diffraction for cuboidal models.

1. Introduction

Scattering light microscopy is a growing field in biomedical optics to investigate characteristic properties of tissue and its microstructures. In a recent publication [1] it was shown that by statistical means of multivariate data analysis scattered light patterns could be correctly assigned to data like scatterer size and local arrangement. This approach could be applicable for classification and process control purposes. However, being primarily based on hyperspectral microscopic measurements, a simulation technique regarding approximate models of biological samples would help to improve quantitative predictions of the scattering results.

In this contribution multispectral simulation results are presented for flat cuboidal sample models. The simulations were performed using the ADDA code [2] which is based on the discrete dipole approximation (DDA) [3]. This numerical method is also suitable to investigate rectangular scatterer geometries. Only simple model geometries as well as assumptions for the refractive index are taken into account here because, in general, no exact parameters can be specified for biological scatterers, and high deviations in preparation are a common drawback. The scattering of monochromatic light, incident at defined angles onto the cuboidal specimen, is calculated for multiple wavelengths. Thus, the total scattering cross section as well as the scattered intensity, integrated over a variable angular range according to the microscope aperture, was calculated and compared to Fraunhofer diffraction [4].

2. Method

In Fig.1 a scheme of the scattering scene is depicted. Dark field illumination light enters the scatterer, which is lying on a substrate, from below in a fixed incident angle α. This

incident light is also limited to a single azimuthal angle which avoids averaging out features of the scattering function. Backscattered light is integrated over an angular range β corresponding to the numerical aperture of the microscope objective. As mentioned above, the scatterer is modeled for simplification as a cuboid in oblique orientation with respect to the incident direction. The conical detection range of the backscattered light is oriented perpendicular to the substrate, i. e. the flat side of the cuboid. We note that the presence of the substrate is neglected here but may be included in next steps using extended DDA codes, e. g. DDA-SI [5]. The DDA simulations have been performed sequentially for each

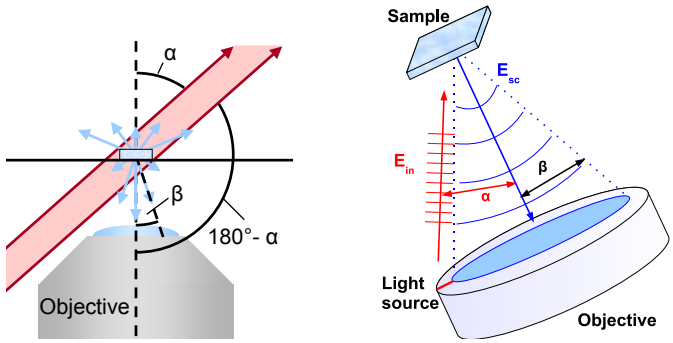

Figure 1. *Left:* Scheme of a scattering sample on the dark field objective. *Right:* Orientation angles of the cuboidal sample.

wavelength. The dipole spacing of the DDA grid was kept constant to avoid step-shaped intensity artefacts due to discretization resolution shifts. This was done by adjusting the 'dipoles per wavelength' resolution where strict convergence criteria were fulfilled even for the smallest wavelength of the range. Since spectral DDA simulations take a lot of computing power and time we tried to compare the scattered intensity distribution, in compliance with Babinet's principle, to Fraunhofer diffraction patterns of an equivalent rectangular aperture, oriented perpendicular or tilted to the incident light direction, to see if accuracy in the diffraction approximation would be sufficient.

3. Results

The size of the cuboids was set to $1500 \times 5000 \times 150$ nm^3 which corresponds to the extent of a small chromosome, its mean refractive index was assumed as $n_s = 1.45$ without absorption. As outer medium vacuum and also microscope immersion oil ($n_m = 1.5$) were chosen. The incident light covering a wavelength range of 400 nm to 700 nm is oriented at tilt angles up to $\alpha = 65$ degrees. In Fig.2 scattering patterns for $\alpha = 25$ degrees and for wavelength $\lambda = 400$ nm are compared to corresponding results of equivalent Fraunhofer diffraction in forward and backward scattering hemispheres, respectively. One can see a similar pattern structure in forward direction, whereas in the backscattering region the diffraction approximation will fail. The deviation increases with the tilt angle of the sample as expected due to its finite extent in incident direction. In addition, it is important to know how many features of the measured intensity are to expect in forward (θ = 0 – 90 degrees)

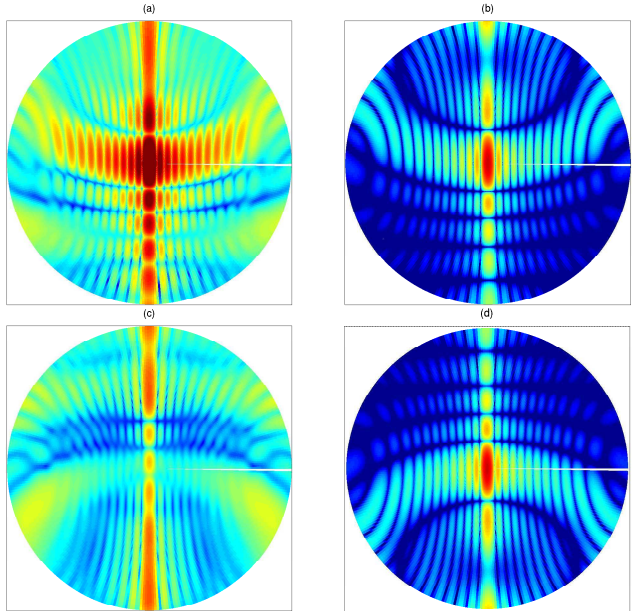

Figure 2. *Upper row (a) and (b):* Forward scattering pattern (DDA) of a cuboid, equivalent diffraction pattern of a rectangular aperture. *Lower row (c) and (d):* Backward scattering pattern (DDA) of a cuboid, equivalent diffraction pattern of a rectangular aperture. Wavelength of incident light $\lambda = 400$ nm, refractive index $n_s = 1.45$ in vacuo, tilt angle $\alpha = 25$ degrees.

and backward (θ = 90 – 180 degrees) scattering directions. We calculated the differential scattering cross section, integrated over the forward and backward hemisphere with respect to the incident direction, against wavelength, see Fig.3 (left). Comparison of the curves shows that oscillations primarily occur in the backward hemisphere, even if the relative refractive index is lowered by immersion oil. A similar integration of diffraction patterns exhibits almost no features (data not shown). Likewise, for each wavelength, the intensity scattered to the angular range of the microscope aperture, see Fig.1, was calculated in forward and backward scattering direction in relation to the substrate. Fig.3 (right) shows the spectral plot normalized to the first wavelength $\lambda = 400$ nm.

4. Conclusion

The influence of the finite thickness of the scatterer as well as high tilt angles of the sample with respect to the direction of incidence require application of precise solutions like DDA for the scattering problem, especially when integrating the scattered light over an angular range. Significant spectral oscillations of the integrated intensity only occur in case of a three-dimensional consideration. Backscattered light contains more spectral features than forward scattered light which is in particular important for evaluation of small subcellular structures.

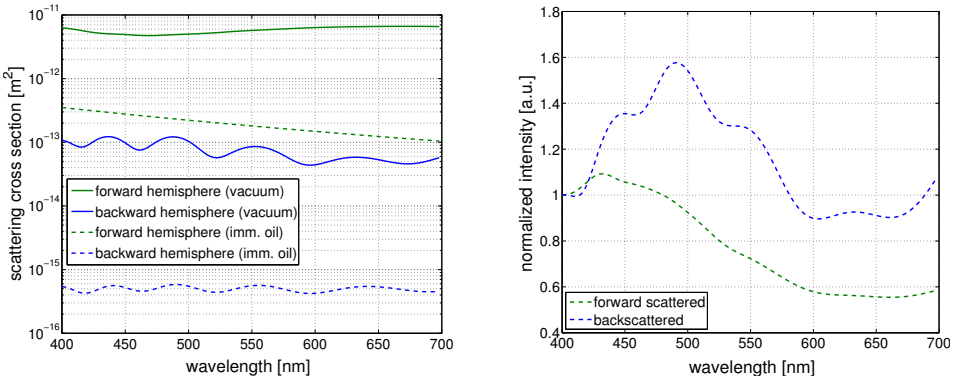

Figure 3. *Left:* Forward and backward hemisphere scattering cross sections of a tilted cuboid ($n_s = 1.45$, tilt angle $\alpha = 65$ degrees) in vacuo and embedded in immersion oil. *Right:* Integrated intensity, scattered in forward and backward direction into the microscope objective (aperture angle $\beta = 48$ degrees in vacuo).

Acknowledgments

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