Spectral encoding of spatial frequency approach for characterization of nanoscale structures

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An approach to acquire axial structural information at nanoscale is demonstrated. It is based on *spectral encoding of spatial frequency* principle to reconstruct the structural information about the axial profile of the three-dimensional (3D) spatial frequency for each image point. This approach overcomes the fundamental limitations of current optical techniques and provides nanoscale accuracy and sensitivity in characterizing axial structures. Numerical simulation and experimental results are presented. © 2012 American Institute of Physics. [<http://dx.doi.org/10.1063/1.4737209>]

Accurate characterization of the sub-surface internal structure of label-free objects, such as biological cells and nanofabricated materials, in their natural environments, with nanoscale accuracy and sensitivity is of great importance in many applications, such as biomedical science, material science, and nanotechnology. The lateral spatial frequency distribution, which characterizes the lateral structures, can be detected from two-dimensional (2D) image with high sensitivity (<10 nm for periodic structures¹), but characterization of nanoscale axial structures remains a significant challenge. Various approaches have been developed to probe 3D structures. Light-scattering spectroscopy (LSS) utilizes spectral or angular dependence of elastic scattering and Mie theory inverse procedure to reconstruct the size of quasi-spherical particles^{2–6} with an accuracy of about 25–100 nm. Wolf developed a physical model within first Born approximation to reconstruct 3D structure based on collection of all accessible spatial frequencies and performing 3D inverse Fourier transform.^{7,8} Different experimental implementations to collect spatial frequencies have been proposed, including changing illumination and collection angles or wavelengths,^{9–13} with the best theoretical resolution of approximately 0.35 of the wavelength. Despite these advances, current techniques are largely hampered by limited accuracy and sensitivity in quantifying 3D nanoscale structures in the axial direction, over-simplified model-based structural characterization and complex instrumentation.

Another modality, based on encoding each spatial frequency component of an object with a corresponding wavelength, has been proposed for real-time super-resolution imaging.^{14,15} In this letter, we present the ability of spectral encoding of spatial frequency (SESF) approach to characterize axial structures of complex 3D objects with nanoscale accuracy and sensitivity. We validate its nanoscale accuracy and sensitivity by numerical simulation, and demonstrate its feasibility to characterize nano-structures by experiments using model systems and biological cells.

An object's structure can generally be described using 3D scattering potential, or its Fourier transform. When a plane wave with a certain spectral bandwidth illuminates the

object at a fixed illumination angle θ (Figs. 1(a) and 1(b)), the accessible Fourier components of the 3D scattering potential (spatial frequencies) in $\mathbf{K}$ -space, under the first Born approximation, can be represented as multiple Ewald's spheres with different radii that depend on wavelength (Fig. 1(a)).⁸ Every point on the Ewald's sphere corresponds to a certain 3D spatial frequency vector (scattering vector),

$$\mathbf{K} = 2\pi(v_x\mathbf{i} + v_y\mathbf{j} + v_z\mathbf{k}), \quad |\mathbf{K}| = \{2ncos[(\theta + a)/2]\}/\lambda, \quad (1)$$

FIG. 1. (a) Illustration of the scattering vector. (b) Spatial frequency representation in $\mathbf{K}$ -space with different wavelengths (from 400 nm, blue color, to 700 nm, red color). (c) and (d) Collection of spectral data in Fourier and image planes. (e) Intensity distribution in 3D $\mathbf{K}$ -space. (f) Spatial frequency vector in image space. (g) and (h) Dependences of (g) absolute value of the 3D scattering vector (dashed lines), axial spatial frequency (solid lines), and (h) maximum uncertainty in determining axial period, on the scattering angles for different wavelengths, at $\theta = 0$.

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whose components can be derived from the following equations:

$$v_x = n(\sin\alpha - \sin\theta)\cos\phi/\lambda, \quad v_y = n(\sin\alpha - \sin\theta)\sin\phi/\lambda, \quad (2)$$

$$v_z = n(\cos\theta + \cos\alpha)/\lambda, \quad (3)$$

where n is refractive index, α is scattering angle (Figs. 1(a) and 1(b)), ϕ is azimuthal angle (Figs. 1(c)–1(f)), and λ is the wavelength. One can reconstruct the 3D scattering potential by 3D inverse Fourier transform of all measured Fourier components in $\mathbf{K}$ -space where integration extends over the accessible range, limited by the numerical aperture (NA) of optical system.⁸ But the structural sensitivity and accuracy are in the diffraction-limited range due to the limited accessible bandwidth of Fourier components.

To overcome the limited accuracy in reconstructing the 3D scattering potential,⁹ our SESF approach directly accesses the 3D spatial frequencies to quantify the 3D structures at each image point. In an optical system, an object's accessible 3D spatial frequencies at a given wavelength, corresponding to the cap of the Ewald's sphere, are projected onto the Fourier plane as 2D spatial-frequency distribution.¹³ This distribution together with wavelengths at each point on the Fourier plane provides information about the 3D spatial frequency vector (Figs. 1(c) and 1(e)). By mapping corresponding 3D spatial frequency to each image location (Figs. 1(d) and 1(f)), it is possible to characterize the entire 3D structure for each specific location within the object.

This concept can be implemented with a simple optical setup, which includes SESF by spatial-spectral filtering (collimated broadband illumination where each wavelength corresponds to a single spatial frequency, selection of the required spatial-spectral frequency range and suppressing zero-order signal), and inverse Fourier transform of the selected spectrally encoded 3D Fourier components via a lens. It is noted that in reflection configuration, the scattering vector $\mathbf{K}$ is only weakly dependent on the backscattering angles within a limited angular range ($\text{NA} \leq 0.5$). At normal illumination, the direction of the scattering vector remains constant for each wavelength at a given point in the Fourier plane. For each wavelength, the magnitude of the scattering vector is close to its axial projection, or its axial spatial frequency v_z (or period $H_z = 1/v_z$) (Fig. 1(g)). We can assume that all scattered light within a limited backscattering angle at a given wavelength describe one particular axial spatial frequency with a small uncertainty. For a moderate NA (e.g., $\text{NA} = 0.5$), the axial spatial frequency of a 3D structure for each image point can be accurately accessed directly at the image plane (Figs. 1(d) and 1(f)) simply by measuring the wavelength distribution at each pixel using a spectral device (Fig. 1(d)) and Eq. (3), without locating its corresponding scattering angle in the Fourier plane. Thus the *axial* Fourier components of the 3D scattering potential can be reconstructed for each image point (Fig. 1(f)).

The SESF approach provides nanoscale accuracy—the closeness of the measured spatial period to the true value—in determining the axial spatial period for each image point. The maximum uncertainty for the determination of axial spatial periods can be estimated using the expression: $\Delta H_z = \lambda(1 - \cos\alpha)/n(1 + \cos\theta)(\cos\theta + \cos\alpha)$. As shown in

Fig. 1(h), at $\text{NA} = 0.5$ (lateral resolution is $0.7 \mu\text{m}$), the maximum uncertainty is 20 nm with the corresponding maximum possible error of $\pm 10 \text{ nm}$ for $\theta = 0^\circ$, $\alpha = 20^\circ$. A smaller NA results in a reduced error.

This approach also provides nanoscale structural sensitivity, defined as follows: An object with axial spatial period H_z undergoes a *change* in the axial spatial period from H_{zA} to H_{zB} due to external perturbation. The smallest change in its axial spatial period δH_z ($\delta H_z = |H_{zA} - H_{zB}|$) that the SESF approach can detect is defined as the sensitivity.¹⁶ The theoretical sensitivity along the axial direction (δH_z) can be estimated using the expression: $\delta H_z = \Delta\lambda/n(\cos\theta + \cos\alpha)$, where $\Delta\lambda$ is the spectral resolution. Examples of sensitivity for different spectral resolutions are presented in Table I. If the wavelength is measured with sub-nanometer accuracy, the potential theoretical sensitivity of SESF approach could reach sub-nanometer scale. Such high accuracy and sensitivity to axial structure can be achieved even with low-NA optics and simple optical instrument, without stringent requirements imposed by other optical instruments such as interferometry.

To validate the nanoscale accuracy of SESF approach, we first numerically construct a 3D object composed of three known mixed-sized nanosphere aggregates. Each nanosphere aggregate unit consists of $10 \times 10 \times 10$ nanospheres with diameter d , where d is 240 nm, 200 nm, and 150 nm, respectively, and refractive index is 1.59 (Fig. 2(a)). To ensure a closer resemblance to experimental configuration, the intra-layers are shifted relative to each other such that nanospheres at a given layer lie in the 2D troughs of the layer below (see insert in Fig. 2(a)). This arrangement is the most natural and stable (lowest potential energy) representation. The periodicity of each single-sized nanosphere aggregate is described by $[d\sin(\pi/3) + 10] \text{ nm}$, corresponding to the axial spatial period of 218 nm, 183 nm, and 140 nm, respectively.

The numerically constructed 3D object (Fig. 2(a)) is illuminated by a plane wave with spectral range of 400–700 nm at spectral resolution of 10 nm. The backscattered waves for each wavelength, within the NA-restricted ($\text{NA} = 0.5$) Ewald's sphere caps, are generated using Born approximation.⁸ The spectral image cube, stacked together as 2D spectral slices for the entire spectral range, is formed after SESF and projection onto the image plane. The spectral data are converted into the axial spatial periods using Eq. (3). The SESF image (RGB image) with axial period profiles for different locations is presented in Fig. 2(b). The axial spatial period profile averaged over the entire image area is presented in Fig. 2(c). The three peaks, corresponding to spatial periods of three nanosphere aggregate units, are clearly present for each of the four selected locations within SESF image (Fig. 2(b)) and in the averaged profile (Fig. 2(c)). The three spatial periods of nanosphere aggregates (219 nm, 183 nm, and 149 nm) in the average profile are

TABLE I. Examples of sensitivity for different spectral resolutions at $\theta = 0^\circ$, $\alpha = 20^\circ$, $n = 1$.

$\Delta\lambda$, nm	10	2	1	0.1	0.05
δH_z , nm	5.16	1.03	0.52	0.05	0.026

FIG. 2. Numerical simulation of SESF approach: (a) structure of the simulated 3D object, illuminated along Oz axis; the insert is a magnified portion of the object. (b) The SESF image (RGB image) of the simulated object, with profiles of the axial spatial periods for four selected locations. (c) Profile of the axial spatial period averaged over the entire image area. The peak location is identified by locating the zero crossings of the derivative of this profile corresponding to the top three maximum peak strengths. (d) Profiles of the axial spatial period averaged over the entire image area for 250 nm (red) and 251 nm (blue) nanospheres.

within the theoretical error of ± 10 nm, when compared to the actual values of the numerical model.

Next, to illustrate the theoretical nanoscale sensitivity of SESF approach, we simulate the SESF formation of one nanosphere aggregate undergoing a change from a diameter of 250 nm to 251 nm (axial spatial period changes from 226 nm to 227 nm), at spectral resolution of 2 nm. The corresponding change in the axial spatial period profile is clearly shown in Fig. 2(d). The difference in the peak locations between these two nano-structures is 1 nm, in agreement with the theoretical prediction of nanoscale sensitivity in Table I.

Further, we conducted an experiment to demonstrate the basic feasibility of SESF approach to characterize the structures of an object with nanoscale spatial periods. In our experiments, we used a reflection configuration at normal illumination. Collimated white-light wave (Xenon 150 W, Oriel), collected by the objective lens with $NA = 0.5$, was filtered in the Fourier plane (an annular filter) to suppress zero-order signal and to provide required bandwidth of spatial frequencies and spectral range. The filtered spectral signal was collected by the imaging spectrograph (Princeton Instrument) coupled with the charge-coupled device (CCD) camera (LucaEM R DL-604, Andor). The spectral resolution was 4 nm. We used nanospheres with three sizes, 147 ± 3 nm, $\delta = 4.3$ nm; 203 ± 5 nm, $\delta = 4.7$ nm; 240 ± 5 nm, $\delta = 3.7$ nm, according to manufacturer's specification, with δ being the standard deviation. The self-assembled

nanosphere aggregate is prepared following a previously published method.¹⁷ Specifically, an aliquot of 10 μ l of diluted monodispersed polystyrene 240 nm nanosphere suspension ($n = 1.59$, Thermo Fisher Scientific) was uniformly smeared onto the glass slide and dried, forming a thin layer of nanosphere aggregates; another 10 μ l of diluted 203 nm was added on top of the previously formed layer till it dried; then the same step was repeated for another 10 μ l of diluted 147 nm. The thickness of each single-sized nanosphere aggregate, measured using an interferometry setup¹⁸ was about 20 μ m.

The SESF image in Fig. 3 shows the intensity distribution of the reflected light integrated along all axial structures. The natural color in the SESF image appears predominantly green for the entire field-of-view, in agreement with that from numerical simulation. In the magnified portion of the selected area, these small structural variations are visualized with non-uniform color distribution, similar to that shown in our simulation (Fig. 2(b)). The axial profiles of the spatial periods in four selected local areas are shown in Fig. 3. The axial profiles in each of the local areas show three distinct peaks (each peak location corresponding to the spatial period as indicated) suggesting that the nanosphere aggregate has indeed three dominant axial spatial periods that are related to the size of nanospheres, similar to our numerical model (Fig. 2). The spatial period of each single-sized axial structure is estimated by $d \sin \gamma + b$, where d is the nanosphere diameter, γ is an angular

FIG. 3. The SESF image of experimentally constructed mixed-sized nanosphere aggregate. Scale bar is 10 μm . The axial structural period profiles are presented for four selected areas. The insert is a magnified portion of the selected area within the object.

parameter quantifying relative inter-layer shifts and b is the inter-layer spacing. According to manufacturer-specified nanosphere sizes of 147 ± 3 nm, 203 ± 5 nm, and 240 ± 5 nm, this theoretical estimate yields the axial spatial periods of 137 ± 3 nm, 186 ± 4 nm, and 218 ± 4 nm, respectively, for $\gamma = \pi/3$ and $b = 10$ used in the numerical model. The corresponding measured axial spatial periods within the experimen-

tal object are 166 ± 4 nm, 176 ± 3 nm, and 187 ± 6 nm, respectively. These values are extracted by averaging each of the three highest-peak locations on the axial spatial period profile over multiple areas within the SESF image to obtain the mean axial spatial periods and their standard deviations. Assuming that the experimental object has the same packing configuration as the numerical model, the measured axial spatial periods deviate from the theoretical estimates by 10–31 nm. However, the perfect packing configuration in the numerical model does not strictly apply to the experimental object. Our experiment is also limited by the difficulty to confirm the actual axial spatial period of the experimental object with nanoscale precision using an alternative technique. Our result, however, illustrates the feasibility of SESF approach to characterize nanoscale structures in a complex 3D object experimentally. The rigorous experimental confirmation of nanoscale accuracy and sensitivity of the SESF approach is needed in future studies.

We further investigate the ability of SESF approach to characterize the 3D structure of biological cells during cell cycle. We use HeLa cell, a common human cervical cancer cell line, arrested at two distinct phases of cell cycle— G_1/S and G_2/M . The protocol has been previously described.¹⁹ Double-thymidine block inhibits deoxyribonucleic acid (DNA) synthesis to arrest cells at G_1/S ; and then nocodazole treatment disrupts microtubules required for cell mitosis after being released from double thymidine to arrest cells at G_2/M . The DNA content in the nucleus at G_2/M doubles that at G_1/S , as confirmed by flow cytometry.^{16,19}

Figures 4(a) and 4(b) show the transmission electron microscopy (TEM) images at G_1/S and G_2/M phases, respectively. To characterize the nanoscale spatial period of cell internal structure with an alternative technique, we extract the spatial period from TEM images of the cell nuclei via a Fourier analysis¹⁶ and averaged over ~ 10 –20 cells, as shown in Fig. 4(c). The axial profiles of the intra-nuclear structure quantified using SESF approach, averaged over 10–20 cells at G_1/S and G_2/M of cell cycle, are shown in Fig. 4(d). Despite the intrinsic difference in sample preparation and imaging principles, the spatial period profile (Figs. 4(c) and 4(d)) of intra-nuclear structures extracted from both TEM images and SESF approach suggests a significant increase in spatial period for cells in G_2/M compared to those in G_1/S .

We demonstrate the ability of SESF approach for comprehensive analysis of 3D structure of a label-free object at the nanoscale at each image point. The SESF approach quantifies the unique structural information of axial spatial period with nanoscale accuracy and sensitivity. Using simple optical instrument, this approach performs wavelength measurements that have better stability than intensity-based techniques. The spectral range can be extended to ultraviolet and infrared regions. This technique is limited by the first Born approximation which only applies to single scattering regime. Various modifications to SESF system, using simple optical instrumentation, can be created per specific application, ranging from probing macro-scale objects with nanoscale sensitivity, to detecting nano-structural changes of microscopic objects, and to quantifying nano-fabricated structures. This approach can be realized even in real time if spectroscopic image can be captured with a single snapshot.²⁰

FIG. 4. Experimental results for quantification of the nanoscale structures within the cell nucleus of HeLa cells at different phases of cell cycle. (a) and (b) TEM images for cells arrested at G_1/S and G_2/M phases; (c) spatial period profile extracted from TEM images, nm; (d) axial spatial period profile quantified using SESF, nm.

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