Label Free Ultra-Sensitive Imaging with Sub-Diffraction Spatial Resolution

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ABSTRACT

In this paper, we show a new way to break the resolution limit and dramatically improve sensitivity to structural changes. To realize it we developed a novel label free contrast mechanism, based on the spectral encoding of spatial frequency (SESF) approach. The super-resolution SESF (srSESF) microscopy is based on reconstruction of the axial spatial frequency (period) profiles for each image point and comparison of these profiles to form super-resolution image. As a result, the information content of images is dramatically improved in comparison with conventional microscopy. Numerical simulation and experiments demonstrate significant improvement in sensitivity and resolution.

Keywords: optical microscopy, label free imaging, nano-sensitivity, super-resolution, spatial frequency.

1. INTRODUCTION

Nowadays modern optical microscopy is one of the most extensively used tools in the life and material sciences. Improvement in resolution and sensitivity to structural alteration is of great importance in biomedical research and clinical diagnosis. But the accurate characterization of 3D structure, especially at the nanoscale, has been challenging. A basic description of an object's structure is 3D scattering potential or its Fourier spectrum. Two fundamental physical limitations prevent us from probing this 3D structure with nanoscale accuracy and sensitivity, first is limited spatial resolution due to partial access of the entire Fourier spectrum; and second is loss of sensitivity and accuracy due to limited resolution in the K-space and the transformation from Fourier to object space.

Recently the far field diffraction resolution limit in the optical range has been circumvented and different methods of super-resolution optical microscopy have been developed [1-5]. The importance of this achievement has been recognized by Nobel Prize for Chemistry in 2014. However, fluorescence based super-resolution techniques can suffer from photobleaching and only function with fluorescent molecules (most of which are toxic and can destroy or lead to artificial results in living biological objects). A few super-resolution techniques for label free imaging have been proposed [6-13].

In parallel with an improvement of resolution, methods to improve sensitivity to structural changes were developed [14-19]. For example, to detect pathological areas within the sample or small structural changes in time, it is more important to provide high sensitivity to structural changes than high spatial resolution.

A new label-free approach to probe three-dimensional (3D) structure at the nanoscale, based on spectral encoding of spatial frequency (SESF), has been developed recently [17-20] and adapted to 3D imaging [25,26]. This has been applied the nano-sensitive optical coherence tomography (nsOCT), based on SESF approach, for visualization of the nano-scale structural changes of the human tympanic membrane in otitis media [27].

Here, we present a brief theory and demonstrate visualization of submicron structure and nano-scale structural changes of different samples.

2. SESF APPROACH

From general scattering theory it is known that if object is illuminated by broad band plane wave then, after scattering from the object, in K-space, all accessible spatial frequencies (Fourier components of the 3D scattering potential of the object) can be represented as a set of Ewald's spheres [28]. Each Ewald's sphere corresponds to one wavelength. Using SESF approach it is possible, using spectral detection, to reconstruct axial spatial frequency or period profiles of the 3D Fourier spectrum of the object and map them to each point within the image (Fig. 1). The theory of the SESF approach was published in [25, 21, 22] and implemented for super-resolution imaging in [23, 24]. It was demonstrated that in reflection configuration, axial spatial frequency components of the 3D Fourier transform of the scattering potential of the object, which provide information

about small, sub-micron structure, can be spectrally encoded as corresponding wavelengths. Amplitudes of such spectrum are very sensitive to structural alterations.

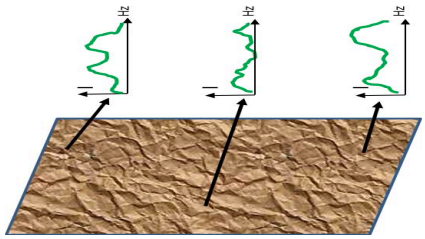

Figure 1. SESF image with axial spatial period profiles reconstructed for three points.

Most optical microscopy techniques have an intensity-based contrast mechanism, where object parameters (absorption, scattering, optical density, birefringence, etc.) are converted into the intensity distribution. So, at each image point there is just one intensity value. In the SESF approach, in contrast to conventional techniques, at each image point we have the axial spatial frequency or period profiles. Instead of just one value it is possible to have hundreds or even thousand values at each image point. So information content of the SESF image dramatically improved that permits to realize super-resolution imaging [29].

SESF images can be formed as color maps of different informative parameters of the axial spatial frequency or period profiles, such as mean spatial period, spatial period at max signal, correlation between profiles, etc.

3. RESULTS

Examples of conventional confocal microscopy and SESF images of stem cells are presented in Fig. 2.

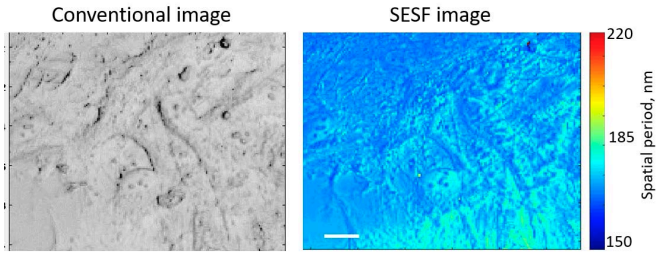

Figure 2. Conventional and SESF images of the stem cells. Scale bar 20 microns.

The wavelength range for imaging was from 405 nm to 635 nm. The SESF image is formed as a color map of the spatial period at max signal, which corresponds to dominant structure within the sample. Beside quantitative information, improvement in resolution using this novel contrast mechanism can be seen from comparison of conventional and SESF images in Fig. 2.

To compare structure at different areas within the sample or detect structural changes in time, the SESF images can be formed as maps of correlation coefficients between axial spatial frequency profile at one point and axial spatial frequency profiles at all other points, or as correlation coefficients between axial spatial frequency profiles within the images, formed at different times. Also, each SESF image can be formed as a map of correlation coefficients between axial spatial frequency profile of numerically synthesized structure and axial spatial frequency profiles at each point of the image.

Figure 3 presents results of numerical simulation of the SESF image formation as a map of correlation with numerically synthesized structure. The sample consists of four repeated areas with different axial structure, the size of each area of the lateral structure being 440 nm × 440 nm. The minimal difference in axial period of the structure at different areas is 10 nm. The image was formed as a convolution with the point spread function (PSF) of the imaging system. The wavelength range of the imaging system was 1230 nm – 1370 nm, resolution 1.6 μm.

In a conventional image it is not possible to resolve areas with different structure because size of each area is about four times smaller than diffraction resolution limit of the imaging system. But axial spatial frequency profiles at different areas can be clearly differentiated (Fig. 3h) enabling resolution of all elements in the reconstructed super-resolution SESF (srSESF) image (Fig. 3d).

Results of a corresponding experiment using custom built scanning microscope with the same parameters as used for simulation, is presented in Fig. 4. In this case, aggregate of polystyrene spheres of 400 nm diameter were used as a sample. A significant improvement in resolution in srSESF image can be clearly seen in comparison with conventional image in Fig. 4.

An example of imaging of collagen tissue is presented in Fig. 5. Here, a piece of collagen tissue was fixed in formaldehyde 4% water solution and placed in a small dish for imaging. Our novel contrast mechanism, realized in srSESF imaging, demonstrates an obvious improvement in resolution in comparison with conventional image.

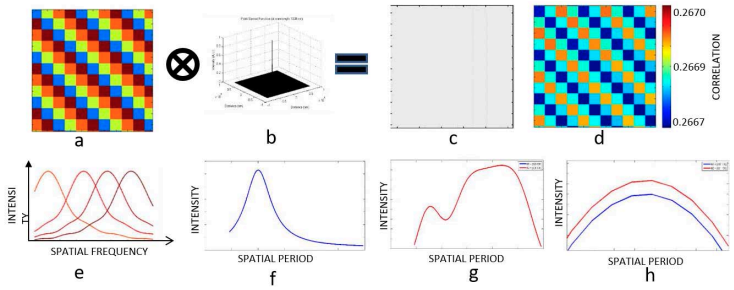

Figure 3. Formation of the SESF image as a result of correlation with numerically synthesized structure. a – sample; b – PSF of the imaging system; c – conventional image; d – srSESF image; e – axial spatial frequency profiles at four areas within the sample; f – profile of the synthesized structure; g - profiles at two areas within the sample in image plane, after convolution with the PSF; h – magnified portion of g where difference between profiles can be clear seen.

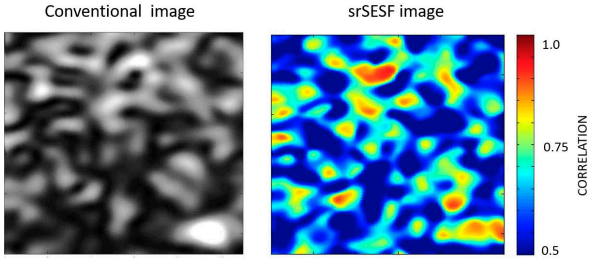

Figure 4. Conventional microscopy and srSESF images of 400 nm diameter spheres. srSESF image formed as a correlation with numerically synthesized structure. The size of imaging area is 10 × 10 microns.

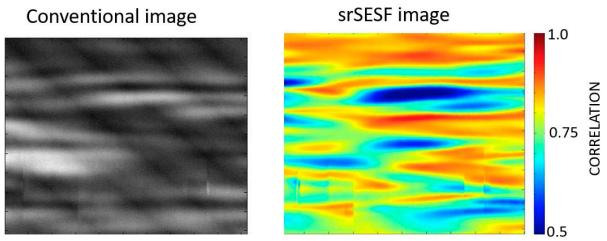

Figure 5. Conventional microscopy and srSESF images of collagen tissue. srSESF image formed as a correlation with numerically synthesized structure. The size of imaging area is 10 × 10 microns.

4. CONCLUSIONS

It was demonstrated that using the SESF approach it is possible to spectrally encode spatial frequencies of the object and translate information about high spatial frequencies through the optical imaging system as different wavelengths independent of resolution. Beside visualization of the dominant structure of the sample with nano-sensitivity it is also possible to compare structures in space or in time by applying correlation between axial spatial frequency profiles. For example, the SESF image can be formed as a correlation with numerically synthesized structure, or as a correlation with axial spatial frequency profile of the pathological structure. As a result, the pathological areas within the sample will be detected and visualized as areas which maximum correlation coefficient with probing pathological structure.

The SESF approach is fond to give a dramatic improvement in information content in comparison with conventional microscopy techniques. It is sensitive to nano- structural changes, yielding a new approach for super-resolution imaging. This form of super-resolution imaging is label free and results demonstrate in 4 times improvement of resolution over conventional microscopy.

Based on preliminary results, the SESF approach has a great potential for many applications in biomedicine as well as in nanotechnology, material science, etc.

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