Achieving High Spatial Resolution Surface Plasmon Resonance Microscopy with Image Reconstruction

Hui Yu,†,‡ Xiaonan Shan,† Shaopeng Wang,† and Nongjian Tao*,†,‡

†State Key Laboratory of Analytical Chemistry for Life Science, School of Chemistry and Chemical Engineering, Nanjing University, Nanjing 210093, China
‡Center for Bioelectronics and Biosensors, Biodesign Institute, Arizona State University, Tempe, Arizona 85287, United States

ABSTRACT: Surface plasmon resonance microscopy (SPRM) is a powerful platform for biomedical imaging and molecular binding kinetics analysis. However, the spatial resolution of SPRM along the plasmon propagation direction (longitudinal) is determined by the decaying length of the plasmonic wave, which can be as large as tens of microns. Different methods have been proposed to improve the spatial resolution, but each at the expense of decreased sensitivity or temporal resolution. Here we present a method to achieve high spatial resolution SPRM based on deconvolution of complex field. The method does not require additional optical setup and improves the spatial resolution in the longitudinal direction. We applied the method to image nanoparticles and achieved close-to-diffraction limit resolution in both longitudinal and transverse directions.

Surface plasmon resonance detection and imaging are powerful methods for studying and quantifying molecular interactions. Using a high numerical aperture objective, high spatial resolution surface plasmon microscopy (SPRM) has been demonstrated and applied for studying biological samples, including cells, bacteria, virus, DNA molecules, liposomes, and proteins. Recently, SPRM-based electrochemical current and impedance imaging techniques have been developed, allowing the study of local electrochemical reactions of heterogeneous surfaces, catalytic reactions of nanomaterials, and cellular processes. The temporal resolution of SPRM can be as fast as μs, which is superior to fluorescence microscopy and scanning probe microscopy. However, its lateral resolution is limited by the finite decaying length of the plasmonic wave along the propagation direction (longitudinal). For example, the SPRM image of a nanoparticle has a parabolic tail of many microns long in the longitudinal direction.

One approach to improve the SPRM spatial resolution is to decrease the decay length by either using short wavelength light to excite the surface plasmons, or introducing metal nanostructures on the metal film. This approach results in less well-defined surface plasmon resonance, and thus loss in the SPRM sensitivity for imaging biological materials and detecting molecular binding processes. Another approach is to obtain a SPRM image by scanning a focused laser beam line by line across the surface. Mechanical scanning of the laser beam in the approach inevitably lowers the temporal resolution that is required for studying fast binding kinetics and surface processes. Finally, a high spatial resolution SPRM image may be reconstructed from images taken at different incident light angles.

EXPERIMENTAL SECTION

Materials and Experimental Setup. The SPRM experiment was performed on an inverted microscope (Olympus IX-81) with a 60× numerical aperture (NA 1.49) oil immersion objective. Light with wavelength of 680 nm from a superluminescence diode was used to excite surface plasmons on a gold thin film (47 nm) on a glass slide, and the plasmonic images were recorded by a CCD camera (AVT Pike F-032B) at a frame rate of 106 frames per second. The images were processed and analyzed by a Matlab program. The 100 nm polystyrene nanoparticles (Microspheres-Nanospheres, Cold Spring, NY), bound to the gold film in 150 mM phosphate buffer, were recorded with SPRM.

Image Processing. Raw SPRM images recorded by the camera were converted to 16-bit tiff format files with a Matlab program. The background noise was removed by subtracting the first image. 2D Fast Fourier Transform (FFT) of the image was performed with the Matlab program to convert the SPRM...
image of nanoparticles from real space to k-space, which revealed a ring-like feature. The radius of the ring was obtained to determine the wavevector of propagating surface plasmon wave.

**RESULTS AND DISCUSSION**

We first present the theoretical basis of the proposed image reconstruction algorithm and then describe the algorithm step by step. Next, we demonstrate the effectiveness of the algorithm by applying it to experimental SPRM images of nanoparticles. We then conclude the paper by discussing the advantages and remaining limitations of the present method.

**SPRM Imaging Mechanism and Reconstruction.**

Rigorous SPRM theories can be found in literature, but here we present a simplified approach. The principle of SPRM imaging of an object (e.g., nanoparticle or virus) near the metal film is illustrated in Figure 1a, showing that a SPRM image is formed by two basic processes (Figure 1b): scattering of surface plasmon wave by the object, which results in a scattered wave denoted by $E_{sc}$ (phase image shown in Figure 1b), and interference of the scattered field ($E_{sc}$) with the propagating surface plasmon wave ($E_p$; phase image shown in Figure 1b).

Accordingly, SPRM image is expressed as

$$I(x, y) \propto |E_p + E_{sc}|^2$$

(1)

where $I(x,y)$ is the SPRM image intensity at location $(x,y)$. In the single scattering regime, we can describe the scattered field $E_{sc}$ as the convolution of the subject, $O$ (spatial distribution of refractive index), and point spread function (PSF), $h$, given by $E_{sc} = O * h$, where $*$ is convolution, $h$ is the field scattered by a point scatterer. $h$ can be represented by a cylindrical wave with amplitude decaying over propagation distance

$$h = \alpha e^{-\gamma r} e^{-ik_0r}$$

(2)

where $\alpha$ is the polarizability of the object (nanoparticles), $\gamma$ is the decaying constant of the surface plasmon wave, $k_0$ is the wavevector of the surface plasmon wave, and $r$ is the distance to the scatterer. In Fourier space, the decaying cylindrical wave described by eq 2 is a ring with radius of $k_0$ and thickness of $\gamma$. The interference of the scattered field for a point-like object and propagating surface plasmon wave results in the characteristic parabolic tail in the SPRM image, which renders the lateral resolution poor, as discussed above.

To improve the lateral resolution of SPRM, we wish to reconstruct the object image ($O$) from the measured SPRM image ($I(x,y)$) with the procedure outlined in Figure 1c. The basic idea is to obtain the scattered field, $E_{sc}$ from $I(x,y)$, and then reconstruct the object image, $O$, via deconvolution of $E_{sc}$ with $h$. We describe each step of the procedures in details below using SPRM image of a 100 nm polystyrene nanoparticle as an example.
example. Figure 2a shows the measured and calculated SPRM images of a nanoparticle. The intensity profiles across the nanoparticle images along the plasmonic wave propagation direction reveals the slow decay in the intensities of both measured and calculated images.

To obtain $E_{sc}$ from $I(x,y)$, we used a filtering method in Fourier space similar to the reconstruction of 3D images in optical holography (Figure 1c). First, we multiply the SPRM image $I$ with the propagating surface plasmon wave, $E_p$, which leads to

$$I \cdot E_p = (|E_p|^2 + |E_{sc}|^2)E_p + |E_p|^2E_{sc} + E_p^2E_{sc}^*$$

where the first term on the right-hand side is a planar wave, the second term is the scattered wave field, and the third term is the conjugate of the scattered wave field. The three terms partially overlap in Fourier space (yellow dashed square in Figure 2b), and the second term ($E_{sc}$) corresponds to a well-defined ring (blue dashed circle at the center of Figure 2b), which determines the image contrast of the object. To isolate the second term ($E_{sc}$) from the other two terms in Fourier space, we apply two filters ($M_1$ and $M_2$) to eq 3, which leads to

$$F{E_{sc}} \sim F(I \cdot E_p) \cdot M_1 \cdot M_2$$

The purpose of $M_1$ is to isolate $E_{sc}$ (the second term) in eq 3 from other terms, which passes only the ring in the Fourier space. $M_1$ takes the form of

$$M_1(k_x, k_y) = e^{-i \frac{(k_x^2 + k_y^2 - k_1^2)\cdot k_1}{k_2^2}}$$

which equals 1 on the ring, and decays rapidly with a constant of $k_1$ (the thickness of the ring) from the ring.

Because the part of the ring within the yellow dashed square in Figure 2b is noisy, we applied another filter $M_2$ as defined by

$$M_2(k_x, k_y) = \begin{cases} 1 & k > k_1 \\ e^{-i(k-k_2)^2/k_2^2} & k < k_1 \end{cases}$$

(6)

to remove the noise within the square, where $k = k_x \cos \theta + k_y \sin \theta$, and $k_1$ and $k_2$ are chosen according to the signal-to-noise ratio around $k_0$, and $\theta$ describes the propagation direction of the surface plasmon wave (Figure 2b). Although the two filters defined above remove part of the scattered wave field, the overall signal-to-noise ratio does not decrease. We will discuss this point later.

With $E_{sc}$ obtained with the procedures described above, and $h$ defined by eq 2, we now turn to the reconstruction of the objective image ($O$), which is related to $E_{sc}$ and $h$ in the Fourier space according to

$$F(O) = F(E_{sc}) / F(h)$$

(7)

The object image ($O$) is given by

$$O = |F^{-1}[F(O)M_2]|$$

(8)

We note that because $F(h)$ is in the denominator of eq 7, which may amplify noise in $E_{sc}$ we applied filter $M_1$ in eq 8 to reduce this noise. The SRPM image in k-space after each step is illustrated in Figure 1c.
Application of the Method to Experimental Images.

To examine the performance of the image reconstruction method, we imaged 100 nm polystyrene nanoparticles. Figure 3a shows a typical SPRM image of multiple 100 nm polystyrene nanoparticles, each with a long parabolic tail. The parabolic tails of the nanoparticles often make it difficult to identify signals from different nanoparticles. Following the procedures described above, we first determined the scattered wave field from the SPRM image with eqs 3–6, and then the propagating surface plasmon wave \( E_p \) from the radius \( k_n \) of the ring in Fourier space. Next, we determined the point spread function \( h \) by computing the wave field scattered by a single nanoparticle with eqs 3–6, using an experimental image of a single nanoparticle as input (e.g., Figure 2a). Knowing \( E_p \) and \( h \), we reconstructed the object image with eq 8.

Figure 3b shows the reconstructed image of Figure 3a, where each nanoparticle appears as a bright spot with the parabolic tail removed. Note that all the nanoparticles remain in the image after reconstruction, even those with low contrast “tails” (i.e., those indicated by the blue arrows in Figure 3a). To evaluate the improvement in the spatial resolution along the propagation direction of surface plasmon wave, we plot the SPRM intensity profiles across the same nanoparticle from the original and reconstructed images in Figure 3c-d. We achieve an improvement in the spatial resolution in the transverse direction (Figure 3d). The achieved \( \sim 270 \) nm resolution in the transverse direction (Figure 3d). The achieved spatial resolutions are \( \sim 300 \) nm (full-width-half-maximum) in both longitudinal and transverse directions, which is close to the diffraction limit of the optical system, which is about 230 nm.

The scattered wave in the present method was calculated by assuming single scattering of the surface plasmon wave by the object, which should be applicable to the study of nanoparticles and viruses as long as they are not too densely packed on the surface. Multiple scattering may not be negligible when the size of the object is large and/or the average distance between objects is small, compared to the wavelength. In those cases, a more sophisticated algorithm based angular spectrum reconstruction techniques may be applied.

CONCLUSIONS

We have demonstrated a simple method to achieve high spatial resolution of SPRM without additional optics or sacrifice of its temporal resolution or sensitivity. Using the method, we have obtained SPRM images of nanoparticles with close-to-diffraction limit spatial resolution in both longitudinal and transverse directions. We anticipate that the method can be applied to the study of nanomaterials, including nanoparticles and nanowires, and biological species, such as bacteria, viruses, and macromolecules.

AUTHOR INFORMATION

Corresponding Author

*E-mail: njtao@asu.edu.

ORCID

Hui Yu: 0000-0002-6927-4451
Xiaonan Shan: 0000-0001-7521-5573
Shaopeng Wang: 0000-0002-2680-0503

Notes

The authors declare no competing financial interest.

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