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Out-of-Focal Plane Imaging by Leakage Radiation Microscopy

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Abstract

Leakage radiation microscopy (LRM) is used to investigate the optical properties of surfaces. The front-focal plane (FFP) image with LRM reveals structural features on the surfaces. Back-focal plane (BFP) image with LRM reveals the angular distribution of the radiation. Herein we experimentally demonstrate that the out-of-focal plane (OFP) images present a link between the FFP and BFP images and provide optical information that cannot be resolved by either FFP or BFP images. The OFP image provides a linkage between the spatial location of the emission and the angular distribution from the same location, and thus information about the film's discontinuity, nonuniformity or variable thickness can be uncovered. The use of OFP imaging will extend the scope and applications of the LRM and coupled emission imaging which are powerful tools in nanophotonics and high throughput fluorescence screening.

Keywords

Leakage radiation microscopy; back-focal plane; out-of-focal plane; surface plasmon-coupled emission

Leakage radiation microscopy (LRM) is a far-field imaging tool which is used for analyzing near-field optical modes both in direct (front-focal plane, FFP) and Fourier (or momentum, back-focal plane, BFP) images [1–7]. It has been successfully implemented with various structures, such as dielectric multilayers for Bloch surface waves (BSW), thin metal film or metal nano-waveguides for surface plasmon polaritons (SPP) [8–13]. In addition to these planar nanophotonic devices, LRM has also been used with imaging of surfaces with nano-scale features. Examples include plasmonic antennas which provide directional emission with wavelength separation and the use of surface gratings to control the angular distribution of Bloch surface wave coupled emission [14–19]. LRM with front-focal plane (FFP)

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imaging has been used to determine the spatial distributions of modes on the surfaces, LRM with back-focal plane (BFP) imaging has been used to determine the angular distribution of the coupled emission or leakage radiation (LR). We use the term leakage radiation (LR) to represent either the scattered incident light or the emission from fluorophores coupled to the surface. Many samples of interest, such as nanohole arrays or biological samples contain structural features in the x-y plane, and because of variable thicknesses or dielectric constants the emission from the features can occur at different angles for either scattered light or coupled emission. The correlation between the location and angular distribution is not present either in the FFP or BFP images. Here we will experimentally demonstrate that the OFP images can provide simultaneous information on the surface topology and the angular distribution of the LR or coupled emission. The use of OFP imaging can result in new optical configurations for parallel sensing or wide-field imaging.

To understand the concept of OFP imaging, it is useful to recall the basis of LRM. In the LRM all the light within the numerical aperture (N.A) of the objective is collected and imaged. This intensity distribution depends on spatial variations on the metal surface. Typically, surface roughness, edges or particles result in increased radiation into the structure under the sample or free-space (air or water) above the sample. In the case of smooth metal films the radiation into the substrate propagates above the critical angle and can be observed with an oil immersed objective with high numerical aperture ($N.A > 1$). Depending on the structure the energy can propagate various distances on the surface, and the radiation intensity is proportional to that of the local point where LR occurs [20]. Thus by mapping the far-field radiation in the FFP, the near-field spatial intensity distribution can be measured. Because the objective and tube lens focuses the light collected at all angles to a specific point the angular distribution of radiation cannot be determined from the FFP image. In BFP imaging the radiation from the entire sample is separated into the angular distribution. For nanophotonic structures the angular distributions provide valuable information about the structure. A common example of the correlation between angle and structure is provided by diffraction gratings where the reflection angles can be used to calculate the grating dimension. The BFP image can be seen as a Fourier transform of the FFP image where the space variables (x, y, z) are changed to the momentum variables (k_x, k_y, k_z). The wave-vector momentum information is contained in the BFP image. The angle (θ, ϕ) of LR into the structure can be derived out with the formula $K_{in-plane}/K_0 = n \cdot \sin\theta$. Where $K_{in-plane}$ is the in-plane wave vector and K_0 is the wavenumber of light in vacuum, n is the effective refractive index of substrate, θ is the polar angle and ϕ is the azimuthal angle. So each spot on the BFP image represents a LR angle (θ, ϕ) [21, 22]. The BFP image contains no information about the spatial distribution of the LR. OFP image act as hybrid state of FFP and BFP, which carries both spatial distribution and wave-vector momentum information.

As a simple example to demonstrate OFP imaging, we show the OFP images of leakage radiation of SPPs coupled from bare silver film. Fluorescent polystyrene spheres (FPSs) is used to serve as point sources for SPPs. We used a 45 nm thick silver film deposited on a glass substrate by the thermal evaporation method. Two FPSs with diameter of 2 μ m were placed on the silver films. An expanded laser beam with wavelength of 532 nm was used to excite these FPSs from the top space. The excited fluorescence would couple with SPP of

the Air/Ag/Glass interface, resulting in the well-known surface plasmon-coupled emission (SPCE) [17, 23 and 24]. The SPCE radiating into the glass substrate was collected by an oil immersed objective (60X, N.A 1.49), and then imaged by a tube lens onto the detector (Neo sCMOS, Andor, UK). A bandpass filter with center wavelength at 600 nm with full width at half maximum (FWHM) of 10 nm was used to filter the fluorescence. At a specific position of the detector along the optical axis the FFP or BFP image can be captured. When the detector was moved from FFP to BFP, a series of OFP images can be obtained, where distance between FFP and OFP is defined as d (labeled on Fig. 1a).

The fluorescence FFP image (Fig. 2a) verifies that there are two fluorescent beads located on the silver film. On the BFP fluorescence image (Fig. 2h), we observed one bright ring, which reveals the momentum of SPP on Air/Ag/Glass interface, but these rings contain no information of the spatial origin of the rings. The OFP images provide a ray tracing like linkage between the spatial origin of the emission and the coupled emission angle (Fig. 2b–2g). An OFP image can be recorded at any location between FFP and BFP. Here, we present the OFP images with 25mm intervals between FFP and BFP in Fig. 2. A smaller interval would result in more accurate tracing. The displacement of the detector from the FFP causes the fluorescence images of the FPSs to change from solid spots to rings, which is due to the directional emission of the fluorescence. With the increasing of d , the two rings become larger and intersect with each other until coincide in the BFP image (Fig. 2h). It should be note that the two FPSs here share the same circumstance, so the leakage of optical modes coupled from these two FPSs possess the same angular spectrum. We don't need to correlate the spatial locations of FFP with its particular angular spectrum here since they share the same one. It is the spatially variant angular spectrum that embody the values of OFP images. This change of angular spectrum may induced by the change of dielectric environment, such as the thickness and refractive index of the dielectric layer coated on the metal film or dielectric multilayer [25, 26] which is normally encountered in high-throughput arrays, clinical diagnostics and fluorescence sensing.

Here, by taking advantage of the relationship of SPPs momentum with the dielectric thickness of silica layer on silver film, we show the potential application of OFP imaging in parallel film thickness sensing. The sample used is as present in Fig. 1b, by using a mask on the 45nm thick silver film, four different thicknesses silica layer were deposited one by one onto four areas of this silver film (1, 2, 3, and 4 on Fig. 1b). The thickness differences are in nanometer scale and cannot be resolved by conventional optical microscopy. Four FPSs were separately placed on each of these silica film. The fluorescence FFP image (Fig. 3a) verifies that a single fluorescent bead is located on each of the four areas of the samples (1, 2, 3, and 4). The FFP image does not reveal the thickness difference of these SiO₂ layers below the FPSs. If we look at the BFP fluorescence image (Fig. 3h), we observed four bright rings, but these rings contain no information of the spatial origin of the rings. Multiple rings can also be observed with thicker dielectric layer. In this case the multiple rings are due to multiple TE or TM modes in the top silica layer [27]. From the FFP and BFP images alone we cannot determine whether the SiO₂ film has a uniform thickness or if the rings are due to additional modes in the structure. OFP images recorded in Fig. 3b–3g provides a way to connect the angular spectrum on BFP images with its corresponding origin of emission on FFP. This collection could be accomplished by a serious of OFP images to trace from FFP to BFP as

done in Fig. 3. Or simply by choosing one particular OFP image which possess distinguishable characters for both FFP and BFP. Taken Fig. 3c for instance, from the spatial location of the four rings here, the origin of these four rings could be determined as labeled. The diameters of ring 1, 2, 3 and 4 could be measured as 346, 360, 364, 374 pixels respectively. The size order of the four rings remains the same on BFP image in Fig. 3i. Then we can correlate the angular spectrum rings on BFP with its corresponding origin on FFP as labeled in Fig. 3i.

Returning to the effect of silica thickness, the wave-vectors (K_{spp}) of the SPP on the Air/SiO₂/Ag/Glass structure can be increased with thickness of the dielectric layer [28, 29]. The increased wave-vector results in the larger resonant angle (or LR angle) of the SPP, corresponding to the larger diameter of the SPCE rings on OFP and BFP. For the bead on bare silver film (Fig. 2), the SiO₂ layer under each FPSs are of the same thickness (0 nm), so their wave-vectors and thus the diameters of the rings are the same. But for the FPSs on Fig. 3a, the SPCE rings are of different diameters, which indicate each excited SPPs have different wave-vectors due to the different silica thickness under each FPS. We confirmed the origin of the four rings in Fig. 3 using reflected light. The incident light was obtained using a white light source and a 600 nm filter with a FWHM of 10 nm. This provided uniform illumination of the back of a high NA objective which was then focused on each of the four areas in Fig. 3. The incident light occurs within all angles accessible within the NA of the objective. The dark rings on the reflected BFP images (Fig. 4a) are due to absorption at the resonance angles of 600 nm light. The resonant angles of the SPP on each area (Fig. 4b) can be derived as 43.4 (area 1), 44.7 (area 2), 46.8 (area 3) and 49.0 degrees (area 4). Based on the principle of surface plasmon resonance sensing [25] and the aid of calculated angular dependent reflectance curves [12, 30], the thickness of each SiO₂ layer can be derived as about 0nm (area 1), 13nm (area 2), 24nm (area 3), and 34 nm (area 4). This thickness can also be derived from the fluorescence BFP image if we focus the laser beam onto the four FPSs one by one, then only one SPCE ring were recorded on each BFP image. From the diameter of each SPCE ring, the thickness can be derived. But with the aid of OFP images, these FPSs can be excited simultaneously, and then four rings appear simultaneously on the BFP image (Fig. 3h, 4c). The corresponding relation of these four rings with the FPSs on the FFP can be judged from the OFP images. From the diameter of the four simultaneously appearing rings on the BFP (Fig. 3h, 3i or Fig. 4c), the LR angle can be derived as about 43.4° (Ring 1), 44.7° (Ring 2), 46.5° (Ring 3) and 48.8° (Ring 4). By comparison with calculated angular dependent reflectance curves, the thickness of each SiO₂ layer can be derived as 0nm (area 1), 12nm (area 2), 22nm (area 3), and 33 nm (area 4), which are consistent with the data measured one by one from the reflected BFP images (Fig. 4a, 4b).

In our experiment, the SiO₂ layers on the four areas (1, 2, 3 and 4, Fig. 1b, Fig. 3a) can be seen as a single sample to be characterized. The fluorescence beads can be seen as the probes labeled on the samples. Our work shows that combination of the FFP, OFP and BFP can be used in parallel (wide-field) detection to correlate the spatial locations with the coupled emission angles. This capability is useful for the emerging combination of biological sensing using substrates with nanoscale features for high throughput sensing such as the use of microcell plates. The SPP and coupled emission are not only sensitive to the

thickness, but also to its local refractive index. So if thickness of the dielectric layer remained the same, but its refractive index varies at different areas, the corresponding SPCE rings from different areas will also be different, which would appear on both OFP and BFP images, similar as the Fig. 3. As a result, the refractive index's variation can be detected simultaneously, which may lead to applications in refractive index images or sensing of individual cells. It should be noted, the proposed method is suitable not only in SPP sensing, it can also be used with BSWs on dielectric multilayers, and metal-dielectric waveguides.

In summary, all the previous reports on optical sensing or detection with LRM were focused on the BFP image that do not contain x-y spatial information, or FFP imaging which does not depend on sample thickness or refractive index. There is no correlation between the spatial location and angular distribution of the emission. Measurement of the OFP images provides insight about the spatial information of the LR or coupled emission, thus establishes the bridge between FFP and BFP. Here, by simply including the long-neglected OFP image, the LRM can be applicable in wide-field imaging, parallel detection or sensing, thereby extending the use of LRM to novel formats and applications.

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Fig. 1. Schematic configuration of the sample and experimental setup. (a) The FPSs on silver were excited by a fiber laser at 532 nm wavelength in reverse Kretschmann (RK) configuration. SPCE at Air/SiO₂/Ag/Glass interface were collected by a high N.A objective, and then imaged by a tube lens onto either FFP or BFP of the LRM. The OFP plane is between FFP and BFP, with distance to FFP as d . A band pass filter with center wavelength at 600 nm and FWHM at 10 nm was used to filter the fluorescence. (b) Fluorescence leakage from four FPSs forms four SPCE rings of different diameter on the BFP using LRM. There are four thicknesses of the SiO₂ layer between the FPSs and silver film (1, 2, 3, and 4).

Fig. 2. Fluorescence detected at different image planes of the LRM. (a) FFP, (b–f) OFP, (h) BFP images. Three FPSs were placed on the bare silver film (45 nm thickness). The dashed circles on (a) represent the positions of the two FPSs. The circular dashed line in (h) represents the Numerical Aperture (N.A) of objective.

Fig. 3.

Fluorescence detected at different image planes of the LRM. FFP (a), (b–g) OFP, and (h) BFP, and (i) enlarged image of the area marked with box on (h). The white dashed lines on (a) represent the edges of the four SiO_2 layers (1, 2, 3 and 4) of different thicknesses. The dashed circles on (a) represent the positions of the four FPSs. From (b) to (g), $d = 25, 50, 75, 100, 135$, and 150 mm respectively. On (c) the number of pixels was used to represent the diameters of the four rings.

Fig. 4.

Measuring the thickness of SiO_2 layer on a silver film. (a) Reflected BFP images of the LRM when a white light was focused on to the four areas (1, 2, 3, and 4 on Fig. 1b) one by one. A band pass filter with center wavelength at 600 nm and FWHM at 10 nm was used to filter the white light. (b) Intensity profiles along the white lines on (a) gives the angular position of the dark ring (or SPR angles). (c) BFP image on Fig. 2h, (d) Intensity profile along the white line on (c) gives the emission angles of the four SPCE rings.