High-speed multispectral imaging of nanoplasmonic array

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Abstract: A multispectral microscopy imaging system is developed for the single-particle scattering spectroscopy of many individual plasmonic nanostructures simultaneously. The system dispenses with the need for the mechanical scanning of sample stage and thus enables high-speed plasmon resonance imaging of nanostructure arrays. The darkfield scattering intensity images of nanoplasmonic structures at individual wavelengths are acquired with a spectral resolution of 2 nm in the wavelength range from 500 nm to 800 nm, and a frame rate of 2 seconds/wavelength. The images are processed afterwards and the plasmon resonance wavelength of every nanostructure within the field of view can be obtained at once. The plasmon resonance wavelengths of more than 1000 Au colloidal nanoparticles and a nanofabricated Au nanowire array are measured within 5 minutes. The presented high-speed spectral imaging system promises the practical application of large-scale high-density nanoplasmonic sensor arrays for label-free biomolecular detections in the near future.

OCIS Codes: (120.6200) Spectrometers and spectroscopic instrumentation; (110.0180) Microscopy; (290.5820) Scattering measurements.

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1. Introduction

Metallic thin-film based surface plasmon resonance (SPR) sensors are widely used in near-real-time label-free chemical and biomolecular sensing [1]; however measuring the characteristic angle in conventional SPR systems requires the mechanical rotation of both the

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light source and detectors, which makes the system difficult to be miniaturized and multiplexed. The largest SPR sensor array demonstrated to date only has around 100 sensing elements [2, 3]. This limitation hinders the application of SPR systems in large-scale genomic and proteomic microarrays in which thousands of elements are usually required [4]. Plasmon resonance spectroscopy of metallic nanostructures has been extensively studied in recent years because the plasmon resonance wavelength of such structures can be affected by biomolecular reactions occurring on their surfaces [5-8]. Metallic nanostructures promise large scale nanoplasmonic biosensor arrays and have several advantages over traditional SPR sensors, including smaller sample volume, greater potential for compact integration and automation, and higher sensitivity. Nevertheless all previous works have been limited to the spectroscopy of several particles at most, and a large array of nanoplasmonic sensors has not been implemented to date due to the limitations imposed by current spectral imaging system. All existing imaging and spectroscopy systems for metallic nanostructures are based upon a microscopy system with a white light illumination source, a darkfield condenser and a polychromator spectrograph [9, 10]. In such a system, the field of view is limited to several microns by the width of the entrance slit to the polychromator even though the field of view of the objective lens is on the order of several hundred microns; therefore only a few nanoparticles or nanostructures that are aligned in a column can be imaged spectrally at once. The polychromator entrance slit is required to limit data pollution for the high-resolution spectroscopy of each individual nanoparticle. Hence in the case of spectrally imaging hundreds or even thousands of sparsely distributed nanostructures the sample stage of the microscopy system must be translated either manually or automatically, which has two disadvantages. First, the spectra of separated nanostructures cannot be simultaneously captured and the acquired data will be from different instants in time, e.g. 20~30 minutes for a 100x100 array, which voids their applicability in the real-time monitoring of multiple biomolecular interactions. Second, the focal plane will tend to drift due to the stage translation which may change the condition of the immersion liquid between the sample and darkfield condenser lens, and thus manual refocusing is often required.

Here we propose and demonstrate a new multispectral imaging system to simultaneously monitor the individual scattering spectra and plasmon resonance wavelength of large numbers of nanostructures distributed within the field of view of a microscopy objective lens without mechanically scanning the sample. The multispectral imaging system currently supports frame rates as high as 2 seconds per frame (wavelength) that could potentially be increased by using a light source with a higher power or an image detector with greater sensitivity, by which the image signal to noise ratio can also be increased. As an exemplary application of our novel imaging system, more than 1000 plasmonic Au colloidal nanoparticles in various sizes are spectrally imaged in 5 minutes with a spectral resolution of 2 nm within a wavelength range from 500 to 800 nm. In another demonstration, a nanofabricated Au nanowire array is also spectrally imaged. The overall imaging time can be further reduced by using a smaller wavelength range or reducing the spectral resolution depending upon the requirements of a particular application.

2. Multispectral imaging system configuration

In contrast with the configurations of previous imaging systems [9, 10], our system consists of a multispectral illumination source synchronized with an intensity imaging camera rather than a polychromatic spectrograph. As shown in Fig. 1, the white light from a 30 W halogen lamp is coupled into a scanning monochromator (SpectraPro 300i, Acton Research) which is controlled by a computer program to output a monochromatic light beam with varying wavelength and specified spectral step size. The spectrum of the light output from the monochromator is measured as the calibration spectrum for further experiments, and the power of monochromatic light outputs is measured to be from 100 to 300 μW / cm^2 / nm. The spectral width of the monochromatic light is measured to be ~2 nm. The monochromatic light beam is then focused and illuminated on the sample by a darkfield condenser (N. A. = 1.2~1.4) with immersion oil (n = 1.58). A 40X microscopy objective lens (N. A. = 0.8) is

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used to collect the scattered light, which is captured by a 512 × 512-pixel CCD camera (Cascade 512B, Roper Scientific). The monochromator and image acquisition control software are integrated and they are synchronized to capture a single image at each wavelength of interest. The image acquisition can be finished in a few minutes and the acquisition time is dependent on the chosen spectral range and resolution.

All images are stored as uncompressed, 16-bit grayscale data files and are analyzed by an image processing program. No noise reduction or smoothing algorithms were applied to the image data prior to analysis. The bright spot regions (typically 1~10 pixels) in each image are recognized by the analysis program as individual nanoplasmonic structures of interest. The mean intensity value of these small regions is extracted from the image at each wavelength as the raw scattering spectra data. The mean intensity value in a large, empty (black) region is also measured at each wavelength as the background spectrum, which is subsequently subtracted from the raw scattering spectra. The difference spectra are then scaled according to the previously-stored spectrum of the light output from the monochromator to yield the final scattering spectra. The process of the image analysis and the spectral data reconstruction is completely automated by the computer program.

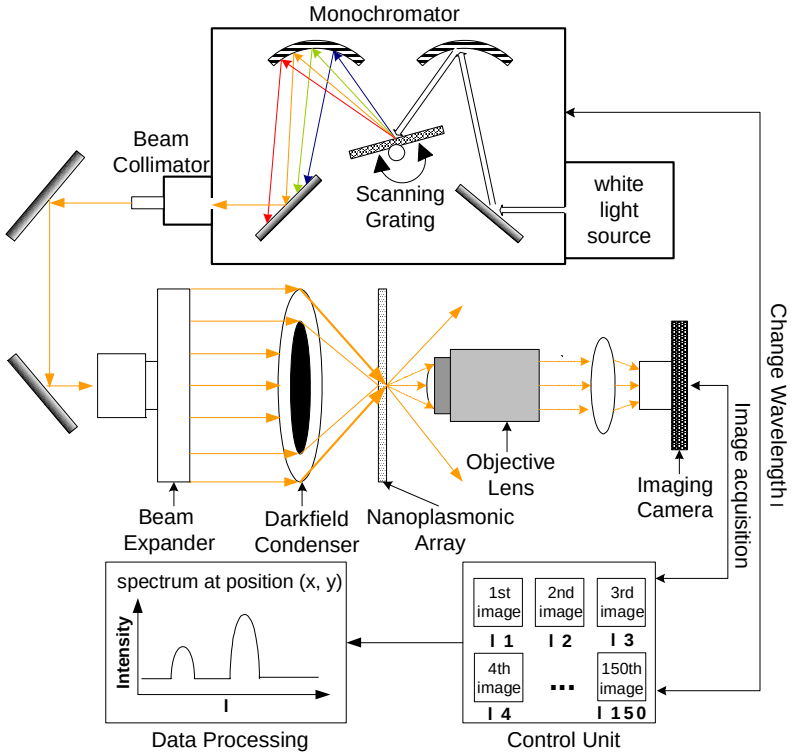

Fig. 1. Configuration of the multispectral imaging system for the scattering spectra measurement of nanoplasmonic arrays.

3. Results and discussion

As a demonstration, we use randomly-dispersed Au colloidal nanoparticles on a glass slide as the imaging sample. The diameters of the Au nanoparticles vary from 20 nm to 80 nm, so their plasmon resonance wavelengths, and thus their scattering colors are different. Fig. 2a shows the true-color scattering image of the nanoparticles within ~1/10 of whole view field of the objective lens. The true-color image is taken in the same darkfield microscopy system but with a white-light illumination source and color camera. Figure 2(b) and 2(c) show the scattering intensity images of the same nanoparticles within the same field of view taken by

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our system at 550 nm and 630 nm, respectively. Figure 2(d) shows the scattering spectra of three representative nanoparticles marked in Fig. 2(a), (b) and (c). The plasmon resonance wavelengths (spectral peaks) of these three nanoparticles are respectively 560 nm, 580 nm and 630 nm, which agree well with their colors (green, yellow, and red) in Fig. 2(a), and their relative intensities in Fig. 2(b) and 2(c). The Au "particle" with red scattering color could be a cluster of a few Au nanoparticles, because the plasmon resonance wavelength of individual Au nanoparticle is shorter than 600 nm according to Mie scattering theoretical predictions. Although only the scattering spectra of three typical particles are shown here, the spectral information for all the other nanoparticles in the field of view are also stored at once and can be reconstructed in the same fashion.

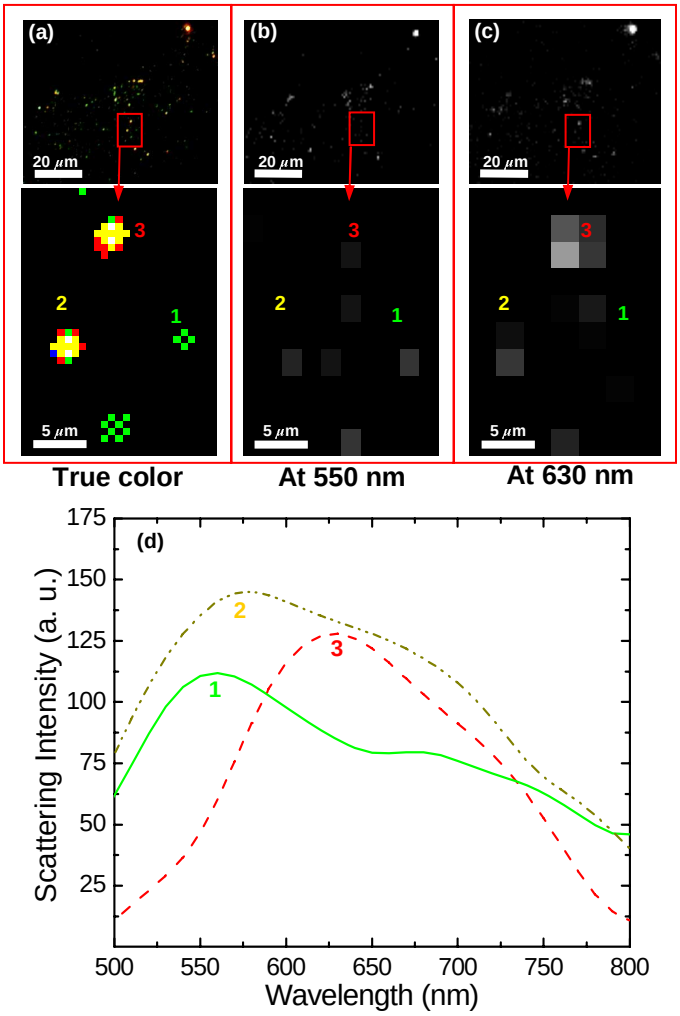

Fig. 2. (a) True-color scattering image of thousands of dispersed Au nanoparticles. For the clarity of the image, a field of 100 μm × 100 μm is cropped from the whole view field of ~ 300 μm × 300 μm. The lower picture is the zoom-in image from the marked area (square) of the upper image. (b) and (c) show the scattering intensity image of the same Au nanoparticles as in (a) with 550 nm and 630 nm monochromatic illumination, respectively. (d) Scattering spectra of three representative particles marked as 1, 2 and 3 respectively in the images.

The ultimate goal of our system is the high-speed spectral imaging of large nanoplasmonic arrays, e. g. 100 × 100 elements, so we imaged a much smaller scale array as a proof-of-concept. The array is comprised of 11 Au nanowires fabricated on a transparent substrate via

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electron beam lithography. The lengths of all the Au nanowires are 70 μm, while their diameters vary from 20 nm to 150 nm. Figure 3(a) shows the scanning electron micrograph of the array. Due to the dependence of plasmon resonance wavelength upon nanowire diameter, the nanowires appear different colors under white light illumination and are shown in Fig. 3(b) when immersed in oil. The narrower nanowires such as 20 nm, 30 nm and so forth primarily scatter green light while the scattering color of wider nanowires are red shifted. Figure 3(c) and 3(d) show the scattering intensity images of the Au nanowires array within the same view field as in Fig. 3(e) at illumination wavelengths of 570 nm and 650 nm, respectively. The relative scattering intensities of each nanowire in these images agree well with their plasmon resonance wavelengths.

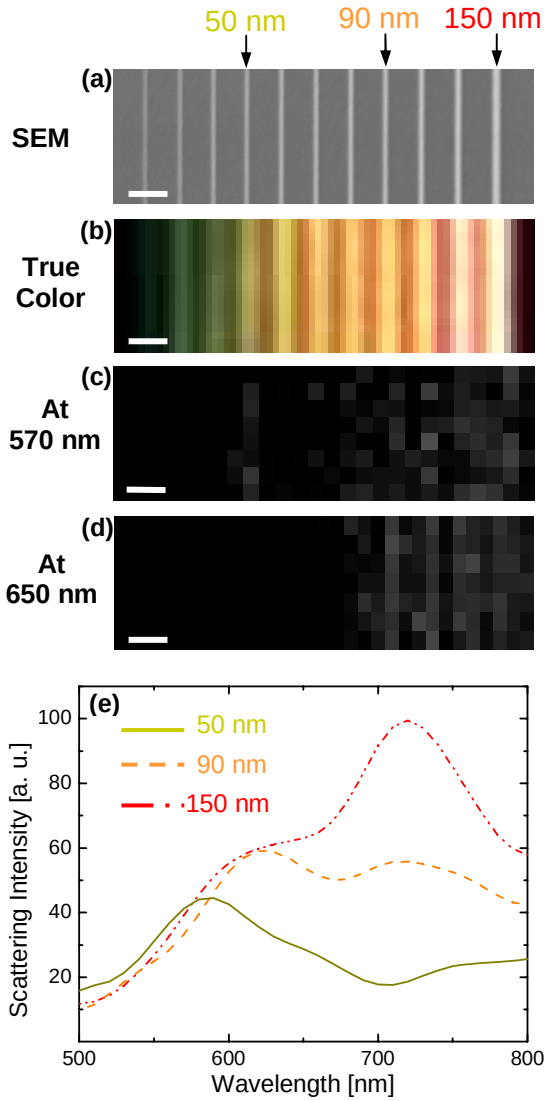

Fig. 3. The scanning electron micrograph **(a)**, true-color scattering image **(b)**, and scattering intensity images with 570 nm **(c)** and 650 nm **(d)** monochromatic illumination of an Au nanowire array. All scale bars stand for 1μm. **(e)** Scattering spectra of three representative Au nanowires.

It should be noted that the scattering intensities of narrower nanowires are much smaller compared to those of the wider nanowires because the Rayleigh or Mie scattering intensity is

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proportional to the sixth power of the scattering length, i.e. the nanowire diameter. Therefore the monochromatic scattering intensities of narrower nanowires such as 20, 30 or 40 nm nanowire are very small relative to the dynamic range of the detector, which compromises the signal to noise ratio in the spectral detection of those nanowires. This problem can be solved by applying a light source with a higher output power or longer image acquisition time. Fig. 3d shows the scattering spectra of three representative nanowires with widths of 50 nm, 90 nm and 150 nm, respectively. Because the distance between each nanowires is only 1 μm which is barely resolved by the 40X microscope objective lens, multiple resonance peaks on the spectra of those nanowires can be observed due to the scatter signal interference from adjacent nanowires.

The spatial resolution of our multispectral imaging system is virtually the diffraction-limited resolution of the microscopy system and thus a 100 × 100 nanoplasmonic array can be clearly resolved both spatially and spectrally by a 40X objective lens with a field of view of ~300 μm × 300 μm. No mechanical stage scanning or rotation operations are required by our system which increases the potential of system miniaturization. Due to the wide plasmon resonance wavelength distribution of the nanostructures studied in this paper, it was necessary to acquire data from 500 nm to 800 nm. However, in a 100 × 100 array of nanofabricated 50 nm Au nanodots designed to study biomolecular binding reactions, a wavelength range from 520 nm to 600 nm would be sufficient, and the spectral data acquisition times could be shorter than 1 minute. Many biomolecular binding and disassociation events require tens of minutes to several hours to finish, and an imaging speed of 1 minute per spectrum would be sufficient for real-time monitoring in those applications. Our multispectral imaging system provides a unique high-throughput solution especially for utilizing very large scale plasmon resonance based genomic and proteomic arrays that would otherwise be impractical due to the limitations of previous spectroscopy systems.

4. Conclusion

We have constructed a novel optical imaging system for high-speed multispectral characterization of plasmonic nanostructure array. Our system does not require the rotation of the light source and detectors as in conventional thin-film based SPR system or the sample stage scanning as in all previous nanoparticle plasmonic imaging systems. More than 10000 elements in a nanoplasmonic array can be simultaneously imaged and their plasmon resonance wavelengths can be probed within a minute, which enables the real-time label-free monitoring of very-large-scale-integrated genomic and proteomic sensor array in the near future.

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