Effective photoexcitation in gold nanowells based on localized surface plasmon

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The localization of the surface plasmon (SP) field in nanospace using wavelength-sized gold wells prepared on a glass substrate and its application to excite fluorophores immobilized on the bottom surfaces have been studied.

Surface plasmons (SPs) are collective oscillations of conductive electrons on flat metal surfaces such as gold and silver usually excited by photoirradiation with a prism coupler.[1] Because the electromagnetic field is strongly localised on the surface and its intensity is remarkably enhanced compared to that of incident light,[1] SPs are a very effective excitation source for fluorescence bioassays[2] as well as photoelectrochemical applications.[3] Recently, researchers' interest has been focused on the handling of SPs using nanostructures to create entirely new prospects for guiding light on the nanoscale.[4–7] Here we studied the excitation of the fluorophores immobilised in the gold 'nanowells', *i.e.*, nanoholes prepared on a glass substrate, by the localised SP field.

The nanowells were prepared by the projection method according to the reported procedure as follows.[5,6] The surfaces of coverslips (Matsunami Glass Inc., $n = 1.51$, 22 × 22 × 0.17 mm^3) were treated with a 2% acetone solution of 3-aminopropyldiethoxysilane for 2 h. After careful washing with acetone, ethanol, and ultrapure water, the amino terminated surfaces were coated with poly-L-lysine by immersion in the 0.1 v/w% solution for 1 h and washed with ultrapure water, and then dried in clean air. The amino-termination and successive coating with poly-L-lysine are essential for the immobilization of fluorophores as well as adhesion to the carboxy-terminated latex beads and the gold film. A dilute suspension of carboxy-terminated polystyrene latex beads with diameters of 430 or alternatively 600 nm (Sejusui Co. and Polybeads Co.) was dispersed on the surface and gold was evaporated for 50 nm at 10^{-5} Pa over the latex beads. After gold evaporation, the gold-covered latex beads were removed by ultrasonication in water. Nanowells with aperture sizes corresponding to the diameters of the latex beads were found on the surface by atomic force microscopy (Fig. 1a). Although isolated single nanowells were dominant, a small number of twin and triplet nanowells due to the aggregation of latex beads were found.

The SP scattering spectra were observed by a fluorescence spectrometer (Perkin-Elmer LS55). The coverslip supporting the nanowells was attached to a BK-7 right angle prism with index matching oil, and p-polarized monochromatic light was irradiated at the incident angle of 55 ± 2.5° for SP generation. Then, transmitted light from the surface was monitored during synchronous scanning of the excitation and emission wavelength from 350 to 800 nm (Fig. 2). When the SPs are generated on a gold film with nanowells, the propagating SPs will encounter nanowells and undergo radiative decay at the edges, resulting in effective transmission of excitation light through the nanowells.[4] A flat gold film without nanowells as a control showed very weak scattering mainly generated by the radiative decay of SPs at the grain structure of the gold surface. On the other hand, both the 430 and 600 nm nanowells showed much more intense scattered light than that of the flat gold film in the wavelength region over 400 nm with the periodic fine structures. The periodic features found in the spectra stem from the multi-reflection between the interfaces of the gold film because the peak distances are roughly the same as the thickness of a gold film.

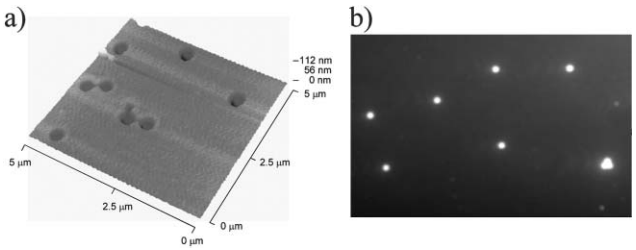

Fig. 1 Microscopic images of the 600 nm gold nanowells; a contact mode AFM (atomic force microscope) image, a); a fluorescence microscope image of the Texas Red-immobilised nanowells excited by p-polarized 532 nm laser light, b).

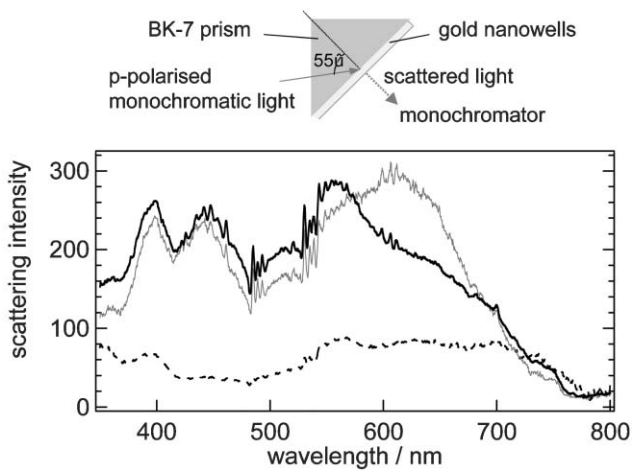

Fig. 2 SP scattering spectra of gold films with 500 nm (solid line) and 600 nm (gray solid line) nanowells, and a flat gold film (dotted line); upper drawing shows optical configuration of the measurement.

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The most remarkable feature of the spectra is the specific enhancements of the scattering efficiencies in the wavelength regions longer than those corresponding to the aperture size of the nanowells. It has been reported that the periodic arrays of subwavelength-sized nanoholes in silver films offer extraordinary transmission of the far-field light due to localization and scattering of SPs.[4,7] On the other hand, the isolated[5] or randomly distributed nanoholes[7] showed similar enhanced light transmissions due to SPs only by the near-field light illumination. The present nanowells were distributed at random and SPs were excited by the evanescent light from a base of a prism, *i.e.*, near-field light illumination. Therefore, the specific enhancement of the scattering spectra was attributable to scattering and localization of propagating SPs by the nanowells. This wavelength specificity suggests that the nanowells effectively scatter SPs having wavelength corresponding to the aperture size because the wavelength of SPs ($\lambda_{sp} = \lambda_{ex}/n \sin\theta$, $n = 1.52$, $\theta = 55^{\circ}$) is somewhat shorter than that of the incident excitation light (λ_{ex}).[1] The remarkable decrease in the scattering intensities at a wavelength region longer than 700 nm suggests that the nanowells do not effectively scatter the SPs having wavelength longer than the aperture size.

Texas Red (TR) having absorption and emission maxima around 600 nm was employed as a fluorophore taking into consideration the wavelength of SPs effectively scattered by the present nanowells.† Another advantage of TR is ineffective excimer formation in a monolayer on a surface. Immobilization of TR on bottom surfaces of the nanowells was conducted by immersion of a coverslip supporting the nanowells into a 70 nM aqueous solution (containing 1% DMSO) of TR succinimidyl ester (TRSE, Molecular Probes Inc.) for 5 min at 25 °C. After the immersion, the surface was carefully washed with ultrapure water and then dried with air. The immersion time was optimized by independent measurement of the absorbance and fluorescence intensity using an amino-terminated coverslip without a gold film. Further immersion induced a remarkable decrease in the fluorescence intensity due to self-quenching. Under this condition, the occupation area of a TR molecule on the glass surface was determined as 2.6 nm^2 based on the absorbance and extinction coefficient of TR. The number of TR molecules immobilized on the bottom surface of a 600 nm well was estimated as 1.1×10^5.

The microscopic measurement of TRSE immobilized 600 nm nanowells was performed by waveguide-mode excitation. Two BK-7 right-angle glass prisms ($5 \times 5 \times 5$ mm^3) were attached to both exposed glass portions of the gold covered area with index matching oil for the preparation of an optical waveguide. The waveguide was set on the stage of an inverted microscope (OLYMPUS IX70) and excitation was conducted by p-polar or alternatively s-polar 532 nm light from a diode pumped Nd^{3+}-YAG laser. The fluorescence image was captured by an objective lens (OLYMPUS, × 60, NA 1.2), a fluorescence cube for Texas Red (OLYMPUS, WIY), and a cooled CCD camera (Diagnostics Inc., SPOT-JR). The fluorescence spectrum was observed by a CCD spectrometer (Ocean Optics Inc., USB-F-2000) by accumulating fluorescence signals from approximately 1000 nanowells. The intense fluorescence was observed from each nanowell (Fig. 1b) and the fluorescence spectrum was characteristic for Texas Red (Fig. 3), indicating the successful immobilization of TR molecules on the bottom surface. The fluorescence intensity decreased remarkably when the excitation light was changed to s-polarization. Because the SPs can be excited only by p-polar light, the result demonstrates that the immobilized TR molecules were excited by the scattered SPs by the nanowells.

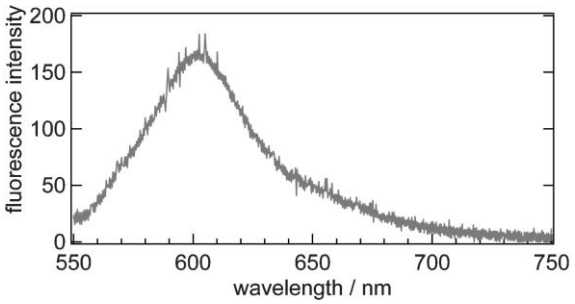

Fig. 3 Fluorescence spectrum of Texas Red immobilized in the 600 nm nanowells observed by an optical microscope with an optical waveguide using a p-polarised 532 nm laser light.

In summary, we emphasize the advantage of the present novel excitation method using nanowells. In this method, the photofunctional molecules were immobilized not on the gold surface but on the glass surface of the nanowells. Because the propagating distance of the SP field into free space from a rim of the nanowell was much longer than the effective distance of energy transfer quenching, *i.e.*, the Förster distance, we could evade energy transfer quenching of the photoexcited states by gold, which is an unavoidable problem of conventional SP excitation. The present method may be a useful strategy for molecular excitation in nanospace and we are now trying to detect hybridization of DNA and antigen–antibody binding in nanowells.

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Notes and references

† Although preliminary liquid phase fluorescence measurement in the nanowells was reported in ref. 6, immobilization of fluorophores at the bottom surface, which is essential for the bioassays, has not been attempted.

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