ELSEVIER

Sensors and Actuators B 22 (1994) 175–180

SENSORS AND ACTUATORS B CHEMICAL

Modelling of particle-enhanced sensitivity of the surface-plasmon-resonance biosensor

P.-T. Leung [a,*], Denise Pollard-Knight [b], Gordon P. Malan [c], Martin F. Finlan [c]

[a] *Department of Physics and Environmental Sciences and Resources Program, Portland State University, PO Box 751, Portland, OR 97207-0751, USA*

[b] *Scientific Generics, King's Court, Kirkwood Road, Cambridge CB4 2PF, UK*

[c] *Amersham International plc, Amersham Laboratories, White Lion Road, Amersham, Buckinghamshire HP7 9LL, UK*

Received 21 February 1994, in revised form 20 May 1994, accepted 25 May 1994

Abstract

A theoretical model is presented for a surface-plasmon-resonance (SPR) biosensor, used to sense particle-enhanced antigen–antibody binding The particles used in this technique, such as colloidal gold, are generally of high optical refractive index We propose a two-dimensional fractal-cluster model to study the effect of coalescence of these particles and compare our results with those obtained from the Maxwell-Garnett model This latter model ignores coalescence and assumes that the particles disperse randomly throughout the binding layer Our results show that the comparison depends critically on the fractal dimension, which is a measure of the clustering among the particles The conclusion is that for colloidal gold, clustering among particles will likely lead to greater signal enhancement, while for other particles, such as polystyrene and titanium dioxide, the enhancement is less certain due to their smaller refractive indices as well as the uncertainty in their fractal dimensions In addition, our results indicate that analysis of the SPR signal using the Maxwell-Garnett model could lead to an overestimate of the binding in this technique

Keywords Biosensors, Particle enhancement, Surface plasmon resonance

1. Introduction

Surface plasmon resonance (SPR) refers to the collective resonant oscillation of the free electrons at a metal–dielectric interface While this phenomenon was first predicted in 1957 [1], optical excitation (for flat surfaces) of SPR was not possible until the attenuation total reflection (ATR) technique was introduced by Otto [2] and Kretschmann [3] in the late 1960s In the ATR approach, the SPR excitation is manifested as a decrease in the reflectance curve for the intensity of incident light (at the 'dip angle') This effect is usually strongest with noble metals such as silver because of their relatively small dissipation coefficient (the imaginary part of the dielectric function) at visible/near-IR wavelengths Since then, optical excitation of SPR has been applied to many different areas of surface science, and in 1983 it was first applied to biosensing at the interface of a noble metal film [4] In most of the SPR-based biosensors, the Kretschmann geometry is usually adopted, with a layer of proteins (e g , antibodies) immobilized on the metal film When binding occurs between the specific agents (e g , certain antigens) in the sample and their immobilized partners, a change in optical properties is induced in the vicinity of the metal, and hence a change in the SPR signal, which is usually accompanied by an increase in reflectance ($\Delta\Re$) of the incident light at an incident angle just a little less than the 'dip angle' The process does not require any labelling and the binding can be monitored in real time Furthermore, by calibrating $\Delta\Re$, one could quantify the concentration of the binding agent in the sample within several minutes of time It was known from the outset that the sensitivity of this biosensor could be very high [4], easily achieving a detection level of nanomolar concentrations for samples of medium molecular weight, such as proteins larger than about 10 000 Da

Since 1983, many groups have started the research and development of this SPR biosensor (see, e g , Refs [5–10]), now, detection down to picomolar concentrations can be achieved for proteins or nucleic acids with high molecular weight Both the noble metals gold [5,6,10] and silver [7–9] have been successfully used as

* Corresponding author

SSDI 0925-4005(94)01306-3

the 'generator' for the surface plasmon and the problem of stabilization of silver in the presence of the biosamples has been overcome [7,8]. Experiments covering a large range of biological binding pairs have successfully been performed; these include, for example, avidin/biotin and α-feto protein/antibody [5], lectin/glycoprotein, DNA/DNA, DNA/RNA, hapten/antibody, hormone/hormone receptor [7,8], dinitrophenyl/bovine serum albumin [9] and monoclonal antibody binding to carboxymethylated dextran hydrogel [10].

2. Enhancement of sensitivity

As with any sensor, the optimization and enhancement of the sensitivity are crucial for the SPR biosensor, and they have been studied intensively since it was first introduced in 1983. Factors affecting the sensitivity of the biosensor can conveniently be classified into two groups: physical (optical) and biochemical factors. While optimization of the properties of the metal film (e.g., film thickness and smoothness, film material, etc.) and those of the prism (e.g., refractive index) certainly belongs to the first group, labelling with an enzyme serves as an example of the second [7]. Furthermore, it has also been reported that increasing the probe light frequency [11], as well as using impure gold and a kind of 'flow-cell design' that optimizes mass transport [10], will enhance the sensitivity of such a sensor. In addition to the optimization of the different components of the biosensor, a powerful technique based on the linkage with optically active particles has also been proposed [12,13]. In this particle-linkage technique, the specific binding agents are bound to certain mesoscopic particles before they are introduced into the sensor. Thus, when binding occurs between these agents and their immobilized partners on the metal film, a larger signal will be induced by the presence of these particles in the sensing layer (within the evanescent field) near the surface of the metal (see Fig. 1). In particular, picomolar concentrations of sheep antibodies linked to polystyrene beads have been detected [12] and recently, enhancement of sensitivity in the detection of antibody to porcine growth hormone linked to colloidal gold particles has been reported [13].

The optimization of this particle-linkage technique depends on certain factors, the effects of which are determined by the system used (e.g., size and refractive index of the particles, number of binding agents attached to each particle, etc.). However, the effect of the *distribution* of the particles is more subtle and highly significant. For example, for a fixed concentration of particles bound through the attached agent to the immobilized layer, there are two extremes for the particle distribution over the layer. In one case the particles may disperse more or less randomly throughout the layer, and in the other case they may coalesce to form local clusters. This coalescence may exist already in the colloidal solution into which the binding agent is introduced to link to the particles, or it may also take place at the sensor interface where binding occurs. In the first instance, although it is possible for the affinity of these 'agent clusters' to be reduced, the fact that enhanced detection is still observed in experiments indicates that this reduction in affinity is not a problem [12,13]. It is the purpose of the present communication to present a modelling study of the effect of this clustering on the sensitivity of the biosensor.

3. Theoretical modelling

The optical sensor system can be modelled as displayed in Fig. 1. The Kretschmann geometry consists of a hemicylindrical prism with a silver film (56 nm) deposited on it. The layer of binding pairs is represented by a dielectric film of refractive index 1.4 and a thickness of approximately 10 nm (i.e., approximate values that might represent an antibody molecule). The mesoscopic particles are assumed to be spherical with radius a and refractive index n. The buffer layer, which is mainly water (refractive index 1.33), is assumed to have infinite extent in the modelling. The light source is taken to be a diode laser of 780 nm wavelength and the complex refractive index for silver at this wavelength can easily be obtained as $0.143 + 5.12\,i$ from a source book [14].

To describe the optical properties of the particle layer (layer 3), we have considered the following two models. Our interest here is only in the low-concentration limit of the binding agent (hence also of the particles), with a submonolayer coverage and volume fraction for the attached particles much smaller than unity.

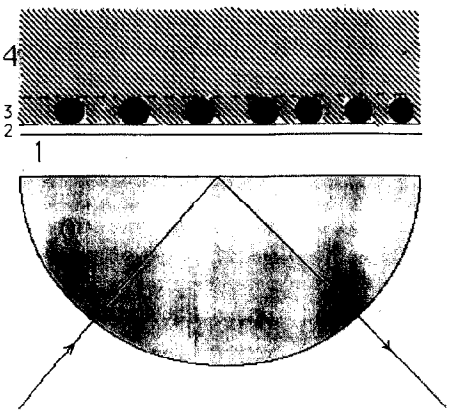

Fig. 1. Schematic set-up of the particle-linkage technique for the SPR biosensor. Refer to the text for details of the individual layers 0, 1, 2, 3, 4.

3.1. Model A: the Maxwell-Garnett model

To model the case when the particles are dispersed throughout the layer, we use the Maxwell-Garnett (MG) model to determine the average dielectric function (ϵ_3) of the particle layer. The MG model is an effective medium theory that is accurate for small particle concentration. For spherical particles of dielectric function $\epsilon = n^2$, ϵ_3 can be given in the following form [15]:

$$\epsilon_3 = \epsilon_m \left(1 + \frac{3f\beta}{1-f\beta}\right) \tag{1}$$

where ϵ_m is the dielectric function of the host medium for the particles. In our case

$$\epsilon_m = \epsilon_4 = 1.33^2 \tag{2}$$

In Eq. (1) f is the submonolayer volume fraction and can be given by $f = c\pi/6$ for spherical inclusions, where c is the percentage coverage; β in Eq. (1) is sometimes called the depolarizing field factor. We thus have, for spherical particles [15],

$$\beta = \frac{\epsilon - \epsilon_m}{\epsilon + 2\epsilon_m} \tag{3}$$

3.2. Model B: the two-dimensional fractal cluster model

For the other extreme when the particles coalesce to form local clusters, we have adopted the differential effective medium formalism of Hui and Stroud for the optical properties of fractal clusters [16]. Despite the simplicity of this model, it has been found that the results obtained from this model agree quite well with those from a more accurate computer-simulation approach [17]. To be applicable to our present situation of a particle layer in a biosensor, the original version of the fractal-cluster (FC) model must be varied a little. Instead of forming three-dimensional (3D) fractal clusters as in Ref. [16], mainly two-dimensional (2D) clusters are formed here when the particles coalesce on top of the immobilized protein layer. Following Ref. [16] closely, we assume at a certain instant during the formation of the 2D (cylindrical) cluster that the size has grown to a radius R (thickness $2a$), the dielectric function of which is $\epsilon(R)$. Then, for an infinitesimal increment in the size of the cluster, one can apply the effective medium theory to obtain [16]

$$\epsilon(R + dR) = \epsilon(R) - 2\frac{f_c'(R)}{f_c(R)}\, dR\epsilon(R)\, \frac{\epsilon_m - \epsilon(R)}{\epsilon_m + \epsilon(R)} \tag{4}$$

where $f_c'(R) = df_c/dR$ with f_c being the volume fraction of the particle in the cluster. For a 2D FC, we have

$$f_c(R) = \left(\frac{R}{a}\right)^{d_f - 2} \tag{5}$$

with d_f (<2) being the fractal dimension of the cluster. Hence one can obtain a differential equation involving $\epsilon(R)$ from Eq. (4) which, on integration, yields the following algebraic equation for $\epsilon(R)$:

$$\frac{\epsilon(R)}{\epsilon(a)}\left[\frac{\epsilon_m - \epsilon(a)}{\epsilon_m - \epsilon(R)}\right]^2 = [f_c(R)]^{-2} \tag{6}$$

This result closely resembles that for the 3D cluster given in Ref. 16. Note that $\epsilon(a) = \epsilon$ and is simply the dielectric function of the particles. Solving Eq. (6) as a quadratic equation for $\epsilon(R)$ and ignoring the solution that is less than unity, one can obtain a unique result for the dielectric function of *one* cluster of the spherical particles when they coalesce. In the case when both roots turn out to be greater than one, the one lying between ϵ and ϵ_m should be chosen. We have to remark that the particles we study here are to a large extent non-dissipative, so that they all have a real dielectric function or the real part is far greater than the imaginary part in their dielectric functions. Even for the case of colloidal gold (see below), we expect the optical properties to be quite different from those of bulk metallic gold due to the presence of the dispersing medium [12]. In the case of particles with complex dielectric functions, the criteria of choosing the unique solution from Eq. (6) will be different and will depend on the sign of the imaginary part of the roots [16]. The ultimate dielectric function for the whole layer of particles when they coalesce to form local fractal clusters is obtained by another application of the effective medium (MG) theory to a *collection of these 2D clusters*, as indicated briefly below.

Let f_v be the volume fraction of these 2D clusters in the layer. Then, obviously, we have $f = f_v f_c$. With this, the average dielectric function for layer 3 (the particle layer) in the particle clustering case can then be given by

$$\epsilon_3 = \epsilon_m \left(1 + \frac{2f_v\beta_c}{1 - f_v\beta_c}\right) \tag{7}$$

where ϵ_m is as in Eq. (2) and β_c is the depolarizing field factor of the 2D cluster:

$$\beta_c = \frac{\epsilon(R) - \epsilon_m}{\epsilon(R) + \epsilon_m} \tag{8}$$

Note that the factor two appears in Eq. (7) as compared to the factor three in Eq. (1). This occurs because the clusters are now cylindrical in shape in the 2D case.

With the dielectric function ϵ_3 described by either of these two models and those functions for the other four layers (0 = glass, 1 = silver, 2 = protein and 4 = buffer (water)) being known, one can calculate the SPR response (i.e., the reflectance ($\Re$) curve) using the usual formulation from Fresnel optics [18].

4. Results and discussion

We have used both Eq. (1) and Eq. (7) to study the effect on the SPR signal when the linked particles disperse uniformly throughout the binding layer and when they coalesce to form clusters, respectively. We have taken colloidal gold as an example for the linked particles [13]. It has been established that colloidal gold in solution does coalesce to show fractal-clustering behaviour [19], and for a 2D cluster the dimension d_f is approximately given by 1.45 [17]. The refractive index for colloidal gold is taken to be 4.86 [12] and is different from that for bulk metallic gold [14]. Fig. 2 shows the calculation of $\Re$ versus incident angle for this case. The cluster size is taken as $R=10a$ and the size of the particle is taken as $a=10$ nm [19]. The broken curve shows the signal without linking the binding agent to any of the particles. The dash–double dotted curves show the shifted reflectance curves for different monolayer coverage (c) of particles in the case with no clustering (i.e., Eq. (1) for ϵ_3). The solid curves, on the other hand, are results for the corresponding values of c when the particles form local fractal clusters (i.e., Eq. (7) for ϵ_3). It is obvious from the results that coalescence among the particles will definitely lead to a larger enhancement in the SPR signal for the biosensor. Furthermore, it can be seen that the enhancement becomes more significant (relative to the particle dispersing case) when the coverage increases in value. This result implies that if one applies the MG model to calibrate the SPR signal obtained in this particle-linkage technique, an overestimate of the concentration of the particles (hence the biosamples) can result, owing to the clustering of these particles. Fig. 3 shows a plot of this possible overestimate versus the true concentration (c) of the particles obtained from analysing the results in Fig. 2. The y-axis represents the quantity $(c'-c)/c$ where c' is the value needed for particle coverage in the MG model that will produce the same 'shift' in the SPR curve as in the FC model with a concentration c for the particles. It is seen that the 'relative overestimate' can be as high as 50% in this case and the variation with c is almost linear. Although the result in Fig. 3 is specific to the many parameters fixed in the modelling that leads to the results obtained in Fig. 2, we expect the qualitative feature of Fig. 3 to have some general validity.

We have also studied the effect of different cluster sizes ranging from $R/a=5$ to $R/a=50$. Here we compare the SPR signals for different cluster radii (R) with all other parameters fixed. We found that the computed reflectance curves, apart from some very minor shifts in dip position and magnitude, are to a large extent very insensitive to R in our model. On the other hand, this signal enhancement due to the clustering of gold particles is very sensitive to the fractal dimension (d_f), i.e., to a certain extent the 'degree of clustering' in our modelling. Fig. 4 illustrates this point by comparing the MG modelling result with the FC results (for

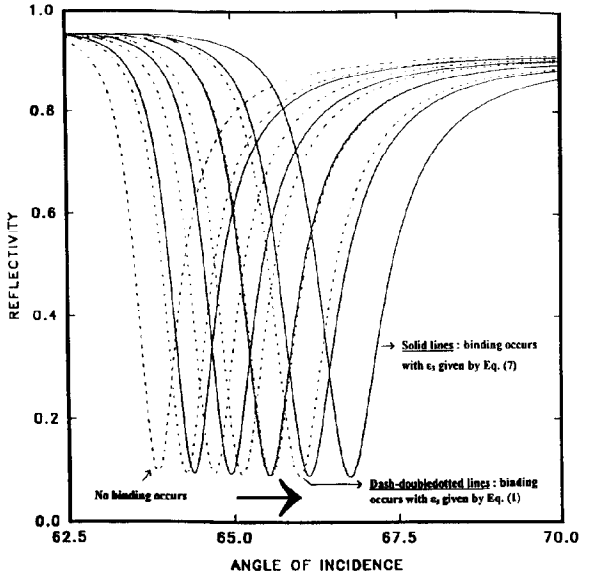

Fig. 2. Curves for SPR reflectance vs. incident angle of the probe light for colloidal gold as the particles linked to the specific binding agents. The broken curve is for binding without any labelling of particles. The dash–double dotted and solid curves are results with particle linkage when the particles disperse and when they coalesce to form fractal clusters, respectively. Curves shift to larger resonant angles in order of higher monolayer coverage: $c=0.05$, 0.10, 0.15, 0.20, 0.25.

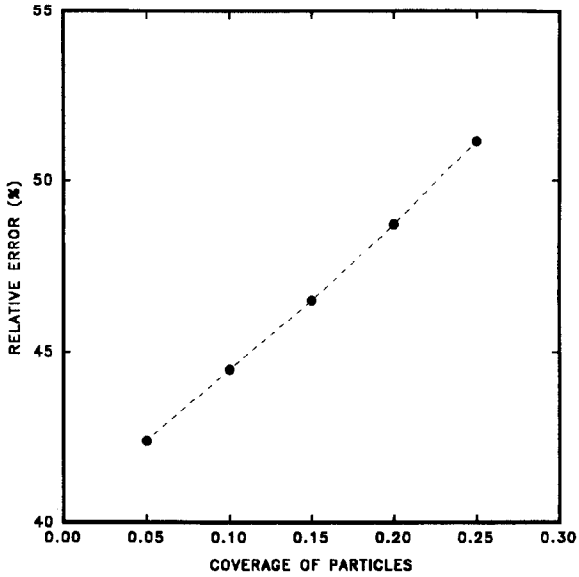

Fig. 3. Illustration of possible relative overestimate in the concentration of the colloidal gold particles as a function of particle coverage. The overestimates arise from ignoring the clustering behaviour and applying the MG model to analyse the SPR signal. All parameters in the models are as fixed in Fig. 2.

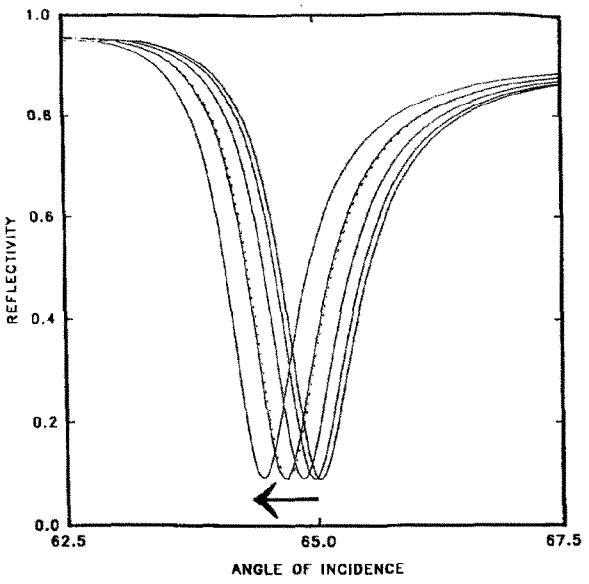

Fig. 4. Same as in Fig. 2, except that c is fixed at 0.10 and d_f is varied for the range of values 1.2, 1.4, 1.6, 1.8, 2.0 for the FC modelling. Note that the SPR curves shift to lower dip angles as d_f increases. The corresponding MG result is shown in dash–double dotted lines and the result with no particles is not shown.

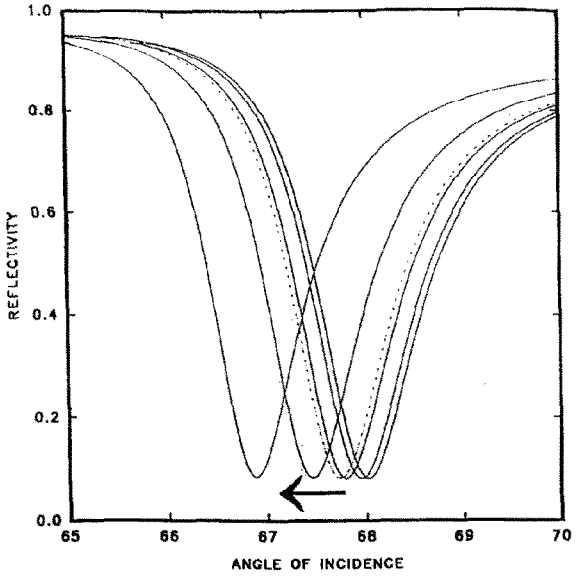

Fig. 5. Same as in Fig. 4, except the results are for TiO_2 particles with refractive index equal to 2.9 [12], diameter equal to 50 nm and c fixed at 0.25.

colloidal gold particles) for different values of d_f at a fixed coverage $c=0.1$. It is seen that the FC curves start to have their minima shifted back towards smaller incident angles as d_f increases from 1.2 to 2.0. Above a certain critical value for d_f (in this case about 1.8), the FC result will lead to a smaller shift in the SPR curve compared to the MG case when binding occurs (note that the curve without any particle linkage is not shown here and is the same broken curve in Fig. 2). Nevertheless, the clustering enhancement for the case of colloidal gold is very likely, since d_f in this case is known to be close to 1.45. We have also modelled the case when polystyrene beads and titanium dioxide are used as the mesoscopic particles. For the latter case, Fig. 5 shows that the FC enhancement will exist if d_f for these particles is not greater than about 1.7 (assuming the particles do form local clusters). For the case with polystyrene beads, FC enhancement is not found for any value of $d_f > 1$. Hence we conclude that this particle-linkage technique will definitely lead to enhanced sensitivity in the SPR biosensor. To optimize this enhancement, one should try to use particles with large refractive indices and preferably, at least in the case of colloidal gold, the particles should tend to coalesce on top of the binding layer rather than dispersing themselves through the layer. In general, the technique must be carefully optimized and controlled in order to achieve greater sensitivities. In particular, without knowing the details of the distribution of the particles on the binding layer, one must calibrate the results for the sample concentration carefully by being aware of the possible effects due to clustering of the particles. Although previous experiments [12,13] have indeed confirmed the capability of enhancing the SPR biosensor sensitivity using this particle-linkage technique, as far as we are aware, a detailed quantitative experiment studying the various effects (especially that from the distribution of the particles) has yet to be carried out. We hope that our present modelling work will stimulate experimentalists to engage in more detailed study of this technique in the future.

Acknowledgements

One of the authors (P.T.L.) would like to acknowledge very useful communication with Dr P.M. Hui of the Chinese University of Hong Kong and the support of the Faculty Development Funds from Portland State University. This is Environmental Sciences and Resources Program Publication No. 296.

References

[1] R.H. Ritchie, Plasmon losses by fast electrons in thin films, *Phys. Rev., 106* (1957) 874.

[2] A. Otto, Excitation of nonradiative surface plasma waves in silver by method of frustrated total reflection, *Z. Phys. 216* (1968) 398.

[3] E. Kretschmann, The determination of the optical constants of metals by excitation of surface plasmons, *Z. Phys., 241* (1971) 313.

[4] B. Liedberg, C. Nylander and I. Lundström, Surface plasmon resonance for gas detection and biosensing, *Sensors and Actuators, 4* (1983) 299.

[5] P.B. Daniels, J.K. Deacon, M.J. Eddowes and D.G. Pedley, Surface plasmon resonance applied to immunosensing, *Sensors and Actuators, 15* (1988) 11.

[6] D.C. Cullen, R.G.W. Brown and C.R. Lowe, Detection of immuno-complex formation via surface plasmon resonance on gold-coated diffraction gratings, *Biosensors, 3* (1987/88) 211; D.C. Cullen and C.R. Lowe, A direct surface plasmon-polariton immunosensor: preliminary investigation of the non-specific adsorption of serum components to the sensor interface, *Sensors and Actuators, B1* (1990) 576.

[7] S.A. Charles, T. Endericks, A.G. Evans, S.E. Garnham, J.C. Irlam, D. Pollard-Knight, M. Downes, P.J. Heaney, M.F. Finlan and P.B. Garland, A biosensor based on surface plasmon resonance – principles, performance and applications, in C.R. Canter (ed.), *Biotechnology and Human Genetic Predisposition to Disease,* Wile-Liss, New York, 1990, p. 219.

[8] D. Pollard-Knight, E. Hawkins, D. Yeung, D.P. Pashby, M. Simpson, A. McDougall, P. Buckle and S.A. Charles, Immunoassays and nucleic acid detection with a biosensor based on surface plasmon resonance, *Ann. Biol. Clin., 48* (1990) 642.

[9] M.T. Flanagan and R.H. Pantell, Surface plasmon resonance and immunosensors, *Electron Lett., 20* (1984) 968; E. Fontana, R.H. Pantell and S. Strober, Surface plasmon immunoassay, *Appl. Opt., 29* (1990) 4694.

[10] S. Lofas, M. Malmqvist, I. Ronnberg, E. Stenberg, B. Liedberg and I. Lundström, Bioanalysis with surface plasmon resonance, *Sensors and Actuators B, 5* (1991) 79; E. Stenberg, B. Persson, H. Roos and C. Urbaniczky, Quantitative determination of surface concentration of protein with surface plasmon resonance using radiolabeled proteins, *J. Coll. Interface Sci., 143* (1991) 513.

[11] R.P.H. Kooyman, H. Kolkman, J. van Gent and J. Greve, Surface plasmon resonance immunosensors: sensitivity considerations, *Anal. Chim. Acta, 213* (1988) 35.

[12] T. Endericks, J. Jackman, A. Allen and D. Pollard-Knight, unpublished.

[13] H. Sadeghi and B. Wang, Enhancement of BIA core sensitivity with colloidal gold labelled secondary antibodies, in R. Granzow (ed.), *BIAsymposium Meet. Review,* Pharmacia Biosensor, Piscataway, NJ, 1992, p. 56.

[14] E.D. Palik (ed.), *Handbook of Optical Constants of Solids,* Academic, New York, 1985, p. 350.

[15] J.C. Maxwell-Garnett, Colours in metal glasses and in metallic films, *Philos. Trans. R. Soc. London, 203* (1904) 385.

[16] P.M. Hui and D. Stroud, Complex dielectric response of metal-particle clusters, *Phys. Rev. B, 33* (1986) 2163.

[17] C.Y. Chang, L.C. Kuo and P.M. Hui, Effects of clustering in binary composites: random fractals, *Phys. Rev. B, 46* (1992) 14505.

[18] See, e.g., O.S. Heavens, *Optical Properties of Thin Solid Films,* Dover, New York, 1965.

[19] See, e.g., P. Meakin, The growth of fractal aggregates and their fractal measures, in C. Domb and J.L. Lebowitz (eds.), *Phase Transition and Critical Phenomena,* Vol. 12, Academic, New York, 1988, p. 335.

Biographies

Pui-Tak Leung received a Ph.D. (1982) in theoretical physics from the State University of New York at Buffalo, USA. He has carried out research in the modeling of various optical phenomena at interfaces, including molecular fluorescence and pulsed-laser interaction with surfaces. He was a visiting scientist at IBM Almaden Research Center (1991–92) and a consultant to Amersham International on the surface plasmon resonance biosensor (1989–92). He has been an associate professor of physics at Portland State University since 1991.

Denise V. Pollard-Knight received B.Sc. and Ph.D. degrees in biochemistry from Birmingham University, UK, and was a Fullbright Scholar at the University of California, Berkeley. She was at Amersham International for four years, where she developed non-radioactive labelling methods and worked as research manager on the surface plasmon resonance biosensor. She then spent three years at Fisons Applied Sensor Technology, Cambridge, as Biosciences Research Manager developing their resonant mirror biosensor. Currently she is Biotechnology Skills-group Manager at Scientific Generics, Cambridge.

P. Gordon Malan received a B.Sc. in chemistry and a Ph.D. in biochemistry from the University of Edinburgh, Scotland. He then spent three years of post-doctoral fellowships at the National Institutes of Health, Bethesda, MD, and the University of Pittsburgh, PA. He was a senior lecturer, Middlesex Hospital Medical School, University of London, working on thyroid endocrinology and immunoassay data-analysis. He has been at Amersham International since 1980, where he carried out R&D work on the surface plasmon resonance biosensor. Currently he is Biosystems and Computing Manager for R&D.

Martin F. Finlan has B.Sc. M.Inst.P. and C.Phy. degrees. He has worked for the Ministry of Defence, Department of Atomic Energy, including 12 years working on thermal and fast reactor developments for the UK AEA. He joined Amersham International (then the Radiochemical Centre) in 1963, where he commissioned and ran cyclotrons for isotope production. He holds about 30 patents, including a super-conducting cyclotron now being manufactured by Oxford Instruments. As Assistant Director of Research, he was jointly responsible for initiating Amersham's surface plasmon resonance biosensor programme in 1985. He has been a consultant on physical sciences to Amersham International since 1990.