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By
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Interaction of Localized Surface Plasmon Resonance
Transducers with Dielectric and Fluorescent Materials

Advisor:
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This work was carried out under the supervision of Prof. Israel Rubinstein in the Department of Materials and Interfaces, Weizmann Institute of Science, Rehovot, Israel

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To Dolev
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<tr>
<td>A.U.</td>
<td>Absorbance units</td>
</tr>
<tr>
<td>APTS</td>
<td>3-Aminopropyltrimethoxysilane</td>
</tr>
<tr>
<td>AuCont</td>
<td>Slide coated with a continuous 100 nm thick gold film</td>
</tr>
<tr>
<td>AuXnm</td>
<td>Slide coated with gold island film, of X nm nominal (mass) thickness</td>
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<tr>
<td>HRSEM</td>
<td>High resolution scanning electron microscopy</td>
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<tr>
<td>LbL</td>
<td>Layer-by-layer</td>
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<tr>
<td>LSPR</td>
<td>Localized surface plasmon resonance</td>
</tr>
<tr>
<td>MEF</td>
<td>Metal enhanced fluorescence</td>
</tr>
<tr>
<td>NP</td>
<td>Nanoparticle</td>
</tr>
<tr>
<td>PAH</td>
<td>Polyallylamine hydrochloride</td>
</tr>
<tr>
<td>PE</td>
<td>Polyelectrolyte</td>
</tr>
<tr>
<td>PSS</td>
<td>Polystyrene sulfonate, sodium salt</td>
</tr>
<tr>
<td>PVD</td>
<td>Physical vapor deposition</td>
</tr>
<tr>
<td>RI</td>
<td>Refractive index</td>
</tr>
<tr>
<td>RIS</td>
<td>Refractive index sensitivity</td>
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<tr>
<td>SEM</td>
<td>Scanning electron microscopy</td>
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<tr>
<td>SP</td>
<td>Surface plasmon</td>
</tr>
<tr>
<td>SPR</td>
<td>Surface plasmon resonance</td>
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<tr>
<td>TEM</td>
<td>Transmission electron microscopy</td>
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<tr>
<td>UV-vis</td>
<td>Ultraviolet-visible</td>
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1 Abstract

Gold nanoparticles (NPs) display an extinction band in the UV-vis-NIR range, attributed to localized surface plasmon resonance (LSPR) with incident light, shifting in response to changes in the dielectric properties of the environment. This effect furnishes the basis for use of such NPs as transducers for sensing purposes. The response to bulk refractive index (RI) changes is quantified as the refractive index sensitivity (RIS). The distance dependent response (plasmon decay length) to layer adsorption, essential to sensing performance, is rarely determined. Here we measured systematically the decay length for a series of gold nano-island films of different average NP size, and found it to be correlated with the RIS, and with NP size. Thus, smaller particles can offer superior sensing performance, despite their lower RIS, thanks to better matching of their smaller sensing volumes with the analyte size.

LSPR transducers can be monitored in both transmission and reflection modes, but the literature contained no comparison of the two modes. We explored this issue for both bulk RI changes and layer adsorption on gold nano-island films of various sizes, and found that reflection mode measurements offer higher responses in every case.

LSPR transducers and sensors are necessarily in competition with other sensing methods, such as the Fabry-Pérot interferometer, which utilizes light interference. We compared the responses of both transducers in various sensing scenarios and found that the LSPR transducers are superior for thin layers (< ~10 nm), while Fabry-Pérot interferometers offer some advantages for thicker recognition interfaces or analytes.

Studies in the literature reported a “long range” plasmon response – plasmon peak shifts in response to layers adsorbed on top of spacers hundreds of nanometers thick, far beyond the expected plasmon decay lengths. We studied this effect, and showed that its origin is in thin film interference. We also managed to extract information regarding the phase shifts of light upon reflection from nano-island films.

Plasmonic structures can enhance or quench fluorescence, depending, among other parameters, on the distance between the metal structure and the fluorophore. We studied the distance dependence of metal-enhanced fluorescence, using a fluorescent metal complex and gold island films. The results show enhancement of the emission in the range of hundreds of nanometers, indicating that the NP films act as mirrors in the far-field. We observed oscillations in the fluorescence lifetime, emission wavelength and linewidth as a function of metal-fluorophore separation.
לנוג-חילקיק (נוסח"ג): קבחי בילעה או חיקה בשיש התוך-אידומ-ואר-כראה-עיל-סוגל, המגיעה

לשניים בתכונת הזריקה לצורכי השבבים, עקר תודעה פלטום מפותחי מומרים (תפמ"ג) של מקדם ההופעה, זה החממה למטרות לחישה. את התוכונה של שניים במקדם השיבוץ של השמירה בשיבוץ פלטום גרוס cáiramids למתקד

שבירה (רמ"ג). התכונה התולדה בברך (מדいただく כתב, ה יחסי לחיות), נמדדת כא萊 של בחום. בנבון, מצוינת עדית, לאール "ל"ג ממוזג שלמ. המעתק העניין נמס על ירי "ל"ג

וב阳县 יוה. לק, חלקיי קלט יולי לפלק נוצרישי משות דוד פל الزوجת Pune מתמדת בקרוב את החית

_ant, ולש הדמות התמקדה של פקיה אחת מתמ"ג. יניק בלקית כתב "מד"ג" במעת ורבד וירדה, החיה ניוזים גמישים הבבキャלה

ולא נמצאה ספרות של פלמק"ג. חקרנו את המספר גם עבור מצבים אלה, והDao NIGHT בל רמ"ש מ蹽 וביר. חלקיקים קטנים י

על עובי השכבה הממדים מצומצם החישה הנפח התאמה טוב יותר, אך ת אורר מתמרי תפמ"ג במצבי העברה וחזרה

חלקיקים גדולים. ניתן לנטה של ביחס ל

שינויי מקדם השבירה. חקרנו את המספר גם עבור ספיחת שכבות על מצעי ננו-חילקיק, צב ליאל שיו, ומוצר

של המפרט וwpdb פלטום עבור משני-חילקיק, צב ליאל שיו, ומוצר

מדידה במדח של החזרה מפותחת התוך-אידומ כל המקראים.

תרומת תפמ"ג מזווית של הכרה בתכונה של antות ד כדי, ואת ה marché מתהל.

האטרכט או, באנטרופורטר פאבר-פר. השו órgão לתכונת של שני גומי התמ"ג במנון תרכות משות. ממון קר מתמי מתמ"ג"ע רגלים, ובשכבות דקור (בולע דוב של

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וס机油 עטיב י_SAVE.

פרוטומום בסקפוץ דווי על תכונה פלטומ"ג "ארוכת טווח" – שיימ-ביש פלטומני

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3 Introduction

3.1 Localized surface plasmon resonance (LSPR): Background

The past 15 years have seen an explosion of research in the field of Plasmonics, with researchers from varied background, such as physics, chemistry, biology and engineering exploring the various principles and applications. The field is concerned with plasmons, which are charge-density oscillations, typically (though not always) in metals. Plasmons come in several varieties – bulk plasmons exist in bulk metals, and are typically of little interest, as coupling into them (and thus exciting and utilizing them) is difficult, though it can be achieved using electron beams, for instance. At the interface between two materials with dielectric constants of opposing signs in the real part, there can exist surface plasmons (SPs), i.e., charge density oscillations confined to the surface and propagating along the interface. SPs can be excited by incident light of appropriate frequency, to form a surface plasmon polariton (SPP), a hybridized excitation of photon and plasmon. As the surface plasmon has a significantly higher momentum than a photon of the same frequency, excitation is achieved though prisms or gratings on the metal surface, which increase the photons’ momentum. This energy-dependent excitation process is termed surface plasmon resonance (SPR), and the coupled energy is later dissipated as re-emitted light and heat. High loss in metals limits the propagating distance of SPPs to a few microns.

Further confinement, in nanoparticles (NPs) or nano-sized features in larger metallic films, support localized surface plasmons. Incident light readily couples to these, in a process termed localized surface plasmon resonance (LSPR), with which the work herein in concerned. We focus on optical measurements, where the interaction is manifested as a strong extinction band in the UV-vis-NIR range, different from the spectrum of bulk metal. The frequency and intensity of the resonance band depend on a variety of factors, including the metal composition, size, shape, and dielectric properties of the surroundings.

In 1908 Gustav Mie expressed the Maxwell equations for the case of spherical NPs in a homogenous medium. The extinction of a film of metallic spheres in the long-wavelength, electrostatic dipole limit can be described by:

$$E(\lambda) = \frac{24\pi^2 N_A a^3 \varepsilon_i^2}{\lambda \ln(10)} \left[ \frac{\varepsilon_i}{(\varepsilon_r + \chi \varepsilon_m)^2 + \varepsilon_i^2} \right]$$

(1)
where $E(\lambda)$ is the extinction (scattering and absorption), $N_A$ is the area density of nanoparticles, $a$ is the radius of the metallic sphere, $\varepsilon_m$ is the dielectric constant of the medium surrounding the metallic nanosphere, $\lambda$ is the wavelength of the incident radiation, $\varepsilon_i$ and $\varepsilon_r$ are the imaginary and real components of the metallic NP’s dielectric function, and $\chi$ describes the aspect ratio of the NP (two for a sphere).

Electromagnetic coupling between the particles can also play a part, in the case of short inter-particle spacing - roughly less than the particle radius.\(^8\) The systems discussed in this work display only weak coupling effects, and this is discussed in section 5.3. For NP ensembles on a substrate, their spectrum will depend, in addition, on the in-plane diameter, out-of-plane height, the substrate dielectric function, and the beam angle relative to the surface.\(^9\) Maximal extinction (i.e., the surface plasmon band) will be observed at a wavelength for which $\varepsilon_i(\lambda)$ is relatively small and $\varepsilon_r(\lambda) = -\chi\varepsilon_m$. Thus, a change in $\varepsilon_m$, due to changing the medium, or binding of adsorbates onto the metallic nanostructure, leads to a shift in the resonance wavelength, and a change in the extinction intensity.\(^10\) This property is widely investigated for use in sensing.\(^6,11-16\)

The extinction band is the result of two distinct phenomena, absorption and reflection (specular and scattered), and both respond to changes in the dielectric constant of the adjacent medium.

### 3.2 LSPR sensing and distance dependence

The common case of sensing adsorbed analyte layers on plasmonic particles is effectively a core-shell system (metal core - dielectric shell). A solution for scattering by a small coated sphere was first formulated by Aden and Kerker in 1951,\(^17\) and later generalized to a multilayer coating by Bohren and Huffman.\(^18\) Extensions of Mie theory for the core-shell case were also formulated, e.g., by Xu et al.\(^19\) and Khlebtsov et al.\(^20\) While a rigorous core-shell approach is more accurate, it was found that the response of an LSPR transducer to layer adsorption can be more easily described by the phenomenological equation:\(^21\)

\[
R = m\Delta \eta \left(1 - e^{-\frac{a}{\Delta \eta}}\right)
\]

where $R$ is the response (wavelength shift, intensity change), $m$ is the refractive index sensitivity (RIS), a parameter of the transducer, $\Delta \eta$ is the difference in the refractive
index (RI) between the adsorbed layer and the medium it displaced (e.g., air, water), $d$ is the layer thickness, and $l_d$ is the plasmon decay length. The RIS can be easily measured by measuring the transducer’s spectrum in solvents of different RI; the effectively infinite layer thickness simplifies Equation 2 to a linear relation $R = m \Delta \eta$. Note that Equation 2, and the derived linear relation, are only the first approximation of the LSPR response to RI change; over a broad RI range, the response function sometimes displays clear nonlinearity, and this can be accounted for by adding a quadratic term. The deviation from linearity, however, is typically small, and Equation 2 usually suffices for practical matters. To afford specificity, an LSPR transducer is commonly coated with a recognition interface, a layer which selectively adsorbs a desired analyte; the equation for this case is then:

$$R = m \Delta \eta e^{\frac{-d_1}{l_d}} \left( 1 - e^{\frac{-d_2}{l_d}} \right)$$  \hspace{1cm} (3)$$

where $d_1$ and $d_2$ are the thicknesses of the recognition interface and analyte layer, respectively. For simplicity, we assume the same RI for both layers; most organic or biological layers have similar RIs of about 1.5. To maximize the response, the RIS must be as high as possible, but the optimal decay length depends on the thicknesses of both the recognition interface and analyte layers.

Over the years, several groups have investigated the plasmon distance dependence for various systems, though only rarely quantified it. In 1996, Liz-Marzán et al. synthesized Au-silica core-shell NPs, and examined the effect of the shell thickness on the spectra. Schmitt et al. later formed Au NP monolayers coated with polyelectrolyte (PE) multilayers, and observed an exponentially diminishing per-layer response. Okamoto et al. coated NP monolayers with the polymer PMMA and quantified the decay length for two particle sizes. In 2001 Malinsky et al. fabricated Ag nano-triangle arrays and found a linear short-range response (up to 2 nm) using alkanethiols of increasing chain lengths. This work was later expanded by Haes et al. to include several particle sizes, and to examine the long range response (up to 40 nm) of those same particles, using a Cu-thioacid multilayer system; they also reported an exponential decay of the response, though again did not quantify the distance dependence. Haes et al. also suggested utilizing different sizes and shapes of particles to tune the decay length for the desired application. Whitney et al. performed a similar study of these systems using atomic layer deposition (ALD) to obtain high-resolution information (in adlayer thickness). In 2002, Xu et al.
formulated an extension of Mie theory for core-shell particles, and noted the superior performance of small particles in certain conditions, due to their stronger field localization.\textsuperscript{19} Nath \textit{et al.} adsorbed a few PE bilayers on multiple types of Au NP monolayers, with diameters varying from 12 to 49 nm, noting a faster decay for smaller particles.\textsuperscript{29} Work done by our group in 2005 by Doron-Mor \textit{et al.} explored the distance dependence of the response in three systems of random Au nano-islands, using a coordination-based multilayer system, previously developed in the group.\textsuperscript{30} The same year, Rindzevicius \textit{et al.} used alkanethiols to study the short-range (up to 2 nm) response of nanoholes in an Au film, and roughly estimated their decay length.\textsuperscript{31} In 2009 Chen \textit{et al.} measured the decay length for high- and low-aspect ratio Au nanodisks.\textsuperscript{32} Kiel \textit{et al.} also utilized PE multilayers to explore the distance dependence for three Au NP diameters, but, again, did not quantify their results.\textsuperscript{33}

Despite these studies, the vast majority of works characterizing LSPR transducers still focus on maximizing the RIS, neglecting the effect of the decay length. In addition, there have been no studies systematically measuring the decay length and RIS for a series of well characterized transducers. For this reason we decided to measure the decay length and RIS for a series of nano-island films (examples are shown in Figure 1), of different average particle size, leading to the important relationships reported in section 5.1.

\textbf{Figure 1.} High-resolution scanning electron microscopy (HRSEM) images of three of the gold island film types used in this work. The samples are of (A) 3, (B) 5 and (C) 10 nm nominal Au thickness, annealed at 580 °C for 10 hr, coated with 2, 3 and 3 nm Cr, respectively, and imaged using the In-Lens secondary electron detector at an accelerating voltage of 15 kV.
3.3 Polyelectrolyte layer-by-layer (LbL) deposition

A substantial portion of this work requires forming layered structures of well-defined thickness on LSPR transducers, either to examine the plasmon response to the layer itself, or to use it as a dielectric spacer, to place some material at a known separation from the transducer. Our main tool to this end is polyelectrolyte layer-by-layer deposition, developed by Decher,\textsuperscript{34} in which alternate layers of oppositely-charged polyelectrolytes (PE) are built in a stepwise fashion, \textit{via} electrostatic interactions. This method is widely-used, and is commonly noted for simplicity and reliability,\textsuperscript{35,36} which has proved true in this work as well. The procedure used for building the polyelectrolyte multilayer coatings is illustrated in Figure 2. The specific choice of polyelectrolyte, in combination with the deposition conditions, determines the thickness of each layer,\textsuperscript{37,38} and whether the layers grow in a linear or exponential fashion.\textsuperscript{39} As the layers are strongly charged, they enable binding of other charged species on the final PE layer, such as metallic NPs (stabilized with charged ligands) or ionic dye molecules.\textsuperscript{40}

![Figure 2. Schematic illustration of the procedure used to build polyelectrolyte multilayers.](image)

3.4 Reflection and transmission measurements

The plasmon band can be monitored using transmission or reflection measurements, with the choice typically dependent on experimental considerations (\textit{e.g.}, substrate transparency, instrument setup), and both modes are often used. However, the information provided by either measurement is not equivalent. Light entering the sample has three possible outcomes – transmission, absorption or
reflection (specular or scattered). The transmitted radiation, exiting the sample at the end opposite the incident light, is all the radiation which was not absorbed or reflected. Thus, transmission (or extinction) measurements combine information about reflection and absorption. The scattering and absorption cross-sections have different intensities and wavelength dependencies, leading to the difference between the results of transmission and reflection measurements. These differences raise the question of whether the plasmon response of the two modes to RI changes will also prove different. In 2003 Khlebtsov et al. modeled the scattering and extinction spectra of Au colloids, with results showing similar responses from either measurement mode. In 2009 Svendendahl et al. compared the performance of LSPR transducers in the transmission and (back) reflection modes for thin layer adsorption, and concluded that there is no difference in the plasmon peak wavelength shift, though the figure shown there does show a slight advantage to the reflection mode. We therefore set out to measure and compare the response in the transmission and reflection modes observed in our Au island film transducers.

3.5 Long-range response

In recent years, several groups have reported a measurable response to layer adsorption on LSPR transducers, for very thick layers, in the range of hundreds of nanometers. This thickness by far exceeds the known electric field decay lengths, as quantified by various groups, including ours. The long-range results showed oscillations in the resonance wavelength as a function of adsorbate layer thickness, beyond the initial exponentially decaying response. The effect was termed “long-range plasmon response”, and its use in sensing was suggested. Several theoretical and computational attempts have been made to explain the interaction, showing, at best, limited success. Following our previous exploration of distance dependence (detailed in section 5.1), we initiated a study of this issue. We decided to explore the phenomenon both experimentally and computationally, explaining it in terms of thin-film-interference – effectively, the system is a Fabry-Pérot interferometer. Concurrent with our work, Saison-Francioso et al. also studied the long-range effects in terms of Fabry-Pérot modes, using a modified Green’s Tensor and FDTD (finite difference time domain) modeling. Our work on this topic led us to the next project, i.e., comparing the use of Fabry-Pérot interferometers and LSPR transducers as sensors.
3.6 Quantification and comparison - LSPR and interference transducers

LSPR sensing, as an emerging technique, must compete with, and ideally outperform, existing optical technologies of comparable experimental complexity. Consideration of the relative merits of sensing methods calls for simple parameters that can quantify the sensing performance of each contender, to make comparisons easier. Over the years, there have been quite a few suggestions for quantification methods. One of the most commonly used options is the figure-of-merit, FoM, which has several definitions in the context of plasmonic sensing. The most common is $RIS/\Gamma$, where RIS is the refractive index sensitivity (plasmonic peak wavelength shift per unit change in the bulk refractive index) and $\Gamma$ is the plasmon linewidth, as full-width at half-maximum (fwhm).\textsuperscript{55} This definition of FoM recognizes that peak shifts are easier to measure the narrower the peak is, but neglects the crucial aspect of distance dependence. In 2009 Unger \textit{et al.} discussed this issue in detail, and suggested a new figure-of-merit, taking into account the field confinement (decay length), sources of noise (instrument, signal and background), and analyte properties.\textsuperscript{56} The same year, Nusz \textit{et al.} developed a model for the molecular detection limit (MDL) of a plasmonic nanorods-based transducer.\textsuperscript{57} The MDL is the minimal number of bound molecules that can be detected, and in Nusz’s model it is dependent on the relative sizes of the sensing and analyte volumes, the uncertainty in peak determination, the spatially dependent RI sensitivity (decay length), and the RI difference between the analyte and the medium. In 2010 Otte \textit{et al.} introduced a figure-or-merit applicable to both LSPR and SPR transducers, to facilitate comparison between the two, finding a somewhat higher sensitivity advantage for LSPR.\textsuperscript{58} The same year, Becker \textit{et al.} formulated a different figure-of-merit, named FOM*, which is based on intensity variation rather than peak wavelength shifts.\textsuperscript{55} The parameter for thin layers, $\text{FOM}^*_{\text{layer}}$, is defined as the relative change in signal intensity, divided by layer thickness, at a single wavelength (chosen to provide the maximal $\text{FOM}^*_{\text{layer}}$). In 2013 Valsecchi \textit{et al.} published a highly detailed discussion concerning the assessment of plasmonic sensor performance.\textsuperscript{59} While their focus is on periodic structures, the quantification methods are valid for all plasmonic transducers.

While studies in the LSPR sensing field typically describe a new type of transducer, or a proof-of-concept for a new sensing scenario (\textit{e.g.}, antibody-antigen,
or protein-carbohydrate), quantitative comparisons with competing technologies are hard to come by. The primary aim of the majority of LSPR transducers is biosensing, and so the response to small analytes, sometimes in sub-monolayer coverage, is of great interest. Early work in our group studied the sub-monolayer sensitivity of several LSPR transducers, and found it to be comparable to that reported for SPR transducers. In 2009 Svendendhal et al. compared the response to thin layer binding (using the biotin-avidin system) of SPR and LSPR transducers, the latter using either transmission or back-reflection. The authors find comparable performance for SPR and LSPR, when measuring the resonance peak shift. They also comment on the irrelevance of the RIS and FoM (RIS/T), both much higher for SPR, for predicting the response for thin layer adsorption, as they neglect the effects of field confinement. The following year Otte et al. compared LSPR and SPR transducers, concluding that LSPR offers 15% better surface sensitivity.

![Figure 3](image.png)

**Figure 3.** Sensing configurations of the interference (left) and LSPR (right) based transducers, where the light is shined from above.

We decided to compare nano-island LSPR transducers to interference-based Fabry-Pérot transducers (also known as etalons), as both are easily fabricated and involve simple optical setups. Fabry-Pérot transducers have been previously compared to surface plasmon resonance (SPR) transducers, and found to offer similar sensitivities. The Fabry-Pérot transducer in our case involved several layers, as shown in Figure 3. Reflected beams from the two interfaces (medium/interference layer, and interference/reflective layers) interfere at the detector. A change in the interference layer thickness, due to analyte adsorption, leads to a change in the phase...
difference between the two beams, and thus a change in the measured light intensity. The relationship between thickness and reflectance is approximately sinusoidal, with a periodicity of $\lambda/2n$, where $\lambda$ is the measurement wavelength and $n$ is the adlayer RI. For the Fabry-Pérot transducer we used computational results to explore a wide range of possible compositions, and compare their performance in several sensing scenarios to that provided by our experimental LSPR transducers.

3.7 Metal-enhanced fluorescence

The interaction between plasmonic structures, e.g., metal nanoparticles (NPs), and fluorescent species has been the subject of considerable research. Proximity of such structures to fluorophores can cause either quenching or enhancement of the fluorescence, as a function of several parameters, including the distance between them, the composition of the metal, the size of the NPs, the properties of the fluorophore itself (e.g., quantum yield), and the overlap of the fluorophore absorption and emission bands with the plasmon band. Metal nanostructures have also been found to decrease self-quenching in nearby fluorophores. In most published data concerning metal-enhanced fluorescence (MEF) with plasmonic NPs, the distance profile spans at most several tens of nanometers, with no interaction at longer distances.

Metal NP films are similar in certain ways to continuous metallic films, and we can take advantage of the considerable theoretical and experimental literature treating the latter. The interaction of fluorescent materials with metallic surfaces has long been a fruitful field of study, and many detailed review articles have been published. Several theoretical approaches have been used to model the interaction, including by Drexhage et al.; Chance, Prock and Silbey; Gersten and Nitzan; and Ruppin. The details and relative merits of the various theoretical models are beyond the scope of this work, and the interested reader is referred to, e.g., the discussion by Klar and Feldmann.

A 1998 review by Barnes provided a comprehensive introduction to the interaction of metal surface with fluorophores, interpreting it in terms of the tuning of the photonic mode density (PMD). The presence of an interface (a metallic or dielectric surface) imposes boundary conditions, causing a reflection of the electromagnetic field. This changes the PMD in the vicinity of the surface, as a function of the phase
difference between the incident and reflected field at the fluorophore layer. If the fields are in-phase, the PMD is increased, and so are the emission rate and intensity. If the fields are out-of-phase, they are decreased. Thus, the emission lifetime should oscillate with metal-fluorophore separation, as the result of an interference effect. At short ranges, the fluorophore can couple to surface plasmon modes, which can then radiate the energy, or decay to heat. This can enhance or quench the emission. At the very closest separations, the emission is quenched due to coupling to lossy surface waves. If the dielectric spacer, separating the metal and the fluorophore layer, is very thick, it can support waveguide modes, to which some radiation can couple. The fluorophore’s emission frequency and linewidth (a function of the damping rate) are also expected to oscillate with spacer thickness, due to the changes in field intensity. The frequency shift and the change in damping rate are related to the in- and out-of-phase components of the reflected field, respectively. The variation in emission frequency is expected to be small, and Barnes discusses shifts in the MHz to GHz range, though very little work is cited on frequency shift effects.

In 1975 Chance et al. studied the modification of the fluorescent lifetime of Eu\textsuperscript{3+} ions close to a metal film, finding oscillations in the lifetime as a function of ion-surface separation.\textsuperscript{85} In a theoretical work published the same year, Chance et al. studied the effect of a partially reflective surface on the emission wavelength,\textsuperscript{86} which the theory for fully reflective surface predicts will oscillate as a function of metal-fluorophore distance. They found that the frequency shift might actually be larger for a partially reflective mirror than for the perfect reflector case. Amos et al. studied the modification of the fluorescence lifetime of Eu\textsuperscript{3+} in the vicinity of thin metal mirrors,\textsuperscript{87} finding a dependence on the metal film thickness, due to coupling to surface plasmon polaritons (SPP) on the far side of the metal film, an effect previously predicted from theory.\textsuperscript{81} In later work, Amos et al. studied the interaction of fluorophores with corrugated metallic surfaces.\textsuperscript{88} In 1984, Garoff et al. investigated the fluorescence spectra of Ru(II) trisbipyridine on silver island films on silica, compared to bare silica, and islands on aluminum. They found changes in the emission spectra between the different cases, but were unable to investigate the distance dependence.

In 1993, Kümmerlen et al. reported interaction at longer ranges, for the case of a silver nano-island film and the fluorescent dye Rhodamine 6G.\textsuperscript{89} These authors report two enhancement maxima – one at short range (5-10 nm), attributed to surface
plasmon based amplification of the local field around the metallic nanoparticles; and a second peak at a larger separation (~60 nm), attributed to an increase in the fluorescence quantum yield, combined with resonant excitation due to a collective action of the nano-islands.

Following our work on distance dependence in LSPR, we were intrigued by a possible connection between the plasmon decay length and the MEF distance dependence profile. To our knowledge, none of the previous studies on MEF used transducers with known plasmon decay lengths. We thus decided to use our well-characterized LSPR transducers, and examine their impact on the fluorescence intensity and lifetime of a fluorophore monolayer, at varying metal-fluorophore separations.
4 Experimental

4.1 Plasmon decay length

Materials. The substrates used were microscope glass cover-slides (Schott AG borosilicate glass D263T No. 3, 22x22 mm², with Tg≈557 °C, supplied by Menzel- Gläser, Germany), cut to 22x8 mm²; and quartz slides (Heraeus Quarzglas, Germany), 1 mm thick, cut to 37x12 mm². Gold (99.99%, Holland-Moran, Israel); SiO₂ pieces for EV-PVD (99.99%, Kurt J. Lasker, USA); polyallylamine hydrochloride (PAH) (56 kDa, Sigma Aldrich); polystyrene sulfonate, sodium salt (PSS) (70 kDa, Polysciences Inc., Warrington, PA, USA); 1-octadecanethiol (ODT) (98%, Aldrich); 2-[methoxy(polyethylenoxy)propyl]-trimethoxysilane, 6-9 C₂H₄O groups (PEG-silane) (90%, Gelest); 3-aminopropyl trimethoxysilane (APTS) (Aldrich); tris(bipyridine)ruthenium (II) oxalate; sodium chloride (Frutarom, Israel); methanol (absolute, Gadot or Biolab, Israel); ethanol (anhydrous, Gadot or Biolab); heptane (anhydrous, Aldrich); chloroform (Gadot); toluene (Gadot); H₂SO₄ (AR 95-98%, Gadot or Biolab); H₂O₂ (30%, Frutarom); and ammonium hydroxide (Frutarom), were used as received. Nitrogen was in-house supplied from liquid N₂. All solutions were prepared using triply-distilled water.

Continuous gold films (AuCont). The slides were cleaned in freshly prepared “Piranha” solution (H₂O₂:H₂SO₄, 1:3 by volume) for 1 h (Caution: “piranha” solution is extremely corrosive and boils upon mixing), washed three times with deionized water, three times with triply distilled water, and finally once with ethanol. The slides were then treated with an “RCA” solution (H₂O:NH₄OH:H₂O₂, 5:1:1 by volume) at approx. 70 °C for 1 h, rinsed, and modified with APTS by overnight immersion in 1% APTS (by volume) in methanol. APTS layers are known to improve the adhesion of gold evaporated on glass substrates. The slides were then washed in methanol, sonicated in methanol three times (5 min each), washed in ethanol and dried under a nitrogen stream. 24 slides were placed on a plate, which was mounted in a cryo-HV evaporator (Key High Vacuum) equipped with a Maxtek TM-100 thickness monitor. The chamber was evacuated to a pressure of 2-3x10⁻⁶ torr, and gold was evaporated onto the slides from a resistively-heated tungsten boat. The plate was rotated during evaporation to achieve homogenous deposition on the slides. 100 nm of gold were deposited at a rate of 0.1 nm/sec. The slides were then annealed at
200 °C for 20 h in a Ney Vulcan 3-550 furnace, using a 5 °C/min heating rate, then left to cool to room temperature inside the furnace. Gold films prepared using this procedure are smooth and can be studied by various surface techniques.91

**Gold nano-island films.** Glass slides were cleaned with Piranha and mounted in the evaporation chamber as described above (without RCA treatment or APTS coating). Gold was evaporated on the slides at a deposition rate of 0.01 nm/sec, to nominal thicknesses of 3 to 10 nm (the nominal thickness is the reading of the evaporator QCM thickness monitor, i.e., the film mass thickness). We refer to these films below as AuXnm, where X is the nominal thickness. Following evaporation, the slides were annealed as detailed above for 10 h at 580 °C (5 °C/min heating rate), then left to cool to room temperature inside the furnace.

**Immobilized Au nanoparticle films.** Citrate stabilized Au NPs were synthesized using known methods, as previously reported.92,93 The NPs were characterized using a Philips CM120 Super Twin transmission electron microscope (TEM). To form NP films, glass slides were cleaned using piranha solution, coated with an APTS binding layer (0.1% v/v in water, 15 min), and incubated in the NP solution overnight.

**Polyelectrolyte (PE) LbL assembly.** The LbL procedure was carried out using the positive PE PAH and the negative PE PSS, 1.0 mM solutions (concentration calculated with respect to the monomer, corresponding to 0.093 mg mL\(^{-1}\) PAH and 0.206 mg mL\(^{-1}\) PSS) in 0.1 M NaCl in triply distilled water. Au films prepared as detailed above were treated for 10 min in a UV/Ozone apparatus (UVOCS Inc. model T10*10/OES/E), with the Au coating facing the UV lamps. The samples were then washed for 20 min in ethanol94 and dried under a nitrogen stream. The slide to be coated was alternatingly immersed in vials containing the PE solutions for 15 min each, starting with PAH. After each adsorption step the slide was rinsed with water, dipped into an aqueous solution of 0.1 M NaCl, and immersed in the other PE solution. After completing the assembly of a number of PE bilayers the slide was washed and dried under a nitrogen stream, measured, and the LbL procedure was resumed. The thickness of each bilayer was determined, using spectroscopic ellipsometry, to be 2.09±0.03 nm. The adsorption and measurements were carried out in a climate-controlled laboratory, at a temperature of 22.5±1.0 °C and a humidity of 50±5 %.

**Characterization.** Extinction spectra at normal incidence were measured using a Varian Carey 50 Probe UV/Vis spectrophotometer. Measurement parameters:
wavelength resolution, 1 nm; scan rate, 300 nm/min; average acquisition time per point, 0.2 sec. For dry measurements, air was used as the baseline; for measurements in a given solvent, a cuvette filled with the same solvent, without a slide, was used as baseline.

PE film thickness on continuous gold slides was measured in air using an Angstrom Advanced PhE-102 spectroscopic ellipsometer, at an angle of incidence of 70°, in the spectral range 300-800 nm, using 1 to 20 nm steps. Ellipsometric data were analyzed using Film Wizard software (Scientific Computing International, California, USA).

High-resolution scanning electron microscopy (HRSEM) images were obtained using a Carl Zeiss Ultra-55 Ultra-high-resolution SEM. Typically, samples were first coated with 2-3 nm of Cr.

**Image analysis.** HRSEM images were analyzed using ImageJ image analysis software (Wayne Rasband, NIH, USA), to determine particle dimensions and size distribution.

### 4.2 Reflection measurements

Sample preparation for this section employed the same methods as described above. Near-normal reflection spectra were measured using an Ocean Optics USB4000XR, USB4000 or USB650 spectrophotometer equipped with a Polychromix MobiLight light source and an Ocean Optics reflection probe.

### 4.3 Long-range response

Samples were prepared and characterized as described above, and coated with SiO$_x$ as outlined below.

**SiO$_x$ coating.** Continuous Au and Au island slides were cleaned in a UV/Ozone apparatus (UVOCs model T10x10/OES/E), dipped in ethanol for 20 min with stirring and dried under a nitrogen stream. As silica shows low adhesion to gold, prior to deposition AuCont slides were coated with APTS, by dipping the slide in a 0.1% v/v APTS solution in methanol for 15 minutes, followed by thorough washing in methanol and drying. Slides were then coated with SiO$_x$ (where $x \leq 2$, as oxygen was not added to the evaporation chamber) using electron-beam assisted physical vapor deposition (PVD), at a deposition rate of 0.15-0.2 nm/sec. The thickness was
monitored in-situ using a quartz crystal microbalance, and accurately determined post-deposition using spectroscopic ellipsometry of coated AuCont samples. This measurement also provided the RI of the coating in the visible range: \( n = 1.46, \ k = 0 \) (negligible absorption).

Calculations. FilmWizard (Scientific Computing International, California, USA) was used to calculate reflection spectra from gold slides with a dielectric coating.

4.4 LSPR and interference transducers

Sample preparation and characterization was performed as described above. Fabry-Pérot interferometers were modeled using Film Wizard software (Scientific Computing International, California, USA).

4.5 Metal-enhanced fluorescence

Au nanoisland films were prepared as described above. For thinner spacers (up to 72 nm), the slides were coated with PE films, terminated by the negatively-charged PSS, to which we then adsorbed the positively-charged fluorescent complex \([\text{Ru(bpy)}_3]^{2+}\) (0.03 M, 1.5 h). For thicker spacers, we used SiO\(_x\) coatings (described above, section 4.3), to which we then adsorbed APTS, PSS and the fluorophore. The control samples were glass slides, cleaned with piranha, coated with APTS, PSS, 5 or 9 PAH/PSS bilayers, and then the fluorophore, all as previously described.

Steady-state fluorescence measurements were carried out using a HORIBA Jobin Yvon Fluorolog-3 spectrofluorometer, at 1 nm resolution, 0.2 sec averaging time, and 5 nm band-pass (excitation and emission). The samples were placed on a solid sample holder, with the excitation light incident on the sample at 30° from the normal, and the light sensor located at 60° off normal to the other side.

Fluorescence lifetime measurements were carried out at Dr. Gerhard Wagenblast’s laboratory (BASF SE, Ludwigshafen, Germany), using a custom-built instrument, comprising a Nd:YAG 355 nm third-harmonic laser as the excitation source, and a multi-channel scaling (MCS) detector. The system was continuously flushed with nitrogen gas during measurements.
5 Results

5.1 Plasmon decay length


In this work we explored the sensing properties of a range of gold nano-island films, ranging in size from approximately 20 nm to 100 nm (in-plane diameter). In particular, we measured the decay length of the plasmon evanescent field, which determines the sensing volume. This is the volume in space surrounding the particle in which changes of the dielectric constant (*e.g.*, due to analyte adsorption) can effect a measurable change in the plasmon spectrum. Determining the value of this parameter is of great importance for practical applications of LSPR transducers, as optimizing the sensitivity entails properly matching the transducer to the analyte and recognition interface dimensions. This study was the first one to systematically determine the decay length for transducers of several average particle sizes, and as a result was the first to show a correlation between the decay length and the refractive index sensitivity (RIS; Figure 4, following the attached paper). The RIS is the value commonly cited when comparing transducer performance. The dependence of the response on these two parameters yielded a novel conclusion – previous researchers focused on maximizing the RIS, suggesting that this will produce maximal responses in sensing scenarios. However, large RIS is correlated with large decay lengths, and sensing volumes. As a thin layer only fills a small fraction of the available sensing volume, it is often advantageous to use a transducer with small RIS and decay length.

This work forms the basis for several of the latter studies, by providing previously unavailable decay length data for a series of easily produced transducers, and by accurately characterizing their sensing properties.
Sensitivity and Optimization of Localized Surface Plasmon Resonance Transducers

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ABSTRACT Gold nanoisland films displaying localized surface plasmon resonance optical response were constructed by evaporation on glass and annealing. The surface plasmon distance sensitivity and refractive index sensitivity (RIS) for island films of different nominal thicknesses and morphologies were investigated using layer-by-layer polyelectrolyte multilayer assembly. Since the polymer forms a conformal coating on the Au islands and the glass substrate between islands, the relative sensitivity of the optical response to adsorption on and between islands was evaluated. The RIS was also determined independently using a series of solvents. An apparent discrepancy between the behavior of the RIS for wavelength shift and intensity change is resolved by considering the different physical nature of the two quantities, leading to the use of a new variable, that is, RIS (for intensity change) normalized to the surface density of islands. In the present system the surface plasmon decay length and RIS are shown to be directly correlated; both parameters increase with increasing average island size. This result implies that a higher RIS is not always beneficial for sensing; maximizing the transducer optical response requires the interrelated RIS and decay length to be optimized with respect to the dimensions of the studied analyte-receptor system. It is shown that, as a rule, transducers comprising larger islands furnish better overall sensitivity for thicker adlayers, whereas thinner adlayers produce a larger response when sensed using transducers comprising smaller islands, despite the lower RIS of the latter.

KEYWORDS: localized plasmon · LSPR · sensitivity · gold · islands · decay length · optimization

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the LSPR band. The transducer is then exposed to the unknown solution; if the latter contains the specific analyte (e.g., an antigen specific to the immobilized antibody), it will bind to the recognition layer, and a change in the spectrum will be detected.6,9 The response in such a scenario is described by eq 2:

$$ R = m \Delta \nu \exp(-d_1/l)(1 - \exp(-d_2/l)) \tag{2} $$

where $d_1$ and $d_2$ are the thicknesses of the recognition interface and the analyte layer, respectively. According to eq 2, to obtain maximal response to analyte binding one has to maximize the RIS $m$ and match the decay length $l$ to the analyte and recognition interface dimensions.

While the quest for maximal RIS is straightforward, the issue of the decay length is more complex. Binding of the analyte takes place at a certain distance from the metal surface, determined by the dimensions of the recognition layer and the analyte. If the decay length is small with respect to the dielectric layer thickness, the rapid exponential decay will lead to a weak response to the binding event. If, on the other hand, the decay length is large compared to the dielectric layer thickness, the analyte will occupy a small fraction of the sensing volume, leading, again, to a weak response. Thus, for optimal response a transducer has to be chosen such that the combined RIS and decay length maximize the response to a given analyte—receptor system.13,14

The decay length in LSPR systems has been previously investigated. Van Duyne and co-workers examined triangular Ag nanoslip films fabricated by nanosphere lithography,15 the response to adsorption of dielectric layers, seen as a red-shift of the extinction peak, was found to be linear with distance in the short-range (several nm),16 and exponentially decaying in the medium range (tens of nm).17 It was found that for equal in-plane dimensions, the decay length is larger for particles with smaller out-of-plane height.18 Work on surface-immobilized Au colloids found a positive relationship between particle size and decay length.13,19 Our group has studied the distance dependence of LSPR extinction in a system comprising coordination-based multilayers of varying thickness adsorbed onto evaporated Au island films.20 In another work a semi-quantitative relationship was shown between distance and LSPR response in biological sensing.11 Interestingly, it was found that in the long-range (hundreds of nm) the response to adsorption of additional layers oscillates between a red shift and a blue shift.21–23

The phenomenological model presented by eq 1 assumes that both parameters defining the optical response (RIS and decay length) are spatially invariant, while calculations24 and recent experiments25 show spatial variations. In the case of island size and shape distribution (as in the present system), the model also does not take into account variations in sensitivity between different islands in the film. In practice we measure a macroscopic area of the island film, thereby averaging over islands and binding sites with possibly different sensitivities and decay lengths. Our earlier work presented evidence for effective averaging in our system, manifested as a linear optical response to binding of small (<2 nm) adsorbates on Au island films, for fractional coverages from ca. 0.01 to a full monolayer.26,27

A common technique for obtaining thin films of well-defined thicknesses in the nanometer range is layer-by-layer (LbL) deposition, in which a layered nanostructure is built up in a stepwise fashion. In the present work we have used the polyelectrolyte LbL approach introduced by Decher and co-workers, involving alternate binding of oppositely charged polyelectrolyte layers via electrostatic interactions.28 This widely used approach is noted for its simplicity and repeatability.29,30 The thickness of the repeated layers depends strongly on the ionic strength of the deposition solution, with higher ionic strengths resulting in thicker layers.31,32

The LbL polyelectrolyte deposition scheme is used here for obtaining dielectric coatings of systematically varying thicknesses, with the aim of determining the decay length and RIS of island-type Au films of different morphologies. The advantages of polyelectrolyte multilayers are simplicity, reproducibility, and a highly controlled thickness. A noted complication is the fact that the resulting layer forms a conformal coating on the islands and on the glass substrate between the islands, thus deviating from the simple exponential model which assumes homogeneous growth on the entire island surface. To justify the use of eq 1 we therefore had to evaluate the relative sensitivity of the transducer to buildup of a dielectric layer on the islands versus between islands.

We set out to obtain systematic data on the sensing parameters (decay length and RIS) of a series of Au island films and study possible relationships between these parameters and physical variables of the islands (e.g., average island dimensions), as well as a possible correlation between the two parameters. Such relationships can be used for rational design and optimization of LSPR transducers for sensing of specific analytes.

RESULTS AND DISCUSSION

Transducer Preparation. Gold island films of 3, 4, 5, 6, 7.5, 9, and 10 nm (nominal thickness) were fabricated by evaporation on glass followed by annealing 10 h at 580 °C, as detailed in the Methods section. We have previously shown that the high-temperature annealing promotes partial embedding and stabilization of the Au islands on the glass,33 while lowering somewhat the RIS due to reduction of the exposed Au area.7 Figure 1 panels A–C show representative high-resolution scanning electron microscopy (HRSEM) images of samples of 3, 5, and 10 nm (Au nominal thickness), covered with...
a thin Cr layer to improve the conductivity. The images were analyzed as described in the Methods section.

Table 1 presents average values of the Au island major and minor axes, the fraction of the image area covered by Au particles, the effective height (calculated as the nominal thickness divided by the coverage, representing material distribution rather than average island height), the particle aspect ratios, the mean interparticle distance (center-to-center), and the particle surface density. Note that the Cr coating may have a marginal effect on the analysis results.

With increase of the film nominal thickness, which is the only independent parameter, the average island dimensions gradually increase, while the coverage tends to become smaller and the average interisland distance becomes larger (Table 1), as a result of gold accumulating into larger islands with a higher volume-to-surface ratio. While the out-of-plane aspect ratio remains rather unchanged, the in-plane aspect ratio shows a tendency to increase at higher nominal thicknesses. The correlations between the various parameters make the evaluation of the impact of individual parameters on the transducer sensitivity (see below) quite difficult.

Island dimensions and interparticle distances show a sharp increase between 6 and 7.5 nm, separating two populations of islands which can be classified according to the morphology of the as-evaporated (unannealed) films: small (isolated, 3 to 6 nm) and large (near-percolated and percolated, 7.5 to 10 nm). The island surface density was calculated from analysis of SEM images.

Figure 2 shows characteristic extinction spectra of Au island films (bare Au, annealed; see Methods). The wavelength of the surface plasmon band is affected by various factors, including interisland distance, island size distribution, aspect ratio, and embedding depth in the glass substrate. The ratio of (average) interisland distance to (average) island major axis increases from 1.2–1.3 for 3–6 nm island films to 1.4–1.5 for thicker films, implying a decrease of the surface plasmon coupling. The latter is expected to result in a blue shift of the surface plasmon band. However, other factors, such as increase of the average island size and aspect ratio, become dominant with increasing nominal thickness, resulting in a near constant wavelength of the surface plasmon band for the small islands and a red-shift of the band for the larger islands (Figure 2).

Numerical modeling using the discrete dipole approximation (DDA) method and taking into account the experimental island size distributions, showed a semi-quantitative agreement between the calculated and measured spectra for the larger islands; that is, the surface plasmon peak shifted to the red and the intensity increased with the nominal thicknesses.34

Polyelectrolyte Multilayers. For polyelectrolyte LbL assembly, slides to be coated were alternatingly immersed in PAH and PSS solutions, as detailed in the Methods section. The first layer of PAH was adsorbed directly on the Au surface, exploiting the interaction of amine groups with Au. A prerequisite for the use of polyelectrolyte LbL assembly for construction of dielectric layers of well-defined thicknesses is that every deposition cycle (PAH/PSS bilayer) adds the same amount of material and the same thickness, for a large number of layers. The PAH/PSS system used in the present work is known to grow in a linear fashion.35 To test this behavior in our hands, polyelectrolyte multilayers were constructed on glass and quartz slides coated with a 20-nm-thick semitransparent continuous Au film. The regularity of the growth can be followed by monitoring the PSS absorption peaks at 194 and 264 nm.
The extinction line (Information) shows the data points and a linear regression of the number of layers. Figure S2 (Supporting Information) presents the multilayers determined and plotted as a function of the numbers of polyelectrolyte layers. The thickness of continuous Au substrates was observed to be the same as that on the work, assuming that the thickness of the polyelectrolyte multilayer (bilayer is 2.09 nm thick. The ellipsometric analysis also provided a value of ca. 1.56 for the refractive index (RI) of the polyelectrolyte multilayer in the visible range. These values were used throughout the reflectance in the visible range with added layers was found to be a shift (as wave-length shift) to ODT and PEG-silane applies to all different sizes, shapes and surface binding sites of the islands. Regarding the first two points, we have previously established the validity of averaging over the different sizes, shapes and surface binding sites of the islands in the ensemble.

To examine the effect of binding on islands versus binding between islands, dielectric monomolecular layers were selectively adsorbed on the Au islands or on the glass between the islands, using 1-octadecanethiol (ODT) and a trimethoxysilane-terminated polyethylene glycol (PEG-silane), respectively. The thickness of ODT and PEG-silane self-assembled monolayers (SAMs) is known to be approximately 2 nm, and their refractive indices are close to 1.5. For each Au nominal thickness, samples were coated either with ODT followed by PEG-silane, or in the reverse order. The procedure is illustrated in Figure 3; note the subtle difference in the final structures in the vicinity of the Au/glass/adlayer interface.

Figure 4 panels A and B show representative spectra of 4 nm (nominal thickness) Au island films before and after coating with SAMs according to the two schemes in Figure 3. In both cases adsorption of ODT (on islands) promotes a considerably larger response compared to assembly of PEG-silane (between islands). The results for a range of Au island sizes (Figure 4C) indicate the following: (i) The difference in the response (as wave-length shift) to ODT and PEG-silane applies to all measured sizes. (ii) The response to a given molecule is always larger when it is adsorbed first; this is consistent with the illustration in Figure 3, showing the different occupancy of the Au–glass–air interface region for the different assembly sequences. (iii) The response generally decreases with increasing island size, reflecting the effect of the growing decay length.

Site-Selective Adsorption. LSPR measurements of LbL polyelectrolyte multilayer formation in the present system involves three kinds of averaging, deriving from the macroscopic nature of the measurements: (i) adsorption at different locations on the Au island surface, possibly having differing sensitivities; (ii) adsorption on islands of different shapes and sizes; (iii) adsorption on the islands and on the glass substrate between the islands. The procedure is illustrated in Figure 3; note the subtle difference in the final structures in the vicinity of the Au/glass/adlayer interface.
as further discussed in the next section. (iv) For a full monolayer coating of the samples, the response is attributed primarily to the SAM fraction adsorbed on the Au islands, while the contribution of the fraction adsorbed on the glass is considerably smaller.

The significance of the results in Figure 4D, showing that the combined response of the two assembly steps is the same in both cases, is in verifying that the system is well-behaved and the assumption of site-selective self-assembly is valid. The results of the site-selective adsorption experiments substantiate the use of polyelectrolyte multilayers for determination of decay length in our system, as elaborated below.

**Effective Decay Length.** To determine the effective decay length and RIS of Au island films of different nominal thicknesses, polyelectrolyte multilayers were deposited on the Au islands in a manner similar to that described above for continuous Au substrates, and transmission spectra were measured after the binding of each bilayer. Four slides were measured for each Au island thickness. The LbL assembly was carried out until the characteristic parameters (plasmon peak wavelength and intensity) showed a tendency to stabilize.

Figure 1D presents a HRSEM plan-view image of a gold island slide coated with a polyelectrolyte multilayer, showing a rather uniform polymer layer around the islands.39 This morphology presents a complication in the use of eq 1, the latter assuming a homogeneous dielectric film around the islands. This morphology presents a complication in the use of eq 1, the latter assuming a homogeneous dielectric film around the islands. However, on the basis of the results in Figure 4D, showing that the combined response of the two assembly steps is the same in both cases, is in verifying that the system is well-behaved and the assumption of site-selective self-assembly is valid. The results of the site-selective adsorption experiments substantiate the use of polyelectrolyte multilayers for determination of decay length in our system, as elaborated below.

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Figure 1D presents a HRSEM plan-view image of a gold island slide coated with a polyelectrolyte multilayer, showing a rather uniform polymer layer around the islands. An isometric view (60° from normal) of the same sample is presented in Figure 1 panels E and F, utilizing different detectors to show, respectively, the surface and the internal composition. The thickness of the 40-layer film, determined from Figure 1D, is 40 ± 4 nm, in agreement with the thickness measured by ellipsometry on continuous gold (Figure S2, Supporting Information).

The images in Figure 1 panels E and F indicate that the polyelectrolyte multilayer forms a conformal coating on the gold islands and the glass surface between the islands.39 This morphology presents a complication in the use of eq 1, the latter assuming a homogeneous dielectric film around the islands. However, on the basis
of the results in the previous section, showing that the major part of the response to a conformal monomolecular coating is attributed to the fraction on the metal islands, it can be shown that the optical behavior of the Au island transducer/polyelectrolyte multilayer system is reasonably well described by eq 1 (see Discussion section and Supporting Information).

Figure 5 shows representative spectra and plasmon peak wavelength and intensity for polyelectrolyte LbL assembly on 3 and 10 nm Au island films, exhibiting the increase in the decay length with nominal thickness. In sensing applications the optical response is usually quantified as the surface plasmon wavelength shift or intensity difference, the latter measured either at the surface plasmon band maximum or at a constant wavelength close to the maximal intensity change. Figure 6 shows the plasmon wavelength shift and extinction intensity change, both at the extinction peaks and at a constant wavelength (570 nm), as a function of polyelectrolyte multilayer thickness, for 3 nm (nominal thickness) Au island films. Data extracted from Figure 5 panels A and B.

Exponential regression of the data in Figure 6 provides the values $3.9 \pm 0.3$ nm and $47.9 \pm 1.6$ nm/RIU for $l_\lambda$ and $m_\lambda$, respectively; $4.4 \pm 0.7$ nm and $0.316 \pm 0.018$ A.U./RIU for $l_{ext}$ and $m_{ext}$ measured at the peak; and $4.8 \pm 0.8$ nm and $0.400 \pm 0.027$ A.U./RIU for $l_{ext}$ and $m_{ext}$ measured at 570 nm. The decay length values calculated at the surface plasmon maximum or at a single wavelength are therefore consistent within the experimental uncertainty. Note that in what follows we use RIS values ($m_{ext}$) measured at the peak.

dielectric layer (see above) gives $\Delta \eta = 0.56$, hence the RIS $m_\lambda$ or $m_{ext}$ can be derived from the same data.
A compilation of data on plasmon peak wavelength and intensity for all the Au thicknesses studied is shown in Figure 7, presented as difference values. Generally, with increased Au thickness (i.e., larger islands), thicker polyelectrolyte coatings are needed to approach saturation (Figure 7), indicating a larger decay length. Note that for films of 6 nm and above, the peak wavelength and intensity reach a maximum and start to decrease after a certain number of layers, with the wavelength lagging behind (Figure 5D and Figure 7, wavelength decrease not seen yet). This phenomenon is related to long-range LSPR effects, seen in our system for assembly of thick polyelectrolyte layers on large Au islands. Another effect which may influence the shape of the curves in Figure 7 is filling of the gaps between islands as more layers are added. These effects, which are not described by eq 1, become important for thick coatings.

To minimize the effect of these deviations, in the curve fitting only data points up to the maximum extinction (Figure 6) were used for the exponential fit of the intensity change. This treatment is justified as our approach in the present work is largely empirical and assumes that eq 1 provides a reasonable description of the system’s optical response. Therefore, the values of the decay length \( l \) and the RIS \( m \) extracted from the exponential regression are to be considered effective parameters, which are important in evaluating sensing capabilities. Understanding the physical basis requires a more elaborate approach which is beyond the scope of the present work.

Average decay length and RIS values, determined using the exponential fit, are summarized in Figure 8 panels A and B. The wavelength decay length and RIS values exhibit a regular increase with increasing nominal thickness and average island size (Figure 8A). The intensity decay length values increase with a tendency to saturate, while the values of the intensity RIS show an initial increase and a decrease for larger islands (Figure 8B). The difference in the values of the wavelength and intensity decay length for larger islands (Figure 8 panels A and B) is likely to be related to long-range localized plasmon interactions, as noted above. The different behavior of the RIS for wavelength (monotonous increase, Figure 8A) and intensity (maximum around 7.5 nm, Figure 8B) is attributed...
primarily to the fundamental difference between the two physical parameters, as further elaborated in the Discussion section.

The results shown in Figure 8 are highly significant, presenting a general correlation between the decay length and RIS, for both wavelength and extinction intensity. This point is further elaborated in the Discussion section. Using the statistical analysis of HRSEM images described above, the relationship between Au island size and decay length is presented in Figure 9 as the wavelength decay length versus the average Au island major axis. Although the experimental error bars are quite large and increase with island size, the figure shows a clear correlation between the two parameters.

**Refractive Index Sensitivity.** The RIS of samples of various Au thicknesses was determined independently by measuring extinction spectra in a series of solvents of varying RI values, which is the common method of determining the RIS of optical transducers. The RI of the different solvents was measured using an Abbe refractometer. The solvents used were (RI in parentheses) methanol (1.328), ethanol (1.359), heptane (1.386), chloroform (1.445), and toluene (1.496). Since the solvent layer thickness is practically infinite relative to the decay length, eq 1 is reduced to \( R = m \Delta \eta \), and the RIS \( m \) can be extracted from the slope of \( R \) versus \( \Delta \eta \), using linear regression and assuming that \( m \) is independent of the RI in the measured range.

Figure 10 shows representative results for a 4 nm Au island film, illustrating the procedure used for obtaining the RIS values: (a) spectra are measured in the various solvents; (b) the peak wavelength and intensity are plotted versus the medium RI, and the RIS values \( m_\lambda \) and \( m_{\text{ext}} \) are determined using a linear fit. Four samples were used for each thickness. While the optical parameters change quite linearly in the different solvents as expected from the linear approximation of eq 1, the experimental points in air (\( \eta = 1.00 \)) deviate from linearity and their inclusion in the calculation requires a higher order treatment. For simplicity, the results in air were not included in the determination of the RIS. From the linear regressions (Figure 10B), it was determined that the RIS (wavelength) is 72.2 ± 2.5 nm/RIU, and the RIS (extinction intensity) is 0.319 ± 0.008 A.U./RIU.

Figure 11 presents the RIS \( m_\lambda \) versus the wavelength of the bare Au plasmon peak, for the different thicknesses. The data suggest a correlation with a minimum at plasmon band wavelengths of ca. 537 nm. A positive correlation between the surface plasmon wavelength and the RIS, with rather large data scatter, was previously shown.\(^7\)\(^,\)\(^42\) A discussion of our current results should take into account various parameters, such as the distribution of island size, shape, and interisland distance, and is outside the scope of the present work.

A comparison of RIS values obtained using the two methods, that is, exponential fit of multilayer results versus direct measurement in different solvents, is presented in Figure 12. For all the nominal thicknesses studied, the RIS values measured using the two methods exhibit the same qualitative behavior. However, \( m_\lambda \) values measured directly are larger by ca. 10—30% than those determined by the exponential fit, whereas \( m_{\text{ext}} \) values measured directly are smaller by ca. 20—40%.

Factors that may be responsible for the differences between the RIS values determined by the two methods include the following: (i) The multilayer thickness and RI extracted from ellipsometry on continuous Au and used for the exponential fit may be somewhat different from the values on Au islands. (ii) The exponential fit includes the difference in RI \( \Delta \eta \) between the
multilayer (1.56, from ellipsometry) and air (1.00), assuming a linear response in this range. However, as discussed above (Figure 10), the experimental data show a linear behavior in the range used for direct RIS measurement (1.328–1.496) but not in the range used for the exponential treatment. (iii) The assumption of a regularly increasing film thickness fails when the growing films on adjacent islands begin to overlap. (iv) Equation 1 assumes that the response is monotonous with increasing film thickness, from zero to infinite thickness (the equivalent of changing the solvent); however, as noted in the introduction, at increased dielectric layer thicknesses the response was found to oscillate.21–23,41 Hence, the simple exponential model used for the polyelectrolyte multilayers may not extend monotonously to the bulk. Given all these factors, the general agreement between the RIS values determined by the two methods is satisfactory.

**Discussion.** We chose to study the characteristic optical properties of LSPR transducers using polyelectrolyte LbL multilayers, as such coatings are easy to prepare, reproducible, and the thickness is well controlled. However, the growth mode of these films introduces a deviation from the model described by eq 1, namely, coating of the glass between the islands and gradual filling of the free space between islands as the multilayer thickness grows. It was therefore essential to show that these deviations have a minor effect on the optical response, thus justifying data analysis using the simple exponential model.

The site-selective adsorption experiments indicated that the response to SAM deposition between the islands is much weaker than the response to similar deposition on the islands. Using these results as well as the known average dimensions of the islands, the dielectric film characteristics, and the measured response to polyelectrolyte binding, the relative contribution of coating of the glass between the islands to the overall response was derived (see Supporting Information for details). The calculation shows that the fraction of the overall response generated by deposition on the glass decreases as the layer thicknesses increases, due to the exponential decay of the plasmon field. Hence, for a 5 nm Au island film, the part of the response attributed to deposition on the glass decreases from ca. 19% to ca. 6% as the layer thickness grows from 1 to 12 nm.

It is therefore concluded that despite the complex process, a simple exponential model describes the optical behavior of the present system in a satisfactory manner, even under conditions where the gap between islands is largely filled by the recognition interface and bound analyte.

The decay length of LSPR transducers has been previously investigated in a wide variety of systems, including nanosphere lithography (NSL) fabricated triangular Ag and Au arrays,18,22,24 chemically synthesized Ag and Au nanoparticles,3 both in solution43 and adsorbed on surfaces,19,40,44 and thermally evaporated Au particles on substrates.20,21,45 Exponential decay of the plasmon field was commonly assumed, and in general larger particles exhibited a longer decay length. Most studies included particles of a single size, and only a few presented actual decay length values.

The need to consider both the RIS and decay length for LSPR transducer optimization is inherent in eqs 1 and 2, and was recognized previously.13,14 This issue was also addressed in the context of comparing the sensitivity of LSPR to that of propagating surface plasmon resonance (SPR),1,12 where the smaller decay

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**Figure 11.** Refractive index sensitivity $n_\lambda$ vs wavelength of the plasmon peak for Au island films of various nominal thicknesses (indicated). Error bars represent the standard deviation of four samples for each Au thickness.

**Figure 12.** Refractive index sensitivity (RIS) values obtained by exponential regression of LbL results and values measured directly using a series of solvents, for wavelength shift (A), and extinction intensity change (B).
length of LSPR transducers is said to compensate for their lower RIS. However, the existence of a direct correlation between the decay length and RIS in localized plasmon systems has not been demonstrated.

As shown in Figure 8A, films with larger islands feature both higher RIS and decay length values. Hence, the two parameters are strongly linked; the experimental correlation is presented in Figure 13. The implications of this correlation are far-reaching in terms of sensing. Common analytes and recognition interfaces (notably, biological molecules) are several nm in size, meaning that choosing transducers with high RIS values inevitably leads to poor matching of the decay length (Figure 13). To maximize the response it is therefore imperative to find the optimal combination of RIS and decay length, which has to be tuned to the specific analyte and recognition layer dimensions.

To demonstrate optimization of the transducer response, Figure 14 shows the incremental change in plasmon peak wavelength and intensity resulting from adsorption of a 2.1-nm-thick polyelectrolyte bilayer (PAH/PSS) on an existing polyelectrolyte multilayer of varying thickness. This exercise simulates binding of a 2.1 nm analyte on a dielectric recognition layer of varying thickness. The figure features graphs calculated by substituting the experimental decay length and RIS values (Figure 8) in eq 2. The simple exponential model is satisfactory in providing a semiquantitative description of the response of these systems, with the wavelength shift showing better reproduction of the experimental data (compare Figure 14 to Figure S3, Supporting Information).

As seen in Figure 14 (most clearly in Figure 14A), the optical response to binding of a 2.1 nm analyte on a dielectric layer (e.g., a biorecognition interface) of a low thickness (lower than ca. 5 nm) is influenced primarily by the decay length, whereas the response for dielectric layers of higher thicknesses follows the RIS trend. In terms of transducer optimization, larger islands are advantageous for systems involving thick layers which occupy much of the sensing volume, hence the large RIS values dominate; on the other hand, small islands exhibit higher sensitivities for thin layers, despite the lower RIS, owing to the better match of the decay length and sensing volume to the dielectric layer thickness. In the case of biological molecules, which are commonly a few nanometers in diameter, better optical response is expected with LSPR systems comprising smaller islands.

Therefore, plots such as those in Figure 14 can be used for rational design, namely, to predict the optimal transducer for detection of a binding event involving specific analyte and recognition layer of given dimensions.

As noted in the introduction, LSPR transducers display spatial inhomogeneity of the sensitivity, while our earlier work showing a linear response to analyte surface coverage indicated effective averaging of this effect in the present system. However, a possible correlation between spatially variable RIS and decay length may be studied in the future and used for further optimization.

The results shown in Figure 8 panels A and B require additional discussion. The values of the wavelength RIS $m_\lambda$ increase with Au layer thickness (Figure 8A), while the corresponding intensity refractive index sensitivity $m_{int}$ reaches a maximum for 7.5 nm films, above which a decrease in the values is observed (Figure 8B).
To understand this apparent discrepancy it is useful to examine the physical basis of these variables. The two experimental parameters, \( m_1 \) and \( m_{ext} \), are commonly treated in the same manner, as both are similarly used for sensing purposes. However, for a physical discussion the fundamental difference between the two becomes relevant. While the peak wavelength is a basic property of the islands which is independent of the quantity of particles in the optical path (other than coupling effects), the extinction intensity is an additive variable which depends directly on the amount of particles sampled. In the present case, a higher surface density of islands will not affect \( m_1 \) (neglecting plasmon coupling effects, which are minor here), but will lead to a higher extinction, and correspondingly, to a higher RIS \( m_{ext} \). Therefore, while for sensing purposes the values of \( m_1 \) and \( m_{ext} \) are the relevant working parameters, for a discussion of physical aspects of the system, including a comparison of different island films, the value of \( m_{ext} \) should be normalized to the surface density of particles. The normalized parameter \( \bar{m}_{ext} = m_{ext}/c \) where \( c \) is the number of islands per unit surface area (see Table 1), is thus a quantity-independent variable, analogous to \( m_1 \) as an intrinsic physical property of the island ensemble.

Values of \( \bar{m}_{ext} \) versus film nominal thickness are plotted in Figure 8C. The normalized values display a generally monotonous increase with Au film thickness, similar to the behavior of \( m_1 \) (Figure 8A). Hence, while the larger islands display larger values of \( \bar{m}_{ext} \), their low surface density results in lower values of \( m_{ext} \).

**CONCLUSION**

We have presented a systematic experimental study on the relationship between refractive index sensitivity (RIS) and plasmon decay length in Au-island-based localized plasmon transducers. The decay length was derived using polyelectrolyte LbL assembly and fitting to a model of exponentially decaying surface plasmon field. Since the polyelectrolyte multilayer covers both the Au islands and the glass substrate between the islands, experiments with site-selective adsorption as well as model calculations were carried out to assess the sensitivity to binding to the top versus the side faces of the islands, establishing the validity of our approach. The RIS was determined from the same exponential regression, as well as from measurements in solvents of different refractive index values. While the wavelength RIS, \( m_1 \), shows a gradual increase with average island size, the extinction intensity RIS, \( m_{ext} \), initially increases with average island size, reaching a maximum and decreasing upon further size increase. This seemingly contradicting behavior emphasizes the different physical nature of the two variables; that is, \( m_{ext} \) is dependent on the number of islands in the sampled area while \( m_1 \) is not. Hence, normalization of \( m_{ext} \) to the surface density of Au islands leads to a similar behavior to that of \( m_1 \). It is concluded that the intensity RIS \( m_{ext} \) is a working parameter relevant to sensing applications; however, for a discussion of the physical significance of the RIS, a surface density normalized value \( \bar{m}_{ext} \) has to be calculated.

The results for a series of Au island films with varying average island size show a near-linear correlation between the RIS and decay length, the two parameters in eq 1 characteristic of a given transducer. This correlation undermines the assumption that in order to achieve higher LSPR transducer sensitivity the RIS has to be maximized. Instead, it is shown that optimization of LSPR transducers requires tailor-design; that is, a combination of RIS and decay length has to be chosen which maximizes the response to a specific analyte and recognition interface of given dimensions. In the case of biorecognition, where the typical molecular size is a few nanometers, small islands exhibiting lower RIS values are actually expected to provide a higher overall sensitivity, as a result of better matching of the decay length. Our conclusions regarding the interplay between RIS and decay length and its effect on system optimization are general and are expected to be relevant to other types of LSPR transducers as well.

**METHODS**

**Materials.** The substrates used were microscope glass coverslides (Schott AG borosilicate glass D263T No. 3, 22 × 22 mm², with \( T_0 ≈ 557 \) °C, supplied by Menzel-Gläser, Germany), cut to 22 × 9 mm², and quartz slides (Heraeus Quarzglas, Germany), 1 mm thick, cut to 37 × 12 mm². Gold (99.99%, Holland-Moran, Israel); polyallylamine hydrochloride (PAH) (56 kDa, Sigma Aldrich); polystyrene sulfonate, sodium salt (PSS) (70 kDa, Polysciences Inc., Warington, PA, USA); 1-octadecanethiol (ODT) (98%, Aldrich); 2-[methoxy(polyethyleneoxy)propyl]trimethoxysilane, 6–9 C₉H₄O groups (PEG-silane) (90%, Gelest); 3-aminopropyl trimethoxysilane (APTS) (Aldrich); sodium chloride (Fruitarom, Israel); methanol (anhydrous, Mallinkrodt); ethanol (anhydrous, Gadot or Biobal, Israel); heptane (anhydrous, Aldrich); chloroform (Gadot); toluene (Gadot); H₂SO₄ (AR, Gadot); H₂O₂ (30%, Fruitarom); and ammonium hydroxide (Fruitarom), were used as received. Nitrogen was in-house supplied from liquid N₂. All solutions were prepared using triply distilled water. **Continuous Gold Films.** The glass slides were cleaned in freshly prepared “piranha” solution (H₂O₂:H₂SO₄, 1:3 by volume) for 1 h, and washed with deionized water, then with triply distilled water, and finally with ethanol. (Caution: “piranha” solution is extremely corrosive and boils upon mixing.) The slides were then treated with an “RCA” solution (H₂O/NH₄OH/H₂O₂, 5:1:1 by volume) at approximately 70 °C for 1 h, to hydroxylate the surface, and modified with APTS by overnight immersion in 10% APTS (by volume) in methanol. APTS layers have been known to improve the adhesion of gold evaporated on glass substrates.46 The slides were then washed in methanol, sonicated in methanol three times (5 min each), washed in ethanol, and dried under a nitrogen stream. A total of 24 slides were placed on a plate, which was mounted in a cryo-HV evaporator (Key High
Vacuum) equipped with a Maxtek TM-100 thickness monitor. The chamber was evacuated to a pressure of 2 – 3 × 10⁻⁶ Torr, and gold was evaporated onto the slides from a resistively heated tungsten boat. The plates were rotated during evaporation to achieve homogeneous deposition on the slides. The 20 nm of gold was deposited at a rate of 0.1 nm/sec; at this thickness, the film is semitransparent and suitable for transmission spectroscopy. The slides were then annealed at 200 °C for 20 h in a Ney Vulcan 3-350 furnace, at a 5 °C/min heating rate, then left to cool to room temperature inside the furnace. Gold films prepared using this procedure are smooth and can be studied by various surface techniques. Discontinuous (Island-Type) Gold Films. Glass slides were cleaned and mounted in the evaporator chamber as described above (without APTS coating). Gold was evaporated on the slides at a deposition rate of 0.01 nm/sec, to a nominal thicknesses of 3 – 10 nm (the nominal thickness is the reading of the evaporator QCM thickness monitor, i.e. the film mass thickness). Following evaporation, the slides were annealed as detailed above for 10 h at 580 °C (5 °C/min heating rate), then left to cool to room temperature inside the furnace.

Polyelectrolyte LbL Assembly. The LBL procedure was carried out using the positive polyelectrolyte poly(allylamine hydrochloride) (PAH) and the negative polyelectrolyte poly(styrene sulfonate), sodium salt (PSS). 1.0 mM solutions (concentration calculated with respect to the monomer, corresponding to chloride) (PAH) and the negative polyelectrolyte poly(allylamine hydrochloride) (PSS) in 0.1 M NaCl in triply distilled water. Au films prepared as detailed above were treated 10 min in a UV/ozone apparatus (UVOCS Inc., model T10*10/OES/E), with the Au coating facing the UV lamps. The samples were then washed 20 min in ethanol and dried under a nitrogen stream. The slide to be coated was alternately immersed in vials containing the polyelectrolyte solutions for 15 min each, starting with PAH. After each adsorption step the slide was rinsed with water, dipped into an aqueous solution of 0.1 M NaCl, and immersed in the other polyelectrolyte solution. After completing the assembly of a number of polyelectrolyte bilayers the slide was washed and dried under a nitrogen stream, measured, and the LBL procedure was resumed. The adsorption and measurements were carried out in a climate-controlled laboratory, at a temperature of 22.5 ± 1.0 °C and a humidity of 50 ± 5%.

Site-Selective Adsorption. Au island slides were treated in the UV/ozone apparatus and washed in ethanol as detailed above. ODT was adsorbed by immersing the slide in a 1 mM ODT ethanolic solution for 1 h. PEG–silane was adsorbed similarly from a freshly prepared 2 mM PEG–silane ethanolic solution, for 2 h. Adsorption of both SAMs was followed by a 20 min wash in ethanol and drying. For each Au thickness studied, four slides were coated with ODT followed by PEG–silane, and four were coated with PEG–silane followed by ODT. Characterization. Extinction spectra at normal incidence of polyelectrolyte multilayers were measured using a Varian Cary 50 probe UV/vis spectrophotometer. Measurement parameters were as follows: wavelength resolution, 1 nm; scan rate, 300 nm/ min; average acquisition time per point, 0.2 s. For dry measurements, air was used as the baseline; for measurements in a given solvent, a cuvette filled with the same solvent, without a slide, was used as baseline. Spectra of SAMs (ODT, PEG–silane) were measured at 0.2 nm resolution using the Varian Cary spectro-photometer or an Ocean Optics USB4000XR spectrophotometer equipped with a Polychromix MobiiLight light source. Polyelec- trolyte film thickness on continuous gold slides was measured in air using an Angstrom Advanced PHE-102 spectroscopic ellipsometer, at an angle of incidence of 70°, in the spectral range 300 – 800 nm, using 1 – 20 nm steps. The high-resolution measurements were used to develop a model, which was then used for analyzing the lower-resolution measurements. Ellipsometric data were analyzed using Film Wizard software (Scientific Computing International, California, USA). High-resolution scanning electron microscopy (HRSEM) images were obtained using a Carl Zeiss Ultra-55 ultrahigh-resolution SEM. The samples were coated with a thin layer of Cr (2 nm Cr on 3 – 5 nm Au, 3 nm Cr on 6 – 10 nm Au), for improved conductivity.

Image Analysis. HRSEM images of Au island films of various nominal thickness were analyzed using ImageJ image analysis software (Wayne Rasband, NIH, USA), to determine particle dimensions and size distribution. The average distance between islands was calculated as the average distance between the particle centers, to the four nearest neighbors. In addition, the surface density of islands for the various Au film thicknesses was calculated; half of the particles crossing edges were counted. These calculations were based on the same HRSEM images used for particle size analysis.

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Supporting Information Available: UV–vis spectra of polyelectrolyte multilayers on Au island films; ellipsometric thickness of polyelectrolyte films; differential wavelength and extinction intensity response to adsorption; site-selective adsorption calculations. This material is available free of charge via the Internet at http://pubs.acs.org.

REFERENCES AND NOTES

Supporting Information

1. Linear growth of polyelectrolyte coatings

A prerequisite for the use the polyelectrolyte LbL assembly for the construction of dielectric layers of a known and predictable thickness is that every deposition cycle (PAH/PSS) add the same amount of material, even after numerous cycles. To test this assumption polyelectrolyte layers were adsorbed (from 1.0 mM polyelectrolyte solutions in 0.1 M aqueous NaCl) onto quartz slides coated with a 20-nm-thick continuous gold film. PSS has two absorption peaks in the UV range attributed to the aromatic ring, at 194 nm and 227 nm. The slides were therefore measured by transmission UV/vis spectrophotometry as well as by spectroscopic ellipsometry.

To examine the effect of drying, the slides were measured at different intervals: slide No. 1 (“s1”) was dried and measured after 10, 20, 30, and 40 layers (corresponding to 5, 10, 15 and 20 bilayers, or deposition cycles); No. 2 (“s2”) – after 20, 30 and 40 layers; No. 3 (“s3”) – after 30 and 40 layers; and No. 4 (“s4”) – only after 40 layers.

Figure S1A shows the change in the extinction (vs. bare gold) for slide No. 1 in the measured spectral range; while there are sharp positive extinction peaks in the UV range, there is mild negative extinction in the visible range. The latter is attributed to anti-reflective nature of the coating in this range. Figure S1B shows the change in extinction (vs. bare gold) for all four slides, indicating that drying the slides during the LbL process has no effect on the extinction peaks. This shows that drying does not affect the amount of deposited material and does not irreversibly change the film structure. Figure S1C
presents the peak intensity at 194 nm and 650 nm vs. number of layers, showing a linear growth of the polyelectrolyte multilayer.

Figure S1. A: Change of the spectrum (reference: bare gold) with the number of polyelectrolyte layers for slide s1. B: Change of the spectrum (reference: bare gold) with the number of polyelectrolyte layers for the four slides. C: Change of the extinction maximum of slide s1 at two wavelengths (indicated).

Figure S2 presents the ellipsometrically-determined thickness of polyelectrolyte films as a function of the number of deposited layers, confirming the linear growth shown in Figure S1C.
Figure S2. Ellipsometric thickness vs. number of polyelectrolyte layers, deposited from aqueous solutions of 1.0 mM polyelectrolyte (PAH, PSS) in 0.1 M NaCl on a continuous gold substrate. The line is a linear regression.

2. Incremental wavelength shift and extinction change

Figure S3. Experimental incremental wavelength shift (A) and extinction change (B) for the adsorption of a 2.1-nm-thick polyelectrolyte bilayer on an existing polyelectrolyte multilayer of varying thickness (data from Figure 10); Au nominal thicknesses are indicated.

3. Model calculation of the response to site-selective adsorption

To assess the contribution of adsorption of a dielectric layer on the glass between the islands to the overall response of Au island transducers, the islands are modeled using the average dimensions measured from HRSEM images. The model considers an individual island, neglecting possible coupling effects; this is a reasonable approximation, as the distance between islands is typically much larger than the decay length. The island is approximated as a cylinder (flat face parallel to the glass surface), with an in-plane radius
equal to half the average of the major and minor axes of the imaged particles, and a height $b$ equal to the effective height in Table 1. The part of the island embedded in the glass does not provide adsorption sites and is not taken into account. The island surface available for adsorption is divided into two sections, i.e., the flat top circle and the cylindrical side. The two sections are assumed to have different RIS value. Figure S4 shows a model Au island being gradually coated with a dielectric polyelectrolyte film covering both the island and the glass substrate.

![Figure S4](image)

**Figure S4.** Schematic representation of a gold island coated with a thin (left) and a thick (right) polyelectrolyte (PE) multilayer. As more layers are deposited (on the gold and on the glass), the part on the glass which is unaccounted for by our model (shown in red) increases in thickness, but its distance from the gold island increases by the same amount.

The areas of the different sections are:

$$S_{\text{top}} = \pi a^2 ; \quad S_{\text{side}} = 2\pi ab ; \quad S_{\text{total}} = S_{\text{top}} + S_{\text{side}} \quad (S1)$$

The response to adsorption on the glass between islands is:

$$R_{\text{glass}} = \frac{2\pi ad}{S_{\text{total}}} m_{\text{side}} \Delta \eta \quad (S2)$$

where $m_{\text{side}}$ is the RIS values for adsorption on the island’s side; $d$ is the adlayer thickness; and $\Delta \eta$ is the change in refractive index between the adlayer and the displaced medium (air in the present work). Note that the exponential term was dropped in Equation S2 as we assume that the distance between islands is much larger than the decay length.

Equation S2 can be rearranged:
As all variables in Equation S3 are known (from site-selective adsorption experiments), \( m_{\text{side}} \) can be calculated for all of the studied island sizes. The values are plotted in Figure S5, together with the average RIS determined experimentally using polyelectrolyte multilayers. The results in Figure S5 suggest that the RIS of the island sides is lower than the average RIS, for all nominal thicknesses measured. While the RIS of the island side may indeed be lower than the average RIS, we assume that at least part of the difference is attributed to the finite distance between islands, neglected in Equation S2.

![Figure S5](image-url)

**Figure S5.** Refractive index sensitivity (RIS) of the island side surface (from site-selective adsorption measurements) and the average RIS (measured using polyelectrolyte multilayers).

Polyelectrolyte multilayers adsorbed in order to determine the decay length cover both the Au islands and the glass surface between islands; however, the concentric growth model used in this work only considers growth on the islands. To assess the deviation from the model, one has to estimate the contribution of the polymer adsorbed on the glass to the overall response. This can be done using our simplified model described above (Figure S4). As polyelectrolyte layers are added, the thickness of the coating on the glass unaccounted for by Equation 1 increases linearly; however, its distance from the island
surface increases by the same amount (for a polymer layer of a thickness $d$, the first $d$ nanometers from the island surface are accounted for by the concentric growth model, whereas the rest of the layer on the glass is not; see Figure S4). Due to the exponential decay of the evanescent plasmon field, the response contributed by this portion of the layer decreases. Hence, one can expect the relative part of the signal attributed to the “unaccounted for” part of the layer on the glass to drop rapidly with increasing adlayer thickness.

As noted above, the data in Figure S5 indicate that the RIS of the island side is equal to or lower than the average RIS. In the calculation below we use a “worst case scenario”, i.e., we assume that the RIS of the island side is equal to the experimental average value. As the RIS and decay length are correlated, we also use the same value of the decay length.

The response to the polymer layer on the gold is described by:

$$R_{Au} = m\Delta\eta \left( 1 - e^{-\frac{d}{\tau}} \right) \quad (S4)$$

Assuming that the coating is thinner than the height of the islands, the response to the part of the adlayer on the glass (beyond the thickness $d$ already included in Equation S4) is:

$$R_{glass} = \left( \frac{2md}{s_{total}} \right) m\Delta\eta e^{-\frac{d}{\tau}} \quad (S5)$$

Figure S6 presents the calculated overall response and its two components, i.e., the contribution of the film on the Au islands and that of the “unaccounted for” film on the glass, for LbL multilayer adsorption (up to 20 nm) on a 7.5 nm Au island film. The calculation was carried out by substituting in Equations S4 and S5 experimental RIS and decay length values determined using polyelectrolyte layers, and using $\Delta\eta = 0.5$.

The fraction of the signal resulting from the extra layer on the glass is plotted, for the same island film, in Figure S7. Its contribution drops from ca. 24% for a 1 nm layer, to ca. 11% for a 20 nm layer.
Figure S8 shows calculated results similar to those presented in Figure S7, for various Au nominal thicknesses. Generally, the greater decay length of larger islands translates to a stronger relative response from the film on the glass and a slowed decay with increasing thickness.

**Figure S6.** Calculated overall plasmon wavelength shift for a polyelectrolyte multilayer of increasing thickness, and its two components (resulting from the film on the Au islands and the extra film on the glass between islands), for a 7.5 nm Au island film.
Figure S7. Calculated fraction of the overall plasmon wavelength shift resulting from “unaccounted for” adsorption on the glass between island, for a polyelectrolyte multilayer of increasing thickness adsorbed on a 7.5 nm Au island film.

Figure S8. Same as Figure S7, calculated for a range of Au nominal thicknesses.
Following our work, in 2012 Tian et al. used PE multilayers to explore the relationship between aspect ratio and decay length for Au nanorods (NR). They found a linear relation between decay length and NR diameter, and decay length and NR length, but no relation between decay length and aspect ratio. While the authors did not report the RIS values, we extracted them, along with the decay lengths, from the decay curves presented in the paper, which we fit to Equation 2. The results show a seemingly linear relation between decay length and RIS for a set of NRs with constant diameter and increasing length (Figure 5A), but no relation for constant length and increasing diameter (Figure 5B). This is to be expected, as the authors monitored the long wavelength, longitudinal plasmon resonance, which is much more sensitive to length compared to width. A linear regression ($R^2=0.93$) of the RIS as a function of decay length for variations in the NR length (Figure 5A) produces the relation:

$$m = -401 \pm 70 \text{nm} \text{RIU}^{-1} + l_d \times (48.9 \pm 6.5 \text{RIU}^{-1})$$

The slope here is vastly steeper than in our results (Figure 4), where the relation is: $m = 58.2 \pm 3.9 \text{nm RIU}^{-1} + l_d \times (2.43 \pm 0.62 \text{RIU}^{-1})$ ($R^2=0.71$). This means that the decay length / RIS correlation factor is not universal, but depends on the nanostructure type.

**Figure 4.** Refractive index sensitivity as a function of decay length, for the various Au nominal thicknesses studied (indicated), with a linear fit (red line); data points are the mean of four samples each, and the errors bars represent standard deviations. Data from our publication above (Kedem et al., *ACS Nano* 2011).
Figure 5. Refractive index sensitivity as a function of plasmon decay length for Au nanorods of various dimensions (indicated, in nm), varying the nanorod length (A; including linear fit) or width (B). Data extracted from reference 96.
5.2 Reflection measurements


The response of LSPR transducers to RI changes is measured using either transmission or reflection spectra. However, no systematic comparison between the sensitivity obtained using the two measurement modes was available. We thus measured the RIS, using solvents of different RIs, of seven different Au nano-island film types, and one immobilized solution-synthesized Au NP film. The extinction and reflection spectra reveal significantly larger peak shifts in the reflection mode, and the RIS for reflection mode is correspondingly higher.

Miller et al., using both theory and calculations, predicted that the RIS is a linearly increasing function of the plasmon peak wavelength. This correlation has been reported by various authors, including our group. It appears that the correlation also extends to the increased RIS of reflection mode measurements (Figure 6; this figure was not published in the paper attached below). The peaks in reflection spectra are wider and flatter, increasing the inaccuracy in peak determination, evidenced by the larger error bars.

In experiments performed later, involving layer adsorption, we found that the plasmon peak wavelength shifts are larger in the reflection mode for various average Au island sizes, over a wide thickness range (Figure 7).

We thus conclude that reflection mode measurements can provide superior sensitivity, and are thus preferable with respect to transmission mode measurement, barring other experimental constraints.

This conclusion is further bolstered by the results presented in section 5.4.
Figure 6. Refractive index sensitivity (RIS), determined by immersion in solvents of different refractive indices, of gold nano-island slides of seven different nominal thicknesses (3-10 nm, indicated) as a function of the plasmon peak wavelength in EtOH, in transmission and reflection modes. Data points are the mean of four samples each, and the error bars represent standard deviations.

Figure 7. Plasmon peak wavelength shift upon adsorption of polyelectrolyte layers, for Au3nm, Au5nm and Au10nm samples (indicated), in both transmission and reflection modes (indicated).
Improved Sensitivity of Localized Surface Plasmon Resonance Transducers Using Reflection Measurements

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ABSTRACT: The refractive index sensitivity (RIS) of a localized surface plasmon resonance (LSPR) transducer is one of the key parameters determining its effectiveness in sensing applications. LSPR spectra of nanoparticulate gold films, including Au island films prepared by evaporation on glass and annealing as well as immobilized Au nanoparticle (NP) films, were measured in the transmission and reflection modes. It is shown that the RIS, measured as the wavelength shift in solvents with varying refractive index (RI), is significantly higher in reflection measurements.

SECTION: Nanoparticles and Nanostructures

Nanostructured metal (e.g., gold, silver, copper) structures exhibit an optical extinction band in the visible range, attributed to excitation of localized surface plasmons (SPs). The SP excitation is sensitive to the metal nanostructures’ surrounding environment, shifting in wavelength and intensity in response to change of the effective dielectric constant of the contacting medium. Such a change may occur upon analyte adsorption on the metal surface, either directly or through a bound recognition layer, making LSPR spectroscopy a viable scheme for chemical and biological sensing.

The optical response of an LSPR transducer to the formation of a dielectric adlayer is often modeled using the simple equation

\[ R = m \Delta \eta \left(1 - \exp\left(-\frac{d}{l}\right)\right) \]  

(1)

where \( R \) is the response (in the present case, wavelength shift), \( m \) is the bulk refractive index sensitivity (RIS), \( \Delta \eta \) is the difference between the refractive index (RI) of the adlayer and that of the bulk medium, \( d \) is the adlayer thickness, and \( l \) is the SP decay length. In the case of an adlayer of a thickness much greater than the decay length (e.g., change of the bulk medium), eq 1 is reduced to

\[ R = m \Delta \eta \]  

(2)

The LSPR extinction band represents two distinct phenomena, absorption and reflection (specular and scattered), both responding to changes in the dielectric properties of the adjacent medium. Extinction (i.e., transmission expressed in log units) and reflection have been used for monitoring adsorption of analytes; however, the two measurement modes have rarely been compared. In a study of bioconjugated Au colloids, it was suggested that a higher sensitivity is achieved by using scattered rather than transmitted light. A recent study concerning reflection measurements from the back side of a sample using p-polarized light, found a similar sensitivity for the two measurement modes.

In the present work, the issue of transmission versus reflection measurements in LSPR spectroscopy is examined using two nanoparticulate Au systems: (i) Au island films prepared by evaporation on glass slides and annealing and (ii) Au nanoparticle (NP) films prepared by immobilization of colloidal Au on glass slides. The first system comprised ultrathin Au island films, 3–10 nm nominal (mass) thickness, resistively evaporated on glass substrates and annealed 10 h at 80 °C. The process results in the formation of Au islands of ca. 20 to 100 nm in lateral dimension, partially embedded in the glass substrate. Utilization of such LSPR transducers in sensing applications was demonstrated using transmission spectroscopy. The second system comprised citrate-stabilized Au NPs immobilized on an aminosilane-modified glass substrate. The NPs are generally oval (average aspect ratio: 1.3), with an average diameter of 23 ± 7 nm along the major axis, comparable to that of the islands in an annealed 3 nm (nominal thickness) Au island film. The peak wavelength of the immobilized NPs in water is located at 529 nm (Figure S1, Supporting Information).

Figure 1 shows high-resolution scanning electron microscope (HRSEM) images of glass slides coated with (i) 3 and 10 nm (nominal thickness) Au films, annealed, and (ii) an immobilized Au NP film. The NP film appears aggregated, resulting from drying of the sample in preparation for the imaging. The transmission spectrum of the Au NP film in solution is characteristic
of an ensemble of isolated NPs, whereas after drying, the spectrum shows an additional broad, red-shifted band indicative of NP aggregates (Figure S1, Supporting Information).

To determine the RIS, we measured the samples in air (RI = 1.000) and in solvents of varying RI values: methanol (1.328), ethanol (1.359), heptane (1.386), chloroform (1.445), and toluene (1.496). Spectra were recorded in the transmission mode and in the specular front-reflection mode at normal incidence. The higher slopes of the reflection data indicate a higher RIS in this mode; the difference between transmission and reflection is more pronounced for the NP films compared with the island films, the former showing a ca. 180% increase in RIS, from 63 ± 2 to 176 ± 8 nm/RIU (Figure 3). Average RIS values in the transmission and reflection modes are shown in Figure 4 for all studied Au island films. In the measured nominal thickness range, reflection measurements afford significantly higher RIS, showing 42% to 105% increase with respect to the corresponding transmission data.

In conclusion, reflection measurements provide a viable mode of operation of LSPR transducers. In the case of evaporated Au island films, reflection measurements furnished substantially higher wavelength sensitivity with respect to transmission measurements. Depending on the island film morphology, the RIS was larger by ca. 42–105% in the reflection mode. The enhancement was even greater with immobilized NP films, showing a sensitivity increase of ca. 180%. The enhanced RIS was achieved using a simple specular reflection configuration. The higher RIS can be advantageous in sensing applications as well as in envisioned LSPR imaging, that is, translating spatial RI variations to images, similar to recent reports on propagating surface plasmon resonance (SPR) imaging and mapping. It should be emphasized, however, that in LSPR sensing of adsorbed analyte layers the plasmon decay length must also be considered (eq 1).

Figure 2. Normalized extinction (A,C) and reflection (B,D) spectra of a 5 nm (nominal thickness) Au island film, annealed (A,B), and an immobilized nanoparticle film (C,D) measured in air (island film) and in various solvents (indicated). The original (not normalized) extinction spectra are presented in Figure S2 (Supporting Information).
in the evaluation of the overall sensitivity. In addition to the enhanced RIS, LSPR reflection measurements may furnish other advantages with respect to transmission, notably in the possibility of studying opaque systems as well as in cases where a reflection geometry is easier to implement.

**EXPERIMENTAL METHODS**

Extinction spectra were measured using a Varian Cary 50 Probe UV/vis spectrophotometer. Spectral reflectance (front reflection) at normal incidence was measured using an Ocean Optics USB2000 fiber optic spectrophotometer with a Polychromix MobiLight light source and an Ocean Optics reflection probe. Au island films of different nominal thicknesses were prepared by evaporation on glass slides and postdeposition annealing, as previously described; the nominal thickness was determined during evaporation using a Maxtek TM-100 thickness monitor. Citrate-stabilized Au NPs were synthesized using known methods, as previously reported. The NPs were characterized using a Philips CM120 Super Twin transmission electron microscope (TEM). To form NP films, glass slides were cleaned using piranha solution, coated with a 3-aminopropyl trimethoxysilane (APTS) binding layer (0.1% v/v in water, 15 min), and incubated in the NP solution overnight. Scanning electron microscope images were obtained using a Carl Zeiss Ultra-55 ultrahigh-resolution SEM. The refractive index of solutions used for determining the RIS was measured by means of an Abbe refractometer.

**ASSOCIATED CONTENT**

Supporting Information. Extinction spectra of an Au NP film under wet and dry conditions; extinction spectra of an Au island film and an Au NP film in various solvents; extinction and normalized reflection spectra of Au films of various nominal thicknesses. This material is available free of charge via the Internet at http://pubs.acs.org.

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**REFERENCES**

7. Bendikov, T. A.; Rabinkov, A.; Karakouz, T.; Vaskevich, A.; Rubinstein, I. Biological Sensing and Interface Design in Gold Island
Supporting Information

Improved Sensitivity of Localized Surface Plasmon Resonance (LSPR) Transducers Using Reflection Measurements

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Figure S1. Extinction spectra of an immobilized Au nanoparticle (NP) film, in the initial wet condition and after drying. Note the appearance of a broad band in the near IR region after drying, characteristic of NP aggregates.

Figure S2. Extinction spectra of a 5 nm (nominal thickness) Au island film, annealed (left) and an immobilized Au NP film (right), taken in air (island film) and in solvents of different refractive indices.
Figure S3. Extinction (left) and normalized reflection (right) spectra of Au island films of various nominal thickness (indicated), taken in air and in solvents of different refractive indices.
5.3 Long-range response

This part of the work was carried out in collaboration with Prof. Takumi Sannomiya (Tokyo Institute of Technology, Tokyo, Japan).


The resonance wavelength and intensity of LSPR transducers are influenced by the transducers’ immediate environment, as a result of the evanescently decaying plasmon electric field, which extends into the medium. Recent works on such systems have reported an oscillatory response of the LSPR peak wavelength and intensity at large nanostructure/adlayer separations, well outside the accepted decay length of the evanescent field. This response was attributed to reflected fields, and a model based on image dipole interactions was proposed. In the present work we investigate in detail the interaction between plasmonic arrays and dielectric overlayers using both experiment and simulation. The long-range oscillatory behavior is attributed to interference effects that modulate the energy distribution between the transmittance, reflectance, and absorbance channels. The observations are accurately modeled using analytical functions based on an asymmetric etalon (Fabry-Pérot interferometer). This treatment allows us to separately extract the reflectivity phase and amplitude of the particle layer at all measured wavelengths by two-dimensional analysis of the extensive acquired data. The complex quantitative data, with emphasis on phase information, provide better understanding of the interaction of light with layered plasmonic structures.

This work is in essence a continuation of the work presented in section 5.1, extending the range of distance dependence research to the long range, interference-dominated regime.
Oscillatory Behavior of the Long-Range Response of Localized Surface Plasmon Resonance Transducers

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Supporting Information

ABSTRACT: Localized surface plasmon resonance (LSPR) entails the resonance of light with collective charge density oscillations in nanostructured metal. The resonance wavelength and intensity are influenced by the nanostructures’ immediate environment as a result of the evanescently decaying plasmon electric field, which extends into the medium. Recent works on such systems have reported an oscillatory response of the LSPR peak wavelength and intensity at large nanostructure/adlayer separations well outside the accepted decay length of the evanescent field. This response was attributed to reflected fields, and a model based on image dipole interactions was proposed. In the present work we investigate in detail the interaction between plasmonic arrays and dielectric overlayers using both experiment and simulation. The oscillatory behavior is attributed to interference effects that modulate the energy distribution between the transmittance, reflectance, and absorbance channels. The observations are accurately modeled using analytical functions based on an asymmetric etalon. This treatment allowed us to separately extract the reflectivity phase and amplitude of the particle layer at all measured wavelengths by two-dimensional analysis of the extensive acquired data. The complex quantitative data, with emphasis on phase information, provide better understanding of the interaction of light with layered plasmonic structures.

1. INTRODUCTION

Nanostructured metal (e.g., gold, silver) surfaces support collective charge-density oscillations, termed localized surface plasmons. Light of appropriate frequency can resonate with the metal structures (localized surface plasmon resonance, LSPR), leading to energy absorption and scattering, exhibited as an extinction band in the UV/vis/IR range. The resonance wavelength and amplitude of the LSPR band are sensitive to the dielectric constant in the vicinity of the metal nanostructures, attributed to an evanescently decaying electric field extending from the metallic nanostructures into the medium. This phenomenon has been widely investigated for use in sensing applications, however, the typically short decay length in LSPR limits the interaction distance between nanostructures (e.g., nanoparticles (NPs)) and their surroundings to the range of a few nanometers to tens of nanometers, depending on several factors, such as the nanostructure size. We have previously studied the distance dependence for a range of gold island transducers.

In the past few years several groups have reported the observation of interaction between NP arrays and dielectric layers hundreds of nanometers apart. This interaction takes the form of oscillations in the resonance peak wavelength as a function of the dielectric adlayer thickness, extending far beyond the interaction range expected from the plasmon decay length. Several theoretical attempts have been made to explain these unexpected oscillations; these reports identified the reflected field as relevant to the effect, but the models only partially reproduce the experimental data, relying on single dipole particle approaches, which cannot fully describe larger particles and ensembles. The effect of the phase shift of light reflected from the NPs, affecting the position of the peaks and dips in the oscillation pattern, was not considered. A very recent report, published during preparation of this manuscript, explored the issue using simulations, attributing the oscillations to Fabry–Pérot modes. Thick dielectric layers have been used previously as spacers or interference layers between a metal NP array and a reflective layer in LSPR sensing systems or in surface-enhanced Raman scattering (SERS), where sensing is actually performed within the range of the field-enhanced evanescent wave.

In the present work we investigate, both experimentally and computationally, the oscillatory behavior of layered systems comprising a dielectric substrate, a plasmonic NP layer, and a thick dielectric layer of increasing thicknesses, coated with dielectric layers of decreasing sizes, coated with dielectric layers of increasing thicknesses, using both experiment and simulation. The large amount of acquired data allows us to present a complete picture of the phenomenon and characterize the complex behavior of a plasmonic array coated with dielectric layers based on an optical interference approach.

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By use of a simple analytical model, we extract the phase and amplitude of the reflectivity of the plasmonic layers by fully fitting the oscillatory curve at all measured wavelengths. In resonant systems, there is a transition from in-phase to out-of-phase oscillations (with respect to the driving force) around the resonant frequency. Our separately acquired phase information, not easily accessible unless a specialized holographic measurement setup is used, provides another dimension for investigating the existing plasmon resonance modes. Lastly, we explore the effect of the dielectric overlayers on the distribution of energy between the transmittance, reflectance and absorbance channels of the NP array. This distribution is a major factor in plasmonic solar cells.

2. EXPERIMENTAL METHODS

Materials. The substrates used were microscope glass cover slides (Schott AG borosilicate glass D263T No. 3, 22 × 22 mm², with Tg ≈ 557 °C, supplied by Menzel-Gläser, Germany), cut to 22 × 9 mm²; gold (99.99%, Holland-Moran, Israel); SiO₂ pieces (99.99%, Kurt J. Lasker, USA); H₂SO₄ (AR, Gadot, Israel); H₂O₂ (30%, Frutarom, Israel); ammonium hydroxide (Frutarom); 3-aminopropyl trimethoxysilane (APTS) (Aldrich); methanol (absolute, Biolab, Israel) and ethanol (anhydrous, Gadot or Biolab) were used as received. Nitrogen was in house, supplied from liquid N₂. All solutions were cleaned in freshly prepared Piranha solution (H₂O₂:H₂SO₄, 5:1:1 by volume) for 1 h, washed with deionized water, then washed with triply distilled water, and finally washed with ethanol. (Caution: “piranha” solution is extremely corrosive and boils upon mixing.) The slides were then treated with an “RCA” solution (H₂O₂:NH₄OH:H₂O₂, 5:1:1 by volume) at approximately 70 °C for 1 h, a treatment commonly used to improve silane binding, and immersed in a 1% (v/v) methanolic APTS solution. APTS layers are known to improve the adhesion of gold evaporated on glass substrates. The slides were then washed in methanol, sonicated three times in methanol (5 min each), washed in ethanol, and dried under a nitrogen stream. Twenty-four slides were placed on a round plate, which was mounted in a cryo-HV evaporator (Key High Vacuum) equipped with a Maxtek TM-100 thickness monitor. The chamber was evacuated to a pressure of 2–3 × 10⁻⁷ Torr, and Au was evaporated onto the slides from a resistively heated tungsten boat. The plate was rotated during evaporation to achieve homogeneous deposition on the slides. 100 nm of Au were deposited at a rate of 0.1 nm/s. The slides were then annealed at 200 °C for 20 h in a Ney Vulcan 3–550 furnace, at a 5 °C/min heating rate, and then left to cool to room temperature inside the furnace. Au films prepared using this procedure are smooth and can be studied by various surface techniques.

Discontinuous (Island-Type) Gold Films. Glass slides were cleaned and mounted in the evaporation chamber as described above, without RCA treatment and with no APTS coating. Au was evaporated on the slides at a deposition rate of 0.01 nm/s to nominal thicknesses of 3, 5, or 10 nm (the nominal thickness is the reading of the evaporator QCM thickness monitor, i.e., the film mass thickness). Following evaporation, the slides were annealed as detailed above for 10 h at 580 °C (5 °C/min heating rate) and then left to cool to room temperature inside the furnace.

SiO₂ Coating. Continuous Au and Au island slides were cleaned in a UV/ozone apparatus (UVOCS model T10 × 10/ OES/E), dipped in ethanol for 20 min with stirring, and dried under a nitrogen stream. As silica has low adhesion to gold, prior to deposition AuCont slides were coated with APTS by dipping the slide in a 0.1% v/v APTS solution in methanol for 15 min, followed by thorough washing in methanol and drying. If this APTS treatment was not utilized, SiO₂ films thicker than ca. 100 nm tended to fracture and peel off of the Au film. Slides were then coated with SiO₂ (where x ≤ 2, as oxygen was not
added to the evaporation chamber) coatings of 30 different thicknesses in the range 15−348 nm, using electron-beam assisted physical vapor deposition at a deposition rate of 0.15−0.2 nm/s. The samples were coated in batches of four, one of each type, for a total of 120 samples. The thickness was monitored in situ using a quartz crystal microbalance and accurately determined post deposition using spectroscopic ellipsometry of coated AuCont samples. This measurement also provided the refractive index of the coating in the visible range: \(n = 1.46\), \(k = 0\) (negligible absorption). Reflectivity measurements of AuCont samples match calculated spectra (using FilmWizard, Scientific Computing International, California, USA) for the various thicknesses, providing further confirmation of the thickness (Figure S5 of the Supporting Information).

**Characterization.** Extinction spectra at normal incidence were measured using a Varian Cary 50 Probe UV/vis spectrophotometer. Measurement parameters: wavelength resolution, 1 nm; scan rate, 300 nm/min; average acquisition time per point, 0.2 s. Reflection spectra were measured at normal incidence using an Ocean Optics USB4000XR spectrophotometer equipped with a Polychromix MobiLight light source and an Ocean Optics reflection probe. Measurement parameters: wavelength resolution, 0.2 nm; acquisition time per spectrum, 0.005 s; 200 spectra to average. SiO\(_2\) film thickness on AuCont slides was measured in air using an Angstrom Advanced PhE-102 spectroscopic ellipsometer, at an angle of incidence of 70°, in the spectral range 300−800 nm, using 10 nm steps. Ellipsometric data were analyzed using FilmWizard software (Scientific Computing International, California, USA). High-resolution scanning electron microscopy (HRSEM) images were obtained using a Carl Zeiss Ultra-55 Ultrahigh-resolution SEM. The samples were coated with a thin layer of Cr (2 nm for Au3nm slides (see below), 3 nm for Au5nm and Au10nm samples are similar and are presented in Figure S1 of the Supporting Information).

3. RESULTS AND DISCUSSION

**Experimental Results.** Our transducers comprised Au island films, fabricated by resistive evaporation of a thin layer of Au onto a glass substrate, followed by high-temperature annealing to form islands partially embedded in the glass.\(^{18,40−42}\) We used Au island films of 3 nm ("Au3nm"), 5 nm ("Au5nm"), and 10 nm ("Au10nm") nominal (mass) thickness (22 ± 5, 34 ± 10, and 114 ± 41 nm average island diameters, respectively; the corresponding in-plane aspect ratio is 1.1 to 1.2), as shown in Figure 1a, and continuous Au films, 100 nm thick ("AuCont"), on glass substrates. Representative experimental transmission spectra of the bare Au island films are shown in Figure 1b, revealing prominent LSPR extinction bands.

SiO\(_2\) films in the thickness range 15−348 nm were deposited on the Au island transducers by electron beam assisted physical vapor deposition, to investigate the effect of a dielectric overlayer. The SiO\(_2\) coatings are smooth and uniform over large areas (panels c and d of Figure 1) with the surface morphology reflecting the underlying island topography.

Experimental transmission and specular reflection (at normal incidence) spectra of the Au island slides before and after SiO\(_2\) deposition enable 2-dimensional mapping of the spectra as a function of wavelength and layer thickness, as shown in panels a and b of Figure 2 for Au5nm samples. The results for Au3nm and Au10nm samples are similar and are presented in Figure S1 of the Supporting Information. In agreement with the literature data,\(^{19−24}\) the transmission spectra (Figure 2a) reveal an initial red-shift and intensity increase of the transmission minimum (i.e., the transmittance is decreased), followed by oscillations for thicker coatings. Figure 2c shows the wavelength shift and intensity change of the transmission minimum as a function of coating thickness, presenting more clearly the oscillations.

To further explore the periodic oscillation of the signal, the reflectance at 500 nm was extracted for all the experimental samples (Figure 3). The oscillations in the reflectance intensity for the different samples share the same periodicity, which holds for all the other wavelengths (Figure S1 of the Supporting Information).

**Simulation.** To investigate in detail these plasmonic/dielectric systems, we performed simulations using the multiple multipole program (MMP), a semianalytical simulation method where the electromagnetic field is expressed by superposition of distributed basis functions, so-called expansions (each is an analytical solution of Maxwell’s equations), and their coefficients are solved at the discretized boundaries by means...
of method of moments.43 MMP is advantageous in our system, consisting of plasmonic structures requiring fine discretization and large dielectric structures.

To emulate our experimental system we used several model systems comprising Au particles of 15, 25, or 35 nm in diameter placed in a square array with periodicities of 50 and 250 nm. The use of spherical particles provides high simulation accuracy in 3D, avoiding triple points where a singularity occurs. Since the scale of our particles is much smaller than the wavelength, dipole-type oscillation dominates, and spherical particles properly model the NPs in the experiments. Under these conditions, no systematic diffraction from the arrays occurs in the visible wavelength range. Also, it is noted that no systematic diffraction occurs in the experiment as the interparticle distance is smaller than the wavelength for small particles and the distribution of the particles has no order for larger particles (Figure 1a). Random scattering in the experiment will be accounted for as absorption.

Figure 4 shows the schematics of the models used. Transmission and reflection intensities are calculated from the far-field components of the Rayleigh expansions located at the medium/coating and coating/substrate interfaces. The coating layer thickness was varied from 60 to 300 nm, at 10 nm intervals. We use the notation of PxxDyy to indicate a periodicity of xx nm and a particle diameter of yy nm. Constant refractive indices were used for the dielectric materials, namely, 1.5, 1.46, and 1.0 for glass, SiO₂, and air, respectively. Literature data were used for the dielectric constants of gold.44 The top surface was assumed to be flat for simplicity, unlike the rough real surface in the experiment. The surface roughness causes incoherent scattering which results in a decrease in the oscillation amplitude. Therefore the absolute reflection amplitude discussed below includes the effect of surface roughness in the experiment.

Figure 5 shows the calculated transmission, reflection, and absorption spectra for the P50D35 model (i.e., particles 35 nm in diameter, with a 50 nm periodicity) as well as the wavelengths and intensities of the transmission minimum. The particles in this model are similar in diameter to those in the Au5nm experimental system, though the experimental particles are flatter. The transmission minimum shows oscillations, as in the experimental system. The calculated spectra of other models exhibit the same basic characteristics, and some are shown in Figures S2 and S3 of the Supporting Information. The transmission spectra of smaller particles are characterized by narrower minima, and the reflection spectra of sparse arrays (e.g., P250D15) are dominated by reflections from the glass.

**Analytical Model.** Our spectra of Au island systems with a clear oscillatory interference effect, as well as models of similar systems, enable analysis of the optical characteristics of this plasmonic/dielectric layered system in a wide wavelength range, thus providing an opportunity to access the amplitude and phase information of the plasmonic structures. Sannomiya et al. have shown that a dense NP layer can act as a mirror by far-field interaction29 and that the NP array introduces a wavelength-dependent phase shift related to the resonance, different from that of a continuous metallic mirror. Our system consists of two interfering reflection sources, at the medium/dielectric layer and dielectric layer/plasmonic layer interfaces.

To investigate this interference, we fit the reflection intensity as a function of coating thickness for the entire thickness range, at each wavelength, of the experimental and model systems. The exact fitting function is based on the modified equation for an asymmetric etalon (Fabry–Pérot interferometer) system:46

\[
I_R = \frac{\left( A - 2 \cos \left( \frac{2\pi nt}{\lambda} + \phi \right) \right)}{1 + R^2 - 2R \cos \left( \frac{2\pi nt}{\lambda} + \phi \right)} R
\]

(1)

where \( I_R \) is the measured reflectance intensity, \( A \) is a parameter related to the reflection coefficient, \( n \) is the coating refractive index (constant, 1.46), \( t \) is the coating thickness, \( \omega \) is the oscillation periodicity, \( p \) is the oscillation phase with respect to coating thickness, and \( R \) is the reflectivity of the etalon (see details in the Supporting Information). The oscillations are approximately sinusoidal for small \( R \), a fact that will be used later. The function easily fits the simulated data (adj. \( R^2 > 0.997 \) for all the models and wavelengths) and the noisier experimental results (adj. \( R^2 > 0.85 \)); examples of the fit are shown in Figure 6. A similar function can be used to accurately fit the transmission spectra (adj. \( R^2 \) ca. 0.8 for the three experimental systems; see the Supporting Information for details on the function).

Panels a, c, and e of Figure 7 present the etalon reflectivity (\( R \)) plots as a function of wavelength, for the three
experimental Au island systems (Au3nm, Au5nm, and Au10nm) and the two classes of modeled systems, i.e., 50 and 250 nm periodicity, with different particle diameters (15, 25, and 35 nm). As expected, the etalon reflectivity is higher for films with larger islands and for denser films (compare panel c with panel e). The peaks for the denser films (the experimental systems and the P50 models) are wider, due to the flat shape of the particle in the experimental systems and to the coupling between the particles in both systems. The effect of coupling is manifested as a wide feature in the red-NIR range (seen mostly in the experimental systems, and hinted at by the wide peak for the P50D35 model), absent in the sparse films (P250 models), which are functionally single particles, without coupling effects. The similarity between the experimental systems and the dense models (P50) indicates interaction between the particles in the experimental films.

Panels b, d, and f of Figure 7 show the phase (p) for the same systems. There is an inflection point around the LSPR peak (530–600 nm for the various systems and models), which is typical of resonances. Here, too, the behavior of the experimental systems and dense models (P50) is similar, and significantly differs from the sparse models (P250), mostly around the red-NIR region. This difference, again, indicates coupling between the particles as well as a smaller effect of reflection from bare substrate between the particles.

The models discussed up to this point included two contributions to the reflections at the NP film, from the Au islands themselves and from the coating/substrate interface, as $n_{(coating)} = 1.46$, and $n_{(glass)} = 1.5$. We can isolate the response of the particles by setting both refractive indices to be 1.46, thus eliminating reflection from the substrate; the phase plot for this case is shown in Figure 8. For the closely spaced P50D35 particles, a less abrupt phase shift around the inflection point is characteristic of strong damping, due to interparticle coupling. The plot for the widely spaced particles of the same size in the P250D35 model does not show this feature. Similar evidence of interparticle coupling is present in the phase plot for Au10nm (Figure 7b). Both the etalon reflectivity (Figure 5) and the phase (Figure 7) show a similar trend for all systems and models.

Figure 5. Calculated (a) transmission, (b) reflection, and (c) absorption spectra and (d) wavelength and intensity of the transmission minimum, as a function of film thickness, for the P50D35 model.

Figure 6. Examples of fitting (a) experimental and (b) simulated reflectance data at a wavelength of 560 nm using the etalon function (eq 1).
7a) and phase (Figure 7b) plots for Au10nm show a secondary feature around 670–680 nm, related to the higher-order modes in large and deformed particles (relative to spheres).

The periodicity ($\omega$) shown in Figure S4 of the Supporting Information is half the wavelength for all the systems, as expected for thin film interference. The plots for the various models all overlap on the diagonal; the plots for the experimental systems show some deviation due to noise, especially at longer wavelengths where the thickness range spans less than half a period, reducing the fitting accuracy. We previously measured the response decay lengths of such nanoisland arrays, and showed them to be very different (approximately 3.2, 5.2, and 21.9 nm for Au3nm, Au5nm, and Au10nm, respectively). In contrast, the oscillation period is identical for all the samples, including experiment and

---

Figure 7. Etalon reflectivity (a, c, e) and phase (b, d, f) derived from the etalon model for the experimental systems (a and b), the P50 model systems (c and d), and the P250 model systems (e and f).

Figure 8. Phase of the reflected light from the model systems, without reflections from the substrate, derived from the etalon model.
simulation; it can therefore be concluded that this oscillatory response is not related to the localized surface plasmon.

**Energy Distribution.** By use of the simulated spectra, one can examine the distribution of light between transmittance ($I_T$), reflectance ($I_R$), and absorbance ($I_A$). Panels a and b of Figure 9 show the fractional intensity of each mode for the P50D35 model at two wavelengths, 400 nm (far from resonance) and 540 nm (at resonance). As the coating thickness changes, the amount of light in either mode is modulated; when the reflectance is at a maximum, both transmittance and absorbance are at a minimum, and vice versa, i.e., the phase of $I_T$ and $I_A$ is opposite to that of $I_R$.

To quantify the average light intensity and oscillation amplitude in each mode, the intensity plots of the three modes at all wavelengths, as a function of coating thickness, were approximated to a sinusoidal function which holds when the reflection coefficient for each layer is relatively small

$$I = I_0 + A \sin \left[ \frac{\pi(x - x_c)}{\omega} \right]$$  \hspace{1cm} (2)

where $I$ is the intensity (reflectance, transmittance, or absorbance), $I_0$ is the average intensity, $A$ is the oscillation amplitude, $x$ is the dielectric coating thickness, $x_c$ is the coating thickness at which the sine term equals zero (representing the phase), and $\omega$ is the oscillation periodicity. Though the etalon function is more accurate, the simplified function is satisfactory for this comparison (adj. $R^2 > 0.97$ for $I_R$ and $I_T$, adj. $R^2 > 0.85$ for $I_A$ for the wavelength range shown in panels c and d of Figure 9), and its results are more straightforward and intuitive.

Because of the very low intensity, the absorbance plots could not be fit beyond 700 nm.

Panels c and d of Figure 9 show the results of applying the simplified sinusoidal model to calculated transmission, reflection, and absorption spectra of the P50D35 model. Figure 9c presents the average intensity of the three modes, revealing a transmission peak between the absorption and reflection peaks. Figure 9d shows the oscillation amplitude, which essentially corresponds to the part of the light that is modulated between the three modes, as the dielectric coating changes in thickness—the amplitude of $I_T$ is equal to the sum of the amplitudes of $I_A$ and $I_R$. At long wavelengths, the majority of light shifted from the reflection mode is transmitted, and the $I_T$ and $I_A$ curves almost converge; around resonance (ca. 540 nm), the absorption mode gains most of the light; at short wavelengths again the transmission mode gains most of the light, though a substantial fraction is absorbed by the Au interband transition.

Thus, the effect of the dielectric coating is to modulate the distribution of light between the three modes—reflection, transmission, and absorption. As can be expected, close to resonance the particles either absorb or reflect most of the light, while far from resonance the reflectivity and absorbance are low, and transmittance is high (Figure 9c). In essence, the observed spectra are a convolution of LSPR spectra and interference-based intensity oscillations in the thickness dimension; the exact phase and intensity of the interference depends on the wavelength, leading to apparent oscillations in the LSPR peak wavelength and intensity for thick coatings.

Figure 9. (a) Fraction of the light reflected, transmitted, or absorbed for the P50D35 model, coated with a SiO$_2$ layer of variable thickness, at 400 nm; (b) same, at 540 nm, around the plasmon peak. (c) Average intensity for the three modes; (d) oscillation amplitudes of the three modes. Data derived using the simplified sinusoidal model (eq 2).
4. CONCLUSIONS

We have analyzed the optical behavior of a plasmonic/dielectric layered system (the so-called long-range LSPR), using both experiment and simulation. The system effectively combines a Fabry–Pérot interferometer with a plasmonic array, enabling extraction of phase information of the plasmon resonance. Our analysis shows that the oscillations in the plasmon peak for thick dielectric overlayers derive from superposition of the plasmon spectra with thin-film interference. The optical design approach employed here is beneficial for this type of system, providing a full analytical description of the observations.

Previous works have suggested use of this long-range response for sensing purposes.\textsuperscript{19–24} The present work shows that the plasmonic NP arrays serve only as a reflective layer, with the sensing capability stemming from thin-film interference. Thus, in actual application of this interference response, the transmission interferometric adsorption sensor,\textsuperscript{49,50} and a variant utilizing plasmonic NPs.\textsuperscript{29} Another implementation, termed iLSPR (interference LSPR), used interference effects to increase the sensitivity of the LSPR transducer.\textsuperscript{27,28} Even very thin dielectric films measurably modulate the reflectance, an effect put to use in differential reflectance spectroscopy.\textsuperscript{51} The effect seen here, i.e., modulation of the energy distribution between reflection, absorption, and transmission, has also been used outside of sensing, for instance, to increase absorption and reduce reflection from metallic films for infrared mirrors and solar absorbers.\textsuperscript{52,53} Plasmonic arrays, displaying strong scattering and absorption, are widely investigated for use in solar cells, where interference effects are of critical importance for achieving high efficiencies.\textsuperscript{34–36}

ASSOCIATED CONTENT

Supporting Information

Details of the etalon model; transmission and reflection spectra and minimum positions for Au3nm and Au10nm samples; calculated transmission and reflection spectra for several models; oscillation periodicity plots for several systems; calculated and experimental reflectivity of coated AuNanom samples. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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REFERENCES

Oscillatory Behavior of the Long-Range Response of Localized Surface Plasmon Resonance Transducers

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Supporting Information

Asymmetric etalon fitting model

The reflectance data for experimental and simulated systems were fitted using the equation:

\[
I_R = \frac{A - 2\cos\left(\frac{2\pi nt}{\omega} + p\right)R}{1 + R^2 - 2R\cos\left(\frac{2\pi nt}{\omega} + p\right)}
\]  

(1)

\[
A = (r_{\text{air/SiOx}}^2 + r_{\text{SiOx/glass}}^2)/R
\]

\[
R = r_{\text{air/SiOx}} \times r_{\text{SiOx/glass}}
\]

where \(I_R\) is the measured reflection intensity, \(r_{\text{air/SiOx}}\) is the reflection coefficient amplitude at the air and SiOx coating interface, \(r_{\text{SiOx/glass}}\) the reflection coefficient amplitude at the SiOx coating and glass interface (including the plasmonic layer), \(n\) is the coating refractive index (constant, 1.46), \(t\) is the coating thickness, \(\omega\) is the periodicity, and \(p\) is the phase.

Similarly, transmission data can be fitted to the following function:

\[
I_T = \frac{B}{1 + R^2 - 2R\cos\left(\frac{2\pi nt}{\omega} + p'\right)}
\]  

(2)

\[
B = (t_{\text{air/SiOx}}^2 \times t_{\text{SiOx/glass}}^2)
\]
where $I_T$ is the reflection intensity, $t_{\text{air/SiO}_x}$ is the transmission coefficient amplitude at the air and SiO$_x$ coating interface, and $t_{\text{SiO}_x/glass}$ is the transmission coefficient amplitude at the SiO$_x$ coating and glass interface (including plasmonic layer).

**Supplementary figures**

![Supplementary figures](image)

**Figure S1.** Experimental transmission (a,b) and reflection (c,d) spectra, and peak wavelength shift and intensity change of the transmission minimum (e,f), for Au3nm (a,c,e) and Au10nm (b,d,f) samples coated with 13-348 nm SiO$_x$ films, shown using a color map.
Figure S2. Calculated transmission (a,c) and reflection (b,d) spectra for the P50D15 (a,b) and P50D25 (c,d) models coated with 60-300 nm SiO$_2$, shown using a color map.
Figure S3. Calculated transmission (a,c,e) and reflection (b,d,f) spectra for the P250D15 (a,b), P250D25 (c,d) and P250D35 (e,f) models coated with 60-300 nm SiO$_2$, shown using a color map.
Figure S4. Oscillation periodicity ($\omega$) for all the studied systems, determined using the etalon model (Equation 1); the plots for the various models practically overlap, with the experimental systems showing some deviations from the diagonal.

Figure S5. Experimental and calculated (using FilmWizard software) reflectance at a wavelength of 400 nm for AuCont slides coated with SiO$_x$ films of increasing thickness. The agreement between the two plots provides further confirmation of the ellipsometrically determined coating thicknesses.
5.4 LSPR and interference transducers


Localized surface plasmon resonance (LSPR) transducers have been widely investigated for use in sensing applications. An alternative approach based on interference from thin films (Fabry-Pérot interferometers) has been previously advanced, offering a rather high sensitivity. Both methods involve easily produced substrates and simple optical setups. Here, the sensitivity offered by typical transducers of the two kinds in several sensing scenarios is compared quantitatively, using experimental and simulated data, and their respective advantages are discussed. To facilitate the comparison a simple sensitivity parameter is proposed. It is concluded that LSPR transducers offer superior sensitivity for analytes and recognition interfaces of small dimensions (up to several nanometers), especially in a wet environment, while the interference transducers become advantageous for thicker layers in dry conditions. For LSPR transducers, significantly higher sensitivity is obtained by using reflection, rather than transmission, measurements.

In this study we draw upon the sensing properties of LSPR transducers, which we characterized in previous studies (sections 5.1, 5.2) to examine realistic sensing scenarios, demonstrating the practicality and strength of systematic characterization.
Comparative assessment of the sensitivity of localized surface plasmon resonance transducers and interference-based Fabry-Pérot transducers

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Localized surface plasmon resonance (LSPR) transducers have been widely investigated for use in sensing applications. An alternative approach based on interference from thin films (Fabry-Pérot interferometers) has been previously advanced, offering a rather high sensitivity. Both methods involve easily produced substrates and simple optical setups. Here, the sensitivity offered by typical transducers of the two kinds in a usual sensing scenario is compared quantitatively, using experimental and simulated data, and their respective advantages are discussed. To facilitate the comparison a simple sensitivity parameter is proposed. It is concluded that LSPR transducers offer superior sensitivity for analytes and recognition interfaces of small dimensions (up to several nanometers), especially in a wet environment, while the interference transducers become advantageous for thicker layers in dry conditions. For LSPR transducers, significantly higher sensitivity is obtained by using reflection, rather than transmission, measurements.

1 Introduction

Localized surface plasmon resonance (LSPR) systems have become a popular research topic in recent years, with the main practical goal being their use as optical transducers in sensing applications [1–6]. Such transducers, comprising ensembles of metallic nanoparticles (NPs), exhibit a strong extinction in the UV-vis-IR range, with the resonance wavelength depending on factors such as NP composition, size, distribution, and shape [7–10]. This extinction is the result of light resonating with charge density oscillations in the NPs. The frequency and intensity of the resonance depend on the dielectric properties of the particles’ environment [11, 12], which may change upon molecular binding to the NPs, either directly or through spacer layers [13–16]. The latter are used in order to afford properties such as selectivity and stability. LSPR transducers are often lauded for requiring only a simple reflection or transmission setup [3, 17, 18], and for their high sensitivity, offering measurable response to sub-monolayer coverage of analytes of interest [19–25].

LSPR transducers have been fabricated using a wide variety of techniques, including thermal evaporation [6, 26, 27], e-beam lithography [28, 29], colloidal lithography [30], nanosphere lithography [31], and nanoparticle immobilization [23, 32], forming random or ordered arrays of various dimensions, utilizing a variety of metals, most often Au and Ag [3]. The transducers are commonly coated with an adlayer comprising a recognition interface, to allow selective binding of specific analytes of interest to the metal surface. As the LSPR response is associated with an evanescently decaying electric field, extending from the metal NP surface into the medium, the existence of a dielectric adlayer reduces the response, more so for thicker layers [13].

The response of LSPR transducers to dielectric layer adsorption is usually described by the phenomenological equation (1) [33]:

$$R = m\Delta\eta \exp\left(-\frac{d_1}{l}\right) \left[1 - \exp\left(-\frac{d_2}{l}\right)\right]$$  \hspace{1cm} (1)

where $R$ is the transducer response (e.g., wavelength shift or intensity change of the LSPR band); $m$ is the refractive index sensitivity (RIS), i.e., peak shift or intensity change per refractive index unit (RIU) change; $\Delta\eta$
is the change in refractive index (RI) of the surrounding medium effected by the adsorbate; \( d_1 \) and \( d_2 \) are, respectively, the thicknesses of the recognition interface and analyte; and \( l \) is the plasmon effective decay length. In this approximation the recognition interface and analyte are assumed to have the same RI.

A structure composed of two layers, where the top layer is at least somewhat transparent and has a different RI from the bottom layer and the medium (e.g., air, solvent) will exhibit reflections from both the top interface (with the medium) and the second interface (between the two layers). These reflected beams will interfere with one another, a phenomenon termed thin film interference [34]. Adsorption of an analyte on the top layer will change the phase of the beam reflected from the second interface, and thus the intensity of the sum of the two beams. Such a system is termed a Fabry-Pérot interferometer, or etalon. The relationship between thickness and reflectance is approximately sinusoidal, with a periodicity of \( \lambda/2n \), where \( \lambda \) is the measurement wavelength and \( n \) is the adlayer RI.

Several groups, in various implementations, have promoted the use of the latter systems for sensing [35–40]. One example is reflectometric interference spectroscopy, advanced by Gauglitz and coworkers [37, 40, 41] and shown by them to be comparable in sensitivity to a commercial surface plasmon resonance (SPR) system [42]. Another notable technique is the transmission interference adsorption sensor, also found to offer a sensitivity comparable to that of SPR [43, 44]. Yet another variant combines plasmonic NPs with an interference layer to improve the sensitivity, a concept named interference LSPR (iLSPR) [36, 45–47].

Both LSPR and interference-based Fabry-Pérot type transducers enable detection of sub-monolayer coverage, and can be made specific using appropriate recognition interfaces. Both techniques require simple transmission or reflection optical setups, and can be prepared and functionalized using similar schemes. However, to our knowledge a clear comparison between these two optical methods is not found in the literature. In the present work we therefore compare quantitatively the sensitivity offered by basic interference transducers with that of LSPR transducers previously studied by us. In the analysis we use experimental data for LSPR transducers and calculated data for interference transducers. This comparison is justified by the fact that simulation of optically uniform multilayer systems is well understood and provides quantitative data on the transducer sensitivity. The calculations for the Fabry-Pérot type transducers are therefore based on known principles and were confirmed experimentally by us elsewhere [48].

2 Experimental

2.1 Materials

Glass microscope cover-slides (Schott AG borosilicate glass D263T No. 3, 22 × 22 mm², \( T_g \approx 557^\circ \text{C} \), supplied by Menzel-Gläser, Germany), cut to 22 × 9 mm² and used as substrates; Au (99.99%, Holland-Moran, Israel); \( \text{H}_2\text{SO}_4 \) (AR, Gadot, Israel); \( \text{H}_2\text{O}_2 \) (30%, Frutarom, Israel); sodium chloride (Frutarom, Israel); polyallylamine hydrochloride (PAH) (56 kDa, Sigma Aldrich); polystyrene sulfonate, sodium salt (PSS) (70 kDa, Polysciences Inc., Warrington, PA, USA); and ethanol (anhydrous, Gadot or Biolab, Israel), were used as received. All solutions were prepared using triply-distilled water; nitrogen was in-house supplied from liquid \( \text{N}_2 \).

2.2 Island-type gold films

Glass slides were cleaned in piranha solution (\( \text{H}_2\text{O}_2 \): \( \text{H}_2\text{SO}_4 \), 1:3 by volume; Caution: “piranha” solution boils upon mixing and is extremely corrosive) for approx. 1 h, washed three times in deionized water, three times in triply-distilled water, once in ethanol, dried under a nitrogen stream, and mounted in the evaporation chamber of a cryo-HV evaporator (Key High Vacuum) equipped with a Maxtek TM-100 thickness monitor. The chamber was evacuated to a pressure of 2–3×10⁻⁶ torr, and gold was evaporated onto the slides from a resistively heated tungsten boat. To achieve homogenous deposition, the plate carrying the glass slides was rotated during the evaporation. Gold films of 3 nm, 5 nm and 10 nm nominal (mass) thickness, henceforth referred to as Au3nm, Au5nm and Au10nm, respectively, were deposited at a rate of 0.01 nm/sec. The Au-coated slides were annealed 10 h at 580°C in a Ney Vulcan 3-550 furnace, at a heating rate of 5°C/min, and then left to cool to room temperature inside the furnace. The annealing process leads to the final Au island morphology and stabilizes the islands [27,32].

2.3 Polyelectrolyte layer-by-layer (LbL) assembly

The LbL procedure was carried out using the positive polyelectrolyte poly(allylamine hydrochloride) (PAH) and the negative polyelectrolyte poly(styrene sulfonate), sodium salt (PSS). 1.0 mM solutions (concentration calculated with respect to the monomer, corresponding to 0.093 mg mL⁻¹ PAH and 0.206 mg mL⁻¹ PSS) in 0.1 M
NaCl, in triply-distilled water. Au island films prepared as described above were treated 10 min in a UV/Ozone apparatus (UVOCs Inc. model T10*10/OES/E), with the Au coating facing the UV lamps. The samples were then washed 20 min in ethanol [49] and dried under a nitrogen stream. A slide to be coated was alternatingly immersed in vials containing the polyelectrolyte solutions for 15 min each, starting with PAH. After PAH the slide was rinsed with water, dipped into an aqueous solution of 0.1 M NaCl (to avoid changing the ionic strength of the next deposition solution with residual water on the slide), and immersed in a PSS solution. After each PAH/PSS bilayer, the slide was rinsed with water, and measured. For spectral measurements, some slides were measured in water, and some were dried under a nitrogen stream to be measured in the dry state. The LbL procedure was then resumed, by first dipping the slide in a 0.1 M NaCl solution and then in PAH. The widely-used LbL scheme produces transparent conformal coatings of controllable thicknesses [13, 50, 51]. The thickness of each PAH/PSS bilayer, under the listed conditions, is $2.09 \pm 0.03$ nm and their RI in the visible range is approximately 1.56, with negligible absorption ($k \approx 0$), as determined by spectroscopic ellipsometry of polyelectrolyte-coated continuous Au films [13]. The adsorption and characterization were carried out in a climate-controlled laboratory, at a temperature of $22.5 \pm 1.0^\circ$C and a humidity of $50 \pm 5\%$.

### 2.4 Characterization methods

Extinction spectra at normal incidence were measured using a Varian Carey 50 Probe UV/Vis spectrophotometer. Measurement parameters: wavelength resolution, 1 nm; scan rate, 300 nm/min; average acquisition time per point, 0.2 sec. Reflection spectra were measured using an Ocean Optics USB4000 spectrophotometer equipped with a Polychromix Mobilight light source and an Ocean Optics reflection probe. Measurement parameters: wavelength resolution, 0.2 nm; acquisition time per spectrum, 0.005 sec; 200 scans to average. High-resolution scanning electron microscopy (HRSEM) images were obtained using a Carl Zeiss Ultra-55 Ultra-high-resolution SEM, operated at 15 kV. The samples were coated with a thin layer of Cr (2 nm for Au3nm slides, 3 nm for others) for improved conductivity.

### 2.5 Simulation

Reflectance values for interference-based transducers were calculated using FilmWizard software (Scientific Computing International, CA, USA).

### 3 Results and discussion

#### 3.1 Sensing scenario

Figure 1 illustrates the general structure of the LSPR and interference based transducers discussed in this work. Light is impinging on the transducers from the top side at normal incidence, while the transmitted or reflected light is measured. In both transducers a glass substrate is employed ($\tilde{n}_1 = 1.45–1.47$ in the wavelength range 400 to 800 nm) and the medium is either air ($\tilde{n}_4 = 1.0$ in the same range) or water ($\tilde{n}_4 = 1.33$ in the same range). In many biosensing applications measurements have to be performed in an aqueous environment, usually a buffer solution. As both transducers utilize the change in RI between medium and analyte, the sensitivity is expected to be lower in water in both cases. The analyte, recognition interface, and interference layer are all approximated using a polyelectrolyte (PE) multilayer assembly, composed of 2.1 nm thick PAH/PSS bilayers (see Experimental section), with a RI $n_3 = 1.56$ and negligible absorption, i.e., $k_3 = 0$; this RI is typical of organic layers. In real cases the RIs of the various layers can differ, changing the reflectivity and introducing multiple reflections, but this effect is small and is neglected in the present discussion. We

![Figure 1](online color at: www.ann-phys.org) Sensing configurations of the interference (left) and LSPR (right) based transducers.
assume a recognition interface 2.1 nm thick and an analyte having the same thickness. These values represent a reasonable choice in the wide range of thicknesses of biological systems [21].

3.2 Evaluation methodology

In either type of transducer, the result of layer adsorption is a change in reflectance or transmittance. In LSPR transducers the measured parameter is commonly the shift of the peak wavelength. This shift translates directly to intensity change at a single wavelength, which is a convenient alternative, as it is readily comparable with the interference transducers and its measurement is simple. The magnitude of the change can be evaluated in two ways– absolute change or relative change. Large relative changes are needed for achieving high sensitivity. Large absolute changes are desired for better stability – optical sensors can suffer from several types of noise, such as environmental light, detector dark noise, instability of the light source output, and scattering particles in the sample (insoluble matter or bubbles in wet measurements). Thus, an optimal transducer will furnish both large absolute and relative changes of the measured signal upon analyte adsorption. To facilitate the comparative evaluation we therefore define a sensitivity parameter which is the product of the two: \( S = \Delta I \cdot \frac{\Delta \lambda}{\lambda} \), where \( \Delta I \) is the absolute change in transmittance or reflectance upon adsorption, and \( I \) is the mean of the transmittance or reflectance before and after adsorption.

3.3 LSPR based transducers

To analyze the sensitivity of LSPR based transducers, we use as a model system gold nano-island films prepared by thermal evaporation and annealing, extensively studied by our group [13,21,26,27,32,52]. These films display an extinction peak in the visible-to-NIR region, ranging from ca. 520 nm to 900 nm, depending on the average particle size. Scanning electron microscopy images of representative samples are shown in Fig. 2. We have previously characterized the response of such films to dielectric layer adsorption using a polyelectrolyte (PE) layer-by-layer assembly scheme [13]. We will discuss samples of three average island sizes, produced by evaporating Au films of 3, 5 and 10 nm nominal (mass) thickness and annealing, henceforth referred to as Au3nm, Au5nm and Au10nm, respectively. The mean island diameters in these sample types are 22 ± 5 nm, 34 ± 10 nm and 114 ± 41 nm, respectively, with an in-plane aspect ratio of 1.1–1.2. These sample types show quite different refractive index sensitivity (RIS) and decay length values [13, 17]. LSPR spectra measured in either transmission or reflection modes will be discussed. While such spectra are most commonly presented in terms of extinction, for the purpose of the present comparison the use of transmittance is more appropriate, being the actual measured physical value.

Figure 3 shows representative spectra for an Au3nm sample, in both the transmission (Fig. 3a) and reflection (Fig. 3b) modes, illustrating the changes upon layer adsorption. The maximal absolute and relative intensity changes occur at very close wavelengths, somewhat red-shifted from the LSPR peak, as the intensity change is accompanied by a red shift of the peak wavelength.

Table 1 lists the transmittance and reflectance values, measured in air, for the three LSPR transducer types (each value is an average of two samples), for the bare Au surface, following adsorption of a 2.1 nm polyelectrolyte layer (simulating a recognition interface), followed by adsorption of an additional identical layer (simulating an analyte). Table 2 is analogous to Table 1, for spectra measured in water. The discussion below focuses on the sensitivity values, which are summarized in Fig. 4. In both media, in transmission and reflection modes, the optimal transducer for this scenario is the Au5nm, furnishing the highest sensitivity values. Measurements in the reflection mode are preferred in all cases, in agreement with our previous results for bulk RI changes [17]. In air, reflection measurements furnish an S value 1.8–2.4 times larger than transmission mode measurements, while in water the advantage is even greater, with a ratio of 3.4–5.9. While transducers with larger particles (Au10nm) ex-
hibit a higher RIS values ($m$ in Eq. 1), they also display longer decay lengths, reducing their response for thin layers, which occupy a smaller fraction of their larger sensing volume. Thus, the optimal LSPR transducer depends on the dimensions of the recognition interface and the analyte layer, a topic previously treated by us [13]. As the LSPR response is strongly wavelength-dependent (Fig. 3), so is the sensitivity value; measurement at a wavelength slightly longer than the surface plasmon resonance peak will yield the largest $S$ value, with longer or shorter wavelengths producing much smaller $S$ values. Figure S1, Supporting Information, shows the wavelength dependence of the $S$ value for measurements in water; the trend for measurements in air is similar. The strong wavelength dependence may require tuning of the LSPR transducer in cases where the usable wavelength range is limited, e.g., in various biological solutions.

### 3.4 Interference based transducers

The structure of the interference transducer is shown schematically in Fig. 1. The analysis performed by Brecht and Gauglitz [40] suggests a way to select a design with superior performance. The two reflected beams (from the air/interference layer and the interference layer/reflective layer interfaces) should have equal intensities, so that under conditions of destructive interference the measured value of $S$ will be maximal. For the design shown in Fig. 1, the two reflected beams should have equal intensities, as discussed in Sect. 3.4. The specific values of the transmittance and reflectance at the wavelengths of interest are shown in Tables 1 and 2.

#### Table 1 Transmittance and reflectance values (row 2, in percent), changes accompanying layer adsorption (rows 3 and 4), and the sensitivity $S$ (row 7), for various LSPR transducers, measured in air. The wavelength (row 1) was chosen as that producing a maximal value of $S$.

<table>
<thead>
<tr>
<th>Wavelength [nm]</th>
<th>Transmittance</th>
<th>Reflectance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Au3nm</td>
<td>Au5nm</td>
<td>Au10nm</td>
</tr>
<tr>
<td>1</td>
<td>571</td>
<td>571</td>
</tr>
<tr>
<td>2</td>
<td>89.21</td>
<td>87.07</td>
</tr>
<tr>
<td>3</td>
<td>82.72</td>
<td>79.07</td>
</tr>
<tr>
<td>4</td>
<td>79.09</td>
<td>73.67</td>
</tr>
<tr>
<td>5</td>
<td>Absolute change for analyte($a$)</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Relative change for analyte($b$)</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>$S$($c$)</td>
<td>16.29</td>
</tr>
</tbody>
</table>

*a Difference between rows 4 and 3. $b$ Ratio of row 5 and the mean of rows 3 and 4, in percent. $c$ Product of rows 5 and 6.

#### Table 2 Transmittance and reflectance values (row 2, in percent), changes accompanying layer adsorption (rows 3 and 4), and the sensitivity $S$ (row 7), for various LSPR transducers, measured in water. The wavelength (row 1) was chosen as that producing a maximal value of $S$.

<table>
<thead>
<tr>
<th>Wavelength [nm]</th>
<th>Transmittance</th>
<th>Reflectance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Au3nm</td>
<td>Au5nm</td>
<td>Au10nm</td>
</tr>
<tr>
<td>1</td>
<td>572</td>
<td>577</td>
</tr>
<tr>
<td>2</td>
<td>88.36</td>
<td>80.22</td>
</tr>
<tr>
<td>3</td>
<td>85.18</td>
<td>76.75</td>
</tr>
<tr>
<td>4</td>
<td>83.78</td>
<td>74.72</td>
</tr>
<tr>
<td>5</td>
<td>Absolute change for analyte($a$)</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Relative change for analyte($b$)</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>$S$($c$)</td>
<td>2.32</td>
</tr>
</tbody>
</table>

*a Difference between rows 4 and 3. $b$ Ratio of row 5 and the mean of rows 3 and 4, in percent. $c$ Product of rows 5 and 6.
ence, the total reflectance will approach zero, thus maximizing the relative changes. The intensity of the reflected light is given by:

\[ I_R(\lambda) = I_1 + I_2 + 2\sqrt{I_1 I_2} \cos \left(2\pi \frac{2nd}{\lambda}\right) \]  \hspace{1cm} (2)

where \( I_1 \) and \( I_2 \) are the intensities of the reflected beams, \( n \) and \( d \) are the RI and thickness of the dielectric layer, and \( \lambda \) is the measurement wavelength. For low reflectance (a few percent) of at least one of the interfaces, the intensity can be approximated by Fresnel’s relation:

\[ R = \left(\frac{n_i - n_j}{n_i + n_j}\right)^2 \]  \hspace{1cm} (3)

where \( n_i \) and \( n_j \) are the RIs on both sides of the interface.

The reflectivity changes in an approximately sinusoidal manner with adlayer thickness, with a periodicity of \( \lambda/2n \); thus, a shorter wavelength will result in a shorter periodicity and a larger reflectance change per unit thickness of the adlayer. We therefore present measurements calculated at two wavelengths, 400 nm – being short but still easy to use in experimental setups, and 800 nm – as this NIR wavelength is within the so-called “optical window” in biological tissue, enabling measurements in physiological liquids.

Figure 5a shows a calculated plot of the intensity of reflected light (at 400 nm) from a glass/Au (100 nm)/PE system in air, as well as the respective sensitivity parameter \( S \), as a function of PE thickness. Figure 5 panels b and c show the reflectance and the sensitivity \( S \) as a function of PE layer thickness, for a glass/ZrO₂ (130 nm)/PE film, at 400 nm (b) and 800 nm (c), in air. The \( S \) plots always show double peaks, corresponding to the negative and positive slopes of the reflectivity vs. PE thickness curves. In the 800 nm reflectivity graph the oscillation period is longer, thus decreasing the slopes and lowering the sensitivity.

Table 3 lists the reflectance values and absolute and relative changes upon PE layer adsorption for transducers with a 100 nm Au, 130 nm ZrO₂, or 84 nm Al₂O₃ film at wavelengths of 400 and 800 nm, in air and in water. The sensitivity values are summarized in Fig. 4b. The response at 800 nm for Au is miniscule, while ZrO₂ and Al₂O₃ produce usable responses. In all cases, the Au based transducer is inferior to ZrO₂, and for transducers with a 100 nm Au film as a high-reflectivity reflective layer.

### Table 3 Reflectance values (row 2, in percent), changes accompanying layer adsorption (rows 3 and 4), and the sensitivity \( S \) (row 5), for interference transducers. The spacer thickness (row 1) was chosen as that producing a maximal value of \( S \).

<table>
<thead>
<tr>
<th>Reflective layer</th>
<th>100 nm Au</th>
<th>130 nm ZrO₂</th>
<th>84 nm Al₂O₃</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Medium</strong></td>
<td>Air</td>
<td>Water</td>
<td>Air</td>
</tr>
<tr>
<td><strong>Wavelength [nm]</strong></td>
<td>400</td>
<td>800</td>
<td>400</td>
</tr>
<tr>
<td>1 Spacer thickness [nm]</td>
<td>59.5</td>
<td>136.6</td>
<td>78.7</td>
</tr>
<tr>
<td>2 Spacer</td>
<td>20.08</td>
<td>94.15</td>
<td>7.06</td>
</tr>
<tr>
<td>3 +2.1 nm “interface”</td>
<td>21.71</td>
<td>94.27</td>
<td>8.23</td>
</tr>
<tr>
<td>4 +2.1 nm “analyte”</td>
<td>23.42</td>
<td>94.39</td>
<td>9.50</td>
</tr>
<tr>
<td>5 Absolute change for analyte (a)</td>
<td>1.71</td>
<td>0.12</td>
<td>0.63</td>
</tr>
<tr>
<td>6 Relative change for analyte (b)</td>
<td>7.58</td>
<td>0.13</td>
<td>2.20</td>
</tr>
<tr>
<td>7 ( S^{(c)} )</td>
<td>12.97</td>
<td>0.02</td>
<td>1.38</td>
</tr>
</tbody>
</table>

*a* Difference between rows 4 and 3. *b* Ratio of row 5 and the mean of rows 3 and 4, in percent. *c* Product of rows 5 and 6.
ues in water, though the latter are associated with very low reflectance values ($\approx 1\%$ or less, row 2 in Table 3), which are sometimes less desirable, due to noise issues in practical implementation. The performance in air is greatly reduced compared to ZrO$_2$, with only a minor improvement in water. This result indicates that the refractive index of the reflective coating is less important than initially anticipated.

The dielectric coating modulates the distribution of light energy between the transmission, scattering (reflection) and absorption modes [48]. For an Au film, at 800 nm the absorption mode is negligible, while scattering is dominant. Thus, adsorption of adlayers produces very little response.

An Au nano-island film (as in the LSPR transducers) can also be used as the reflective layer in interference transducers. For the case of $n_3=1.46$ (SiO$_x$ coating), the reflectivities for an Au5nm reflective layer, measured at 400 nm, are listed in Table S1, Supporting Information (together with experimental details). The $S$ values for the Au5nm system also depend on the wavelength, with the highest values obtained by measuring near the resonance wavelength. The $S$ values thus obtained are close to those calculated for a 100 nm Au reflective film (Table S1, Supporting Information).

The $S$ values for LSPR and interference-based transducers are shown in Fig. 4, for adsorption of a 2.1 nm thick analyte on top of a 2.1 nm thick recognition interface, as previously discussed. Comparing these values, and the values in Table S1, Supporting Information, it is clear that the best sensitivity values of the interference transducers examined here are lower than those measured for the LSPR transducers under similar conditions.

Figure 3 (online color at: www.ann-phys.org) Transmission (a) and reflection (b) spectra of a representative Au3nm sample, measured in air. The dashed vertical lines indicate the wavelengths producing the largest $S$ values (see Table 1).

Figure 4 (online color at: www.ann-phys.org) (a) Sensitivity values in air and in water for the LSPR transducers; $T$ and $R$ denote transmission- and reflection-mode measurements. (b) Sensitivity values in air and in water for the interference-based transducers, measured at wavelengths of 400 and 800 nm (indicated).
and the calculated reflectance of a 130 nm ZrO$_2$ interference transducer with a 78.7 nm spacer, for increasing PE analyte thickness on a 2.1 nm recognition interface, measured in air. Sensitivity values for the same transducers are presented in Fig. 6b. For the LSPR transducers, increasing the analyte thickness shows a limiting (sub-linear) behavior, attributed to the decay of the plasmon electric field [13]. For the interference transducer, increasing the analyte thickness, up to ca. 20 nm, results in a linearly increasing response (slope = 0.81%/nm, R$^2$ = 0.99965). The LSPR transducers maintain their sensitivity advantage even for 20 nm thick analytes, although their lead is gradually lost for thick analytes.

Thicker recognition interfaces pose no difficulty for the interference transducers, as the combined thickness of the interference layer and recognition interface can

3.5 Analyte and recognition layer thickness

For the comparison discussed above, we assumed that the analyte and recognition interface are each 2.1 nm thick; however, these layers can be of different thicknesses, as is the case with certain biological analytes and interfaces. Figure 6a shows the experimental reflectance of an Au5nm LSPR transducer at a wavelength of 590 nm, and the calculated reflectance of a 130 nm ZrO$_2$ interference transducer with a 78.7 nm spacer at a wavelength of 400 nm, for various analyte thicknesses on top of a 2.1 nm recognition interface, measured in air. Sensitivity values for the same transducers are presented in Fig. 6b.
We carried out a comparative study of the sensitivity of LSPR transducers, increasing the separation between the plasmonic array and the analyte greatly reduces the response. For such LSPR transducers and a 2.1 nm thick analyte, a 10.5 nm recognition layer will produce a sensitivity value of 12.00 (data not shown), slightly below that of the interference transducer (Table 3). Thus, for thick interfaces the interference transducers can offer better results.

3.6 Transducer geometry

Examination of the LSPR and interferometric transducers presented in Fig. 1 shows that the geometry of the sensing scheme is quite different: in LSPR transducers, the analyte is bound solely to the metal islands, while the interferometric sensor provides a spatially uniform sensing surface. This difference may imply an additional advantage of LSPR transducers in kinetic experiments, where diffusion to spatially separated binding sites (Au islands) is enhanced compared to a uniform planar diffusion (interferometric sensor), analogous to the behavior of microelectrode arrays [53].

4 Conclusions

We carried out a comparative study of the sensitivity of a variety of LSPR and interference based (Fabry-Pérot) transducers to dielectric layer adsorption, under dry and wet conditions, and explored the effects of the measurement wavelength as well as the thickness of the analyte and recognition interface. A sensitivity parameter $S$ was introduced, taking into account both the relative and the absolute changes of the optical signal upon analyte adsorption. In the sensing scenario examined here (i.e., the analyte and recognition layer are both 2.1 nm thick, typical of common biological systems), LSPR transducers offer large absolute and relative changes, resulting in considerably higher $S$ values compared to the interference transducers, by a factor of 4.7 in air and 8.9 in water, for the best performing transducer of each type. The analysis also shows that for LSPR transducers the use of reflection measurements provides better sensitivity than transmission measurements by a factor of 1.8–2.4 in air and 3.4–5.9 in water, in agreement with our previous report [17]. The difference stems from the lower intensity of the reflected light, resulting in higher relative changes.

The advantage of LSPR transducers decreases when thick analytes are used, and more so for thicker recognition interfaces. The response of LSPR transducers is significantly higher for thin analytes and recognition layers, while showing a limiting behavior for thicker analytes; the latter is inherent to LSPR transducers. On the other hand, interference based transducers produce a linear response even for thick analytes, and are insensitive to the recognition layer thickness.

The maximal response of LSPR transducers is measured in the vicinity of the resonance wavelength (usually somewhat red shifted with respect to it); the latter can be tuned by adjusting the particle size and composition (e.g., Ag particles exhibit shorter resonance wavelengths). Larger particles generally exhibit a resonance at longer wavelengths and display higher RIS values, but also show longer decay lengths, which tend to reduce the sensitivity, especially for thin analyte layers [13]. Measurement wavelengths far from resonance can be used, but at a cost of reduced sensitivity (see Fig. S1, Supporting Information). Interference based transducers can be effective over a wide wavelength range, using different interference layer thicknesses, although longer wavelengths offer a reduced sensitivity compared to shorter wavelengths. This also requires a reflective layer with appropriate reflectivity at the desired wavelength. While single wavelength measurements suffice for the present discussion, both types of transducers can benefit from applying more advanced analysis techniques, taking advantage of changes to the entire spectrum upon analyte adsorption [24].

Overall, LSPR transducers are clearly advantageous for thin (several nm) analytes and recognition interfaces, especially in wet media. Interference transducers gain advantage with thicker layers (beyond ca. 10 nm), and more so when the usable measurement wavelengths are constrained.

Our choice of Fabry-Pérot type interference transducers as a reference technology for evaluation of the analytical performance of LSPR based transducers was motivated by the fact that both systems require simple optical setups and are based on similar measurements. Quantitative comparison of LSPR transducers to other competing technologies would be beneficial in enhancing our understanding of the strengths and weaknesses of the LSPR approach and thus contribute to its implementation in sensing applications.

Supporting information for this article is available free of charge under http://dx.doi.org/10.1002/andp.201200165.

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Key words. LSPR, localized plasmon, sensor, nano-islands, sensitivity, interferometry.

References

Comparative Assessment of the Sensitivity of Localized Surface Plasmon Resonance Transducers and Interference-Based Fabry-Pérot Transducers

Ofer Kedem, Alexander Vaskevich*, Israel Rubinstein*

Department of Materials and Interfaces, Weizmann Institute of Science, Rehovot 76100, Israel

Supporting Information

Wavelength dependence of the sensitivity of LSPR transducers

Figure S1. Sensitivity values for LSPR transducers as a function of measurement wavelength in (a) transmission and (b) reflection modes, in water. The trend for measurements in air is similar.

SiO$_x$ coated samples

Thirty Au5nm samples were coated with different thicknesses of SiO$_x$ (x ≤ 2, n = 1.46), from 15 to 348 nm, using electron-beam assisted physical vapor deposition (EB-
PVD). The reflectance was measured for each thickness, showing an approximately sinusoidal relation between reflectance and thickness at each wavelength. To assist in further analysis, the plot was fit to a sinusoidal function:

\[ R = R_0 + A \sin \left( \frac{\pi(t - t_0)}{\omega} \right) \]

where \( R \) is the measured reflectance, \( R_0 \) is the average reflectance, \( A \) is the oscillation amplitude, \( t \) is the coating thickness, \( t_0 \) is the phase, and \( \omega \) is the oscillation period. Using this function, we produced reflectance values with 0.1 nm resolution of the coating thickness, from 15 to 350 nm. From this high-resolution data we extracted the optimal responses for analyte adsorption, this time for a 2.1 nm thick analyte with \( n_3 = 1.46 \). Reflectance values for a 100 nm Au film were calculated for \( n_3 = 1.46 \) and the results for both types are shown in Table S1.

**Table S1.** Reflectance values (row 2, in percent), changes upon layer adsorption, \( n=1.46 \) (rows 3 and 4), and the sensitivity \( S \) (row 7), for interference transducers, measured in air. The spacer thickness (row 1) was chosen as that producing a maximal value of \( S \).

<table>
<thead>
<tr>
<th>Reflective layer</th>
<th>Au5nm</th>
<th>Au5nm</th>
<th>100 nm Au</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wavelength [nm]</td>
<td>400</td>
<td>550</td>
<td>400</td>
</tr>
<tr>
<td>1 Spacer thickness [nm]</td>
<td>90.4</td>
<td>125.7</td>
<td>74.6</td>
</tr>
<tr>
<td>2 Spacer</td>
<td>7.12</td>
<td>9.85</td>
<td>28.38</td>
</tr>
<tr>
<td>3 +2.1 nm “interface”</td>
<td>7.56</td>
<td>10.50</td>
<td>29.52</td>
</tr>
<tr>
<td>4 +2.1 nm “analyte”</td>
<td>8.02</td>
<td>11.16</td>
<td>30.68</td>
</tr>
<tr>
<td>5 Absolute change for analyte(^a)</td>
<td>0.46</td>
<td>0.67</td>
<td>1.17</td>
</tr>
<tr>
<td>6 Relative change for analyte(^b)</td>
<td>5.91</td>
<td>6.19</td>
<td>3.89</td>
</tr>
<tr>
<td>7 ( S )(^c)</td>
<td><strong>2.72</strong></td>
<td><strong>4.14</strong></td>
<td><strong>4.55</strong></td>
</tr>
</tbody>
</table>

\(^a\) Difference between rows 4 and 3. \(^b\) Ratio of row 5 and the mean of rows 3 and 4, in percent. \(^c\) Product of rows 5 and 6.
5.5 Metal-enhanced fluorescence

*This part of the work was carried out in collaboration with Dr. Wendel Wohlleben (BASF, Ludwigshafen, Germany).*

For this work, we set out to study the distance dependent interaction (emission enhancement or quenching) between plasmonic metal nanoparticles (NPs) and fluorescent species. Our initial motivation was to look for a possible connection of plasmon decay length and the metal-enhanced fluorescence distance dependence. We used two types of gold nano-island films, of ca. 20 nm and ca. 100 nm average in-plane particle diameters (denoted here as Au3nm and Au10nm, respectively), to represent two vastly different particle sizes, with different resonance wavelengths and decay lengths. Figure 8 shows HRSEM images of some of the samples used.

![HRSEM images](image-url)

**Figure 8.** High-resolution scanning electron microscopy (HRSEM) images of (A) pristine Au3nm and Au10nm samples, 15 kV accelerating voltage; (B) fractured Au10nm sample coated with 100 nm SiOx, 10 kV; (C) same, combining the backscattered and in-lens SE detectors (50/50 ratio), to highlight the gold islands, 10kV. Samples were coated with 2 nm (Au3nm) and 3 nm (all else) of Cr for improved conductivity during imaging. The images in A and B were recorded using the in-lens SE detector.
The fluorophore used was the fluorescent complex ruthenium (II) trisbipyridine, [Ru(bpy)$_3$]$^{2+}$, and as spacers we used polyelectrolyte multilayers for thin spacers (2-72 nm), and electron-beam assisted physical vapor deposition (EB-PVD) deposited SiO$_x$ for thicker layers (up to 348 nm). The fluorescence intensity and lifetimes of these samples were compared with control samples, i.e., glass slides coated with PE and the fluorophore, without Au islands.

**Figure 9.** (A) Normalized fluorescence emission and excitation for [Ru(bpy)$_3$]$^{2+}$ in solution, emission spectrum measured while exciting at 454 nm, excitation spectrum determined by measuring the emission at 605 nm, and scanning over different excitation wavelengths; (B) normalized extinction spectra of Au3nm and Au10nm, coated with 21 nm PE, and normalized fluorophore emission in solution; (C) emission spectra for a glass control and for Au10nm samples coated with the fluorophore on 2 nm and 42 nm PE spacers; (D) fluorescence intensity, relative to control samples without gold islands, for Au3nm and Au10nm samples, coated with varying thicknesses of PE or SiO$_x$ (indicated).

**Results.** Figure 9 presents the fluorescence excitation/emission spectra of the fluorophore in solution (A), the spectral overlap between the Au films’ SP bands and the fluorophore emission in solution (B), an example of quenched or enhanced fluorescence of the fluorophore on Au10nm (C), and the distance dependence of the fluorescence intensity for the two transducer types (D). We find a maximal (fivefold)
enhancement for Au10nm with a spacer thickness of about 60 nm, followed by a slow decrease, reaching the control levels around 300 nm. For Au3nm we find mostly quenching, even at large separations.

The fluorescence emission spectra exhibit variations in the peak wavelength and spectral width (full width at half-maximum, fwhm), as a function of spacer thickness (Figure 10). The variation for Au10nm samples (Figure 10 panels B, D) appears to be a sinusoidal oscillation, whereas the data for Au3nm samples (Figure 10 panels A, C) is not clear enough to permit such a conclusion.

![Figure 10](image)

**Figure 10.** (A,B) Fluorescence emission peak wavelength and (C,D) full-width at half-maximum (FWHM) for (A,C) Au3nm and (B,D) Au10nm samples, coated with varying thicknesses of PE or SiOx (indicated).

Figure 11 presents the fluorescence spectra for Au3nm (A) and Au10nm (B) samples at two spacer thicknesses, clearly demonstrating the large shift in peak wavelength. The variation of both plasmon peak wavelength and emission peak wavelength causes the spectral overlap to change as a function of thickness; this is
demonstrated for two Au10nm samples of different spacer thickness in Figure 11 panels C and D.

Figure 11. (A,B) Normalized emission spectra for two spacer thicknesses (indicated) for (A) Au3nm and (B) Au10nm samples; (C,D) normalized emission and extinction spectra for two fluorophore-coated Au10nm samples, with (C) a 40 nm PE spacer and (D) a 148 nm SiO\(_x\) spacer, demonstrating the varying spectral overlap.

The interaction of fluorophores with metallic surfaces or metallic NPs can result in three main effects – changes to the radiative rate, the non-radiate rate, and the excitation cross-section of the fluorophore.\(^{79}\) The steady-state fluorescence intensity measurements cannot distinguish between the possible causes of intensity changes, and so fluorescence lifetime measurement are required.

We therefore measured the fluorescence lifetime for several samples. Examples of fluorescence decay curves are shown in Figure 12A.

The decay curves were fitted to a bi-exponential model:

\[
I = I_0 + A_1 \exp\left(-\frac{t}{\tau_1}\right) + A_2 \exp\left(-\frac{t}{\tau_2}\right)
\]  

(4)

where \(I\) is the fluorescence intensity, \(I_0\) is the baseline intensity, \(A_1\) and \(A_2\) are the amplitudes of the two exponentials, \(t\) is the time, and \(\tau_1\) and \(\tau_2\) are the lifetimes. Of the
numerous definitions of the mean or effective lifetime, we chose to adopt the definition used by Zhang et al.: \(^{100}\)

\[
\bar{\tau} = \frac{A_1 \tau_1^2 + A_2 \tau_2^2}{A_1 \tau_1 + A_2 \tau_2}
\]  

(5)

**Figure 12.** (A) Fluorescence decay curves for three Au10nm samples, coated with spacers of different thicknesses (indicated) and [Ru(bpy)$_3$]$^{2+}$; (B) the fast decay component lifetime $\tau_1$, (C) the slow decay component lifetime $\tau_2$, and (D) the mean fluorescence lifetime, $\bar{\tau}$ vs. spacer thickness.

Figure 12 panels B-D show the two lifetime components and the mean lifetime for the samples studied. Two glass control samples gave the following mean values: $\tau_1 = 118 \pm 5$ nsec, $\tau_2 = 1250 \pm 60$ nsec, $\bar{\tau} = 1190 \pm 60$ nsec. Both $\tau_2$ and $\bar{\tau}$ of the gold slides are shorter than the controls for essentially all the thicknesses. However, their qualitative behavior is similar for both island types, being generally shorter for thicker spacers, which display weaker fluorescence intensities. $\tau_1$ for Au10nm is longer than that of the controls, and for Au3nm – shorter than the controls. However, this component has only a small effect on the mean lifetime.
**Discussion.** While most studies in the field of metal-enhanced fluorescence present a distance dependence enhancement ranging up to only 20 or 30 nanometers, effects beyond this range were reported by several groups. Specifically, Kümmelon et al.\textsuperscript{89} report a long-range interaction, with peak intensity around 60 nanometers. This matches our own results, though we do not observe a peak at close separations, while they do. A strong distance dependence is also mentioned for the emission peak wavelength, but this is not elaborated upon.

While the samples we used are composed of gold islands, not smooth films, these islands can act as a mirror by far-field interaction, as was previously reported.\textsuperscript{101,102} As mentioned earlier, metal mirrors were reported to cause large oscillations in the fluorescent lifetime of nearby fluorophores, as a function of metal-fluorophore separation.\textsuperscript{85,87} The lifetime plots in those studies exhibit an initial maximum at quite short separations (~20 nm), followed by a decrease, with a second peak around 200 nm. Those values match our results quite well, especially considering that a NP array introduces a phase shift in the reflected field which is different from that introduced by continuous metal films, as we have shown in section 5.3.

Our results show a strong oscillation of the emission peak wavelength and peak width (fwhm). While such an oscillation is predicted by theory, the magnitude of the measured shift is much larger than that discussed elsewhere. Barnes, in his detailed review, mentioned shifts in the MHz to GHz range,\textsuperscript{84} while our measured shifts are in the THz range (a wavelength shift from 605 to 610 nm is about 4 THz in the frequency domain).

In the present work, fluorescence lifetime measurements revealed a shortened fluorescence lifetime for almost all samples, compared to the controls (glass slides without Au islands); however, there seems to be no correlation between fluorescence intensity and lifetime – the lifetimes for Au3nm and Au10nm samples are similar, despite the substantial difference in intensity. The relationship between fluorescence lifetime and intensity is not trivial, as there are several mechanisms at work. Aussenegg et al. explored this issue, and listed three mechanisms for the interaction of a metal surface with fluorescent molecules:\textsuperscript{103} (i) enhancement of the absorption, which increases the intensity but leaves the lifetime unchanged; (ii) enhancement of the emission, which shortens the lifetime, and can increase the intensity (for dyes with quantum yield < 1); (iii) dissipative loss to surface waves, which shortens the lifetime.
and decreases the intensity. More in-depth analysis will be needed to fully understand these results.
6 General Discussion

The presented works explore the interaction of Au nanoisland film based LSPR transducers with dielectric and fluorescent species. The interaction with dielectric species (or dielectric overlayers) is treated within the framework of sensing performance, examining the distance dependence, different measurement modes, and quantification of the sensing response compared to a competing technology.

The issue of decay length, previously studied only in isolated cases, or mentioned in passing, was studied systematically for the first time. Whereas most published works focus on increasing the refractive index sensitivity (RIS) at all costs, our results indicate that this approach is of limited value. We found that, compared to high-RIS transducers, transducers exhibiting relatively small RIS values can provide significantly higher responses to analyte adsorption, thanks to better matching between their plasmon decay length and layer dimensions. Our results show that the RIS and decay length are correlated in a near-linear manner. This correlation should be taken into account in quantification of LSPR transducer response using Equation 2, 

\[ R = m\Delta\eta(1 - exp(-d/l_d)) \]

where the parameters \( m \) and \( l_d \) are not to be considered as independent variables. The slope of the curve of this correlation is not constant, though, and seems to depend on the type of nano-structure; this is evidenced by the significantly steeper slope in the decay length / RIS correlation for gold nanorods, in our analysis of Tian et al.’s work. Thus, perhaps an alternative form of Equation 2 would incorporate a structure-dependent decay length / RIS correlation factor. More work, on other nanostructure types, is needed to gather sufficient experimental evidence to advance this change, and learn of the physical meaning of such correlation factors. The RIS is also correlated with the plasmon peak wavelength, as predicted by Miller et al.,\(^{97}\) and also confirmed by us (section 5.2); thus, the three parameters – RIS, plasmon peak wavelength, and decay length, are all linked. What is the physical meaning of this? What is the nature of this triple correlation? These are still open questions, but ones that we hope will be further treated both experimentally and theoretically.

Variations in the decay length might explain a curious finding in one of our group’s early publications on LSPR sensing. In 2002 Kalyuzhny et al. studied the response of gold island films (different in some details from the ones studied in the current work) to monolayers and sub-monolayers of various organic molecules.\(^{60}\)
They compared the performance of thermally annealed and unannealed films, and found that the unannealed samples provided much better sensitivities (e.g., a detection limit of 0.7% vs. 20% monolayer coverage for one type, unannealed and annealed, respectively). One possible explanation is that the unannealed films exhibit shorter decay lengths, which would enhance their response for thin monolayers, as studied there.

An issue which we did not address here, but which we think would be of interest for future research, is that of the recognition layer density. Two of the possible effects are: (i) When the recognition molecule (e.g., receptor) is smaller than the analyte (e.g., protein), in a dense recognition monolayer, some binding sites are blocked by analytes bound to adjacent sites. In such cases, a sparser recognition monolayer can result in larger analyte coverage, and a stronger signal. (ii) Even in the absence of steric interference, a closely packed receptor layer can display decreased affinity towards the analyte, compared to solution experiments. This could be due to an inability of the receptor-analyte complex to reach the minimal-energy conformation when in such a close-packed layer. Thus, controlling for recognition layer density, using either kinetic control or spacer molecules, can increase the response, as was shown in LSPR biorecognition systems studied by our group.¹⁰⁴

To further explore the sensing performance, we studied the differences between sensing in the transmission and reflection modes. Our results show that the reflection mode provides significantly higher responses, both for bulk RIS, and for thin layer adsorption.

Following our interest in reflectivity, we found the literature reports on long-range plasmon response curious, and proved the phenomenon to be the result of thin-film interference. The observed spectra were in effect a convolution of plasmonic effects and Fabry-Pérot modes; the latter effect shifted the plasmon peaks at long ranges, creating the illusion of a long-range plasmonic response. This study produced a way to extract the phase shift incurred by light reflected from a plasmonic nanoparticle film, demonstrating an inflection point in the phase around the resonance wavelength, as expected for a resonant system. Another product of the work is an analysis of the effect of a dielectric coating on the distribution of energy between the absorbance, transmittance and reflectance modes. One possible use of this analysis is in the incorporation of plasmonic nanoparticle (NP) layers in solar cells, where the above-mentioned energy distribution can greatly enhance or diminish the efficiency.
The sensitivity of interference spectra to thin layer adsorption, observed in the long-range study, piqued our interest in interference sensors, and led us to compare the performance of LSPR transducers with interference-based transducers, for a variety of scenarios. To facilitate the comparison, we proposed a new sensitivity parameter, which we hope will be further used by other researchers. Using this parameter, we quantified the advantages offered by LSPR transducers over interference-based transducers for detection of thin layer adsorption in both wet and dry conditions. We also quantified the advantage of reflection mode measurements, in line with our previous findings for bulk RIS. In addition, we demonstrated the limited LSPR response for thicker layers, due to the decay of the evanescent field, as well as the advantage in using transducers with smaller RIS and decay length. We hope that this study will prompt other researchers to invest effort in the comparison of LSPR transducers to alternative methods, in order to highlight relative strengths and weaknesses.

Our interest in the LSPR decay length led us to wonder about the decay length’s relation to other plasmonic phenomena, and we chose to investigate metal-enhanced fluorescence (MEF). We were interested in examining the distance dependence profile of MEF, to see whether it correlates with the plasmon decay length that we previously measured. While the distance dependence of MEF has been previously studied, it was never for NP films with a known plasmon decay length, whereas we had a series of well characterized films. Surprisingly, we found a very different distance dependence, with measurable effect on the fluorescence even for very large metal-fluorophore separations (even around 200 nm, while the decay length for the Au10nm films used is only ~20 nm). This long-range effect was previously shown for metal mirrors, and explained by the presence of reflected fields interfering and modifying the photonic mode density (PMD). This phenomenon is related to the long-range plasmonic effect that we studied above, as both are based on reflected fields.

Fluorescence lifetime measurements revealed a shortened fluorescence lifetime for almost all samples, compared to the controls (glass slides without Au islands); however, there seems to be no correlation between fluorescence intensity and lifetime – the lifetimes for Au3nm and Au10nm samples are similar, despite the substantial difference in intensity. We also observed an oscillation in the emission wavelength as a function of metal-fluorophore distance; such an effect was previously hypothesized, though at a significantly smaller oscillation amplitude. Further analysis and
theoretical work are needed to properly understand these results and put them in context, an effort that we are currently pursuing in collaboration with a theoretical group.

Throughout the study of sensing systems we pursued the goal of quantifying sensing performance as accurately as possible and identifying specific scenarios important in real-world sensing, to obtain the best possible transducer performance and maximal sensitivity in our gold island system. In general, throughout this work, we tried to focus on quantitative work, large sample sizes, and the production of robust datasets, to enable the drawing of strong conclusions.

It is my sincere hope that these results will advance both the application and basic understanding of LSPR systems, in sensing, interaction with fluorophores, and other possible applications.
7 References


Declaration of independent research

The work presented in this thesis is the result of my original and independent research, under the supervision of my advisor, Prof. Israel Rubinstein, and of Dr. Alexander Vaskevich, the group’s staff scientist.

For all projects, Prof. Rubinstein and Dr. Vaskevich guided and supervised the work, suggested experiments, measurements and analyses, and co-wrote the papers. One exception is the metal enhanced fluorescence project, in which Dr. Vaskevich was not involved. Unless otherwise noted, I performed all the experiments, characterizations and analyses, suggested research directions together with Prof. Rubinstein and Dr. Vaskevich, and co-wrote the papers.

Several of the projects were performed in collaboration with other researchers, and their contributions are outlined below.

1. In the decay length project, Alexander B. Tesler produced custom SEM samples holders, allowing me to image tilted samples.
2. In the long-range project, Prof. Takumi Sannomiya modeled the system, suggested analyses for both modeled and experimental results, and co-wrote the paper.
3. In the metal-enhanced fluorescence project, Dr. Wendel Wohlleben performed several of the experiments, and suggested some analyses and measurements.